

COMPARATIVE TRANSCRIPTOME ANALYSES OF A STANDARD STRAIN
AND CLAVULANIC ACID OVERPRODUCING INDUSTRIAL STRAIN OF *S.*
CLAVULIGERUS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY



BY
GÖZDE ÇELİK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOTECHNOLOGY

SEPTEMBER 2021

Approval of the thesis:

**COMPARATIVE TRANSCRIPTOME ANALYSES OF A STANDARD
STRAIN AND CLAVULANIC ACID OVERPRODUCING INDUSTRIAL
STRAIN OF *S. CLAVULIGERUS***

submitted by **GÖZDE ÇELİK** in partial fulfillment of the requirements for the
degree of **Master of Science in Biotechnology, Middle East Technical University**
by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Candan Gürakan Gültekin
Head of the Department, **Biotechnology**

Prof. Dr. Gülay Özcengiz
Supervisor, **Biological Sciences, METU**

Assoc. Prof. Dr. Çağdaş Devrim Son
Co-Supervisor, **Biological Sciences, METU**

Examining Committee Members:

Prof. Dr. Semra Kocabıyık
Biological Sciences, METU

Prof. Dr. Gülay Özcengiz
Biological Sciences, METU

Prof. Dr. Mustafa Akçelik
Biology, Ankara University

Assoc. Prof. Dr. Çağdaş Devrim Son
Biological Sciences, METU

Assoc. Prof. Dr. Can Özen
Biotechnology, METU

Date: 10.09.2021



I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Gözde Çelik

Signature :

ABSTRACT

COMPARATIVE TRANSCRIPTOME ANALYSES OF A STANDARD STRAIN AND CLAVULANIC ACID OVERPRODUCING INDUSTRIAL STRAIN OF *S. CLAVULIGERUS*

Çelik, Gözde

Master of Science, Biotechnology

Supervisor : Prof. Dr. Gülay Özcengiz

Co-Supervisor: Assoc. Prof. Dr. Çağdaş Devrim Son

September 2021, 171 pages

Streptomyces clavuligerus is the producer of the medically important β -lactam antibiotics, including cephamicin C (CC) and potent β -lactamase inhibitor clavulanic acid (CA). In this study, comparative transcriptome analysis between an industrial stain, namely DEPA (CA overproducer strain which was developed by several rounds of classical random mutagenesis), and a reference strain NRLL3585 was performed by using the genome-wide RNA-seq technique. Our aim was to understand and improve the knowledge about the complex mechanisms of clavulanic acid overproduction and find out novel targets for use of gene technologies (rational approach) for industrial strain development. According to RNA-seq analysis, structural genes of CA biosynthesis, arginine biosynthesis, G3P synthesis, clavam biosynthesis, and other additional genes/gene clusters that have some role in CA production as well as several regulatory genes like AdpA, TetR/AcrR, and CarD were found to be meaningfully up- or -downregulated. Transcriptomic findings as were also integrated to those of a recent comparative proteome analysis via nanoLC/MS MS obtained in our laboratory. Positive correlation between integrative

transcriptomic and proteomic analysis provided more precise insights into the mechanisms of CA overproduction in *Streptomyces clavuligerus* DEPA.

Keywords: *Streptomyces clavuligerus*, clavulanic acid overproduction, genome-wide RNA seq analysis, proteome, integrated -omic analysis



ÖZ

S. *CLAVULİRUS*'UN STANDART BİR ŞUSU İLE KLAVULANİK ASİTİ ÇOK YÜKSEK MİKTARDA ÜRETEBİR ENDÜSTRİYEL ŞUŞ ARASINDA KARŞILAŞTIRMALI TRANSKRİPTOM ANALİZİ

Çelik, Gözde
Yüksek Lisans, Biyoteknoloji
Tez Yöneticisi: Prof. Dr. Gülay Özcengiz
Ortak Tez Yöneticisi: Doç. Dr. Çağdaş Devrim Son

Eylül 2021, 171 sayfa

Streptomyces clavuligerus, sefamisin C (SC) ve güçlü β -laktamaz inhibitörü klavulanik asit (KA) dahil olmak üzere tıbbi açıdan önemli β -laktam antibiyotiklerinin üreticisidir. Bu çalışmada, ardışık klasik mutajenezle geliştirilmiş endüstriyel bir KA aşırı üretici suş olan DEPA ile referans suş olan NRLL3585'in karşılaştırmalı transkriptomik analizi için genom çapında RNA dizi analizi yapılmıştır. Amacımız, klavulanik asit üretiminin karmaşık düzenleyici mekanizmaları hakkındaki daha fazla bilgi edinmek ve gen teknolojileri kullanılarak yeni üretici suşlar geliştirilebilmesi (rasyonel yaklaşımlar) için yeni hedefler göstermektir. RNA dizi analizi sonuçlarına göre, KA biyosentezi genleri, arginin biyosentezi, G3P sentezi, klavam biyosentezi ve KA üretiminde herhangi bir rolü olan diğer ek yapısal genler / gen kümeleri, bunlara ek olarak da, AdpA, TetR/AcrR ve CarD gibi temel düzenleyicilerin ekspresyon seviyeleri anlamlı biçimde artmış ya da azalmıştır bu. Daha sonra, transkriptom sonuçlarının, daha önce laboratuvarımızda yapılmış olan nanoLC/MS MS temelli karşılaştırmalı proteomik analizi sonuçları ile entegrasyonu gerçekleştirilmiştir. Entegre (bütünleştirici) transkriptomik ve proteomik analizlerden elde edilen pozitif korelasyon,

Streptomyces clavuligerus'ta KA aşırı üretimi ve regülasyon mekanizmaları hakkında edindiğimiz bilgileri doğrulamıştır.

Anahtar Kelimeler: *Streptomyces clavuligerus*, klavulanik asit, genom çapında RNA sekans analizi, proteom, entegre -omik analiz





To My Family

ACKNOWLEDGMENTS

Firstly, I would like to express my sincere gratitude to my supervisor Prof. Dr. Gülay Özcengiz for all her endless support, excellent guidance, and constructive feedbacks throughout my studies. Through this journey, she has always encouraged me and helped me improve myself into my best version with the light she shed on my path and her inspiring comments. I am also grateful to my co-supervisor Assoc. Prof. Dr. Çağdaş Devrim Son for all his support and trust.

I would like to thank Assoc. Prof. Dr. Aslıhan Kurt Kızıldoğan and her Ph.D. student Çiğdem Otur for all their kind hospitality in Ondokuz Mayıs University, their ceaseless support and helps on my work. I am also thankful to Prof. Dr. Servet Özcan and his team in Kayseri for their help with proteomic analyses.

It is a pleasure to thank my labmates Caner Aktaş, Meltem Kutnu, Sergen Akaysoy, Duygu Keser, Ozan Ertekin, Nazlı Hilal Türkmen, İlayda Baydemir, and Naz Kocabay. They made these three years very productive and full of fun with their cooperation, understanding, patience, and precious friendship for me.

I am very thankful to my dearest friends Utku Günçe, Bensu Polat, Ecem Özatlan, Orçun Gökçen, İrem Akın, Yeşim Yıldız and Sergen Akaysoy from the bottom of my heart. Even if the miles between us, they have brought joyfulness to me even at the worst times and never spared their limitless friendship, love, and support for any time and anything.

Special thanks to my grandmother Leman Çelik for all the advice and support she gave me to stand on my feet as a woman. I also appreciate my aunt Gülümser Ak and my cousin Dilek Tükel for their generous and kindful help whenever I need.

Lastly but most importantly, I am endlessly thankful for my superheroes, my mother Hatice Çelik and my father Mete Çelik, also my sister Tuğçe Mutaş, my brother-in-law Volkan Mutaş and my dearest little nephew Toprak Mutaş. I have always felt their infinite support on my back, limitless trust, proudness, and unconditional love for me. I would never have accomplished this thesis without their warm hearts, very deep trusting embrace, and always smiling faces.

TABLE OF CONTENTS

ABSTRACT	v
ÖZ.....	vii
ACKNOWLEDGMENTS	x
TABLE OF CONTENTS	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTERS	
1 INTRODUCTION.....	1
1.1 The Genus <i>Streptomyces</i> and Its Life cycle	1
1.2 <i>Streptomyces clavuligerus</i> and Production of Secondary Metabolites.....	3
1.2.1 β -lactam Antibiotics	4
1.2.1.1 Developing Resistance Against β -lactam Antibiotics.....	6
1.2.2 Clavulanic Acid as a β -lactamase Inhibitor.....	6
1.2.2.1 Clavulanic Acid Gene Cluster.....	7
1.2.2.2 Biosynthesis of Clavulanic Acid.....	10
1.3 Regulation of Secondary Metabolism	11
1.4 Transcriptomics	14
1.4.1 Next-Generation Sequencing (RNA-sequencing)	17
1.5 Aim of the Present Study.....	20
2 MATERIALS AND METHODS	21

2.1	Bacterial Strains	21
2.2	Media and Culture Conditions	21
2.3	RNA Extraction and Purification	22
2.4	Transcriptomic Analysis	23
2.4.1	Total RNA Isolation	23
2.4.2	RNA Sequencing	23
2.4.3	Bioinformatic Analyses	23
2.5	Real-Time Quantitative Polymerase Chain Reaction	24
2.5.1	Total RNA Isolation	24
2.5.2	cDNA Synthesis with Reverse Transcription PCR (RT-PCR)	24
2.5.3	Real-Time Quantitative PCR (RT-qPCR)	25
2.5.4	Relative Quantification of qRT-PCR Data Based on Efficiency Correction	28
2.5.5	Statistical Analysis	29
2.6	Correlation Analysis	29
3	RESULTS	31
3.1	Summary of Clean Data from RNA-sequencing Analysis of <i>S.</i> <i>clavuligerus</i> NRRL3585 and DEPA strains	31
3.1.1	Pathway Analysis of Differentially Expressed Genes	33
3.1.2	Functional Classification of Differentially Expressed Genes	37
3.1.3	Differentially Expressed Genes Data Analysis	39
3.1.3.1	The Most Significantly Upregulated Genes in DEPA Strain	55
3.1.3.2	The Most Significantly Downregulated Genes in DEPA Strain	59
3.1.3.3	Secondary Metabolites Biosynthesis, Transport, and Catabolism	63

3.1.3.4	Energy Production and Conversion	66
3.1.3.5	Amino Acid Transport and Metabolism	67
3.1.3.6	Carbohydrate Transport and Metabolism.....	68
3.1.3.7	Lipid Transport and Metabolism.....	68
3.1.3.8	Coenzyme Transport and Metabolism	69
3.1.3.9	Nucleotide Transport and Metabolism.....	70
3.1.3.10	Inorganic and Organic Molecule Transport	70
3.1.3.11	Transcription/ DNA Replication, Recombination and Repair/ Translation, Ribosomal Structure, and Biogenesis.....	72
3.1.3.12	Posttranslational Modification, Protein Turnover, and Chaperones	73
3.1.3.13	Cell Wall/ Membrane/ Envelope Biogenesis	74
3.1.3.14	Cell Cycle Control, Dell Division, Chromosome Partitioning & Cell Motility & Chromatin Structure and Dynamics	74
3.1.3.15	Signal Transduction Mechanism.....	75
3.1.3.16	Defense Mechanism	76
3.1.3.17	Functions Unknown	77
3.2	Gene Expression Analysis for Validation of RNA-seq Results	79
3.3	General Characterization of Nano-LC/MSMS Data	83
3.4	Integrative Analysis of Transcriptomic and Proteomic Data	84
3.4.1	Comparison of the RNA-seq and nano-LCMS/MS data.....	113
3.4.1.1	Secondary Metabolism.....	113
3.4.1.1.1	Clavam Gene Cluster	113
3.4.1.1.2	CA-CC Supercluster	114
3.4.1.1.3	Others in Secondary Metabolism.....	115

3.4.1.2	Energy Production and Conversion	117
3.4.1.3	Amino Acid Transport and Metabolism	118
3.4.1.4	Translation, Ribosomal Structure, and Biogenesis.....	120
3.4.1.5	Carbohydrate Transport and Metabolism	121
3.4.1.6	Lipid Transport and Metabolism	122
3.4.1.7	Posttranslational Modification, Protein Turnover, and Chaperones	122
3.4.1.8	Cell Wall/ Membrane/ Envelope Biogenesis.....	123
3.4.1.9	Nucleotide Transport and Metabolism	124
3.4.1.10	Transcription, DNA Replication, Recombination and Repair.	125
3.4.1.11	Coenzyme Transport and Metabolism.....	127
3.4.1.12	Cell Cycle Control, Cell Division, Chromosome Partitioning	127
3.4.1.13	Signal Transduction Mechanism	128
3.4.1.14	Intracellular Trafficking, Secretion, and Vesicular Transport.	129
3.4.1.15	Inorganic/Organic Molecule Transport	129
3.4.1.16	Defense Mechanism.....	130
3.4.1.17	Functions Unknown.....	130
3.4.2	Correlation Analysis	132
4	CONCLUSION	135
	REFERENCES	141
APPENDICES		
A.	Compositions and Preparation of Culture Media/Buffers/Solutions	167
B.	Original Clean Data from Genome-wide Comparative RNA-seq	171

LIST OF TABLES

TABLES

Table 1.1 ORFs found in CA gene cluster and their functions	9
Table 2.1 Reverse transcription PCR components and the protocol	25
Table 2.2 RT-qPCR master mix preparation	26
Table 2.3 Reaction protocol of RT-qPCR	26
Table 2.4 Primer sequences	27
Table 3.1 Statistics of RNA-seq data	32
Table 3.2 Reorganized Transcriptome Data	41
Table 3.3 The top highly expressed genes in <i>S. clavuligerus</i> DEPA	58
Table 3.4 The most significantly downregulated genes in <i>S. clavuligerus</i> DEPA ..	62
Table 3.5 Detailed information about genes that are chosen for data validation. ...	81
Table 3.6 Integration of transcriptomic and proteomic data	85

LIST OF FIGURES

FIGURES

Figure 1.1 <i>Streptomyces</i> spp. life cycles.....	3
Figure 1.2 Derivatives of β -lactam antibiotics with their chemical structure and the producer microorganism.....	5
Figure 1.3 Clavulanic acid gene cluster and its paralog gene cluster.....	8
Figure 1.4 Biosynthetic pathway of clavulanic acid.....	11
Figure 1.5 Transcriptomic methods used over time.....	15
Figure 1.6 Workflow of RNA-sequencing experiment.....	18
Figure 1.7 Data analysis workflow of RNA-seq analysis.....	19
Figure 3.1 Volcano plot of differentially expressed genes.....	33
Figure 3.2 KEGG Pathway analysis of <i>S. clavuligerus</i> DEPA.....	35
Figure 3.3 The most enriched pathways of <i>S. clavuligerus</i> DEPA.....	36
Figure 3.4 GO functional classification of <i>S. clavuligerus</i> DEPA.....	38
Figure 3.5 The most enriched GO terms of <i>S. clavuligerus</i> DEPA.....	39
Figure 3.6 RT-qPCR results of selected a) upregulated genes b) downregulated genes.....	80
Figure 3.7 Venn Diagram representing the identified proteins via LC-MS/MS analysis of <i>S. clavuligerus</i> DEPA and NRRL3585 strains.....	83
Figure 3.8 Correlation analysis of the transcriptomic and proteomic data.....	132

LIST OF ABBREVIATIONS

ABBREVIATIONS

CA	:	Clavulanic Acid
PBPs	:	Penicillin-Binding Proteins
CC	:	Cephameycin C
G3P	:	Glyceraldehyde-3-Phosphate
PSR	:	Pathway-Specific Regulators
SARP	:	<i>Streptomyces</i> Antibiotic Regulatory Proteins
ORF	:	Open Reading Frame
TCSs	:	Two-Component Systems
EST	:	Expressed Sequence Tags
SAGE	:	Serial Analysis of Gene Expression
CAGE	:	Cap Analysis of Gene Expression
RNA-seq	:	RNA-sequencing
TSB	:	Tryptic Soy Broth
SA	:	Starch-Asparagine
NCBI	:	National Center for Biotechnology Information
SRA	:	Sequence Read Archive
FPKM	:	Fragments Per Kilobase Million
KEGG	:	Kyoto Encyclopedia of Genes and Genomes

GO	:	Gene Ontology
DEGs	:	Differentially Expressed Genes
DEPs	:	Differentially Expressed Proteins
RT-qPCR	:	Real-Time Quantitative Polymerase Chain Reaction
NRPS	:	Non-Ribosomal Peptide-Synthase
PKS	:	Polyketide Synthase
NIS	:	NRPS-Independent Siderophores
SDR	:	Short-chain Dehydrogenase/Reductase
FASII	:	Fatty Acid Synthase Type II System
MFS	:	Major Facilitator Superfamily
PEP	:	Phosphoenol Pyruvate
SMC	:	Secondary Metabolite Gene Cluster

CHAPTER 1

INTRODUCTION

1.1 The Genus *Streptomyces* and Its Life cycle

The genus *Streptomyces* belongs in the phylum *Actinobacteria* that is one of the most prominent taxonomic groups under the Bacteria domain. *Actinobacteria* are known as Gram-positive, filamentous, spore-forming bacteria with high GC content (70-74 mole %) in their genomes (Barka *et al.*, 2016).

Due to mycelial *Streptomyces*' way of growth, i.e., branching, and morphogenetic development, they look like filamentous fungi rather than bacteria. In contrast to their superficial characteristics fungi, *Streptomyces* spp. have basic bacterial traits. For example, their chromosome is organized like prokaryotic nucleoids, they have a peptidoglycan cell wall and are affected by antibacterial agents (Barka *et al.*, 2016; Flärdh, 2003).

There are three critical reasons for why *Streptomyces* constitute an important research area and take massive scientific attention. Firstly, over 95% of the soil population of *Actinobacteria* are composed of *Streptomyces* spp. which are phylogenetically very widespread. Secondly, in the soil, the members of the genus *Streptomyces* show important recycling activities; for example, enormous hydrolytic exoenzymes produced by *Streptomyces* help decomposition of insoluble organic debris from plants and fungi for the carbon cycle. Lastly, *Streptomyces* are able to produce a number of valuable secondary metabolites (Barka *et al.*, 2016). Some of these secondary metabolites are antimicrobials, antifungals, herbicides, anticancer compounds, antiviral and antiparasitic agents. From biotechnological point of view, the genus *Streptomyces* is of major pharmaceutical importance, given that two thirds

of all industrially manufactured antibiotics are synthesized by members of this genus (Demain, 2002; Hayashi *et al.*, 2013; Weber *et al.*, 2003).

Streptomyces spp. have a complicated life cycle to get adapted and survive harsh conditions in soil. The life cycle begins with the germination of spores. In the vegetative phase of growth, spores form long filamentous hyphae called substrate mycelium (Jones and Elliot, 2018). In response to nutrient depletion, the vegetative phase is followed by the aerial growth phase, an important phase for secondary metabolite production. In the aerial phase, hyphae extend away from the substrate mycelium into the air. Next, the aerial hyphae undergo massive septation to create a series of uninucleoid compartments. Finally, these compartments differentiate to create spore chains and after a while, the aerial hyphae are matured and septated to become pigmented spores produced for dispersal (Claessen *et al.*, 2006; Jones and Elliot, 2018). Recent research about the life cycle of *Streptomyces* spp. brought about a different growth model called 'exploration'. In the exploration growth model, colonies mimic the polymicrobial communities. Explorer cells have properties of both vegetative hyphae and aerial hyphae, and their growth is lateral, not vertical (Jones and Elliot, 2018). The classical and exploration life cycle of *Streptomyces* spp. are shown in Figure 1.1.

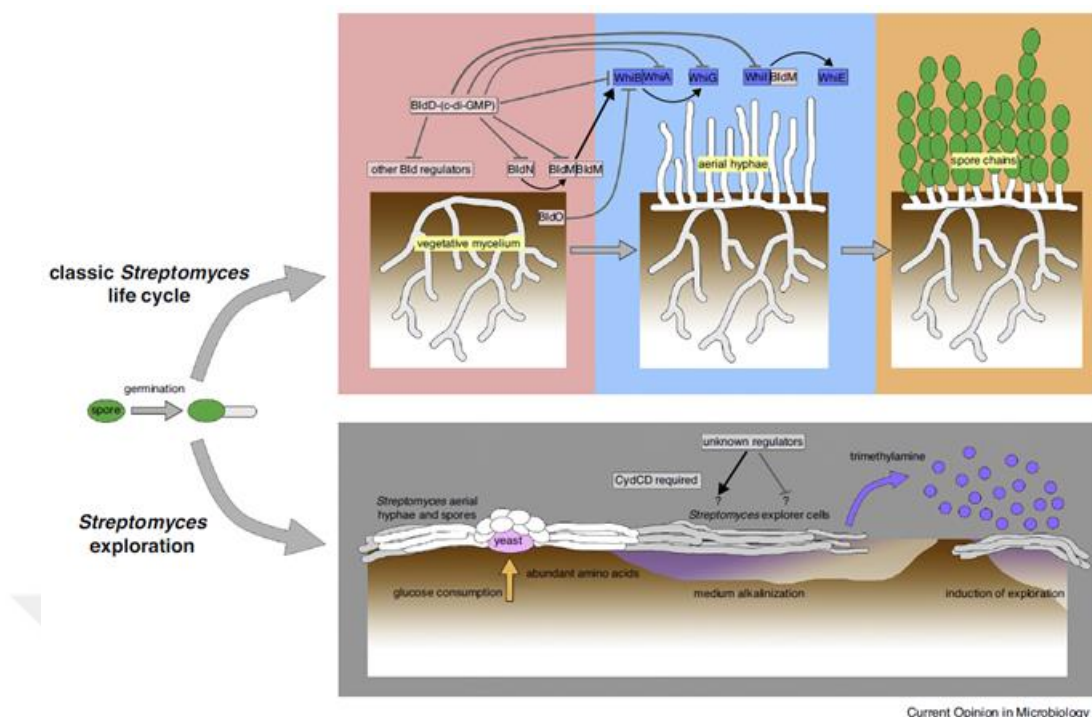


Figure 1.1 *Streptomyces* spp. life cycles (Jones and Elliot, 2018).

1.2 *Streptomyces clavuligerus* and Production of Secondary Metabolites

In the first place, *S. clavuligerus* was first isolated from South American soil samples (Higgins and Kastner, 1971). The strain was named NRRL 3585 by The Agricultural Research Service Collection, on the other hand, ATCC 27064 by the American Type Culture Collection, Rockville, Md. In 2010, two important genome sequencing studies of *S. clavuligerus* were published. The genome of NRRL3585 strain has been sequenced by Song et al. (2010) and Medema et al. (2010). The genome sequencing studies collectively showed that *S. clavuligerus* has one linear chromosome (6,736,475 bp, 72.69% GC), one giant linear plasmid, pSCL4, (1,796,117 bp, 71.85% GC) and 3 additional linear plasmids, pSCL1 (10,266 bp, 71.96% GC), pSCL2 (149,326 bp, 70.07% GC), pSCL3(442,792 bp, 70.77% GC) (Song et al., 2010; Medema et al., 2010). 30 secondary metabolite gene clusters were found in

the chromosome, and 28 other secondary metabolite gene clusters were found in the pSCL4 (Medema *et al.*, 2010; Hwang *et al.*, 2019), already known that *S. clavuligerus* produces a vast amount of clinically significant secondary metabolites. For example, it produces two different types of β -lactam compounds: sulfur-containing ones (isopenicillin N, desacetoxyccephalosporin C, and cephamycin C), showing strong antibiotic effects and oxygen-containing ones (clavams including β -lactamase inhibitor clavulanic acid) with weak antibacterial activity, but potentiate the antibacterial effects of the other antibiotics. It is also able to produce additional non- β -lactam antibiotics like N propionylholothin, tunicamycin, and holomycin with a pyrrothine structure (Thai *et al.*, 2001; Okamura *et al.*, 1977; Kenig and Reading, 1979; Li and Walsh, 2010).

1.2.1 β -lactam Antibiotics

β -lactam antibiotics are historically important. Their history began with the discovery of penicillin by Alexander Fleming. β -lactam molecules, which have a wide variety of chemical derivatives, have a strong bactericidal effect on the different kinds of species (Kong *et al.*, 2009). All derivatives of the β -lactam antibiotics have a four-membered β -lactam ring in their chemical structure (Figure 1.2). Penems/penams, cepheems/cephams, carbapenems, and monobactams are the main sub-classes of β -lactam antibiotics. In the structure of penicillin, β -lactam ring is fused to another ring, namely five-membered thiazolidine ring. According to the second ring structure, β -lactams are specifically named as penam/penem antibiotics

if fused to a five-membered ring or named as cepham/cephem antibiotics if fused to a six-membered structure (MacKenzie, 2007).

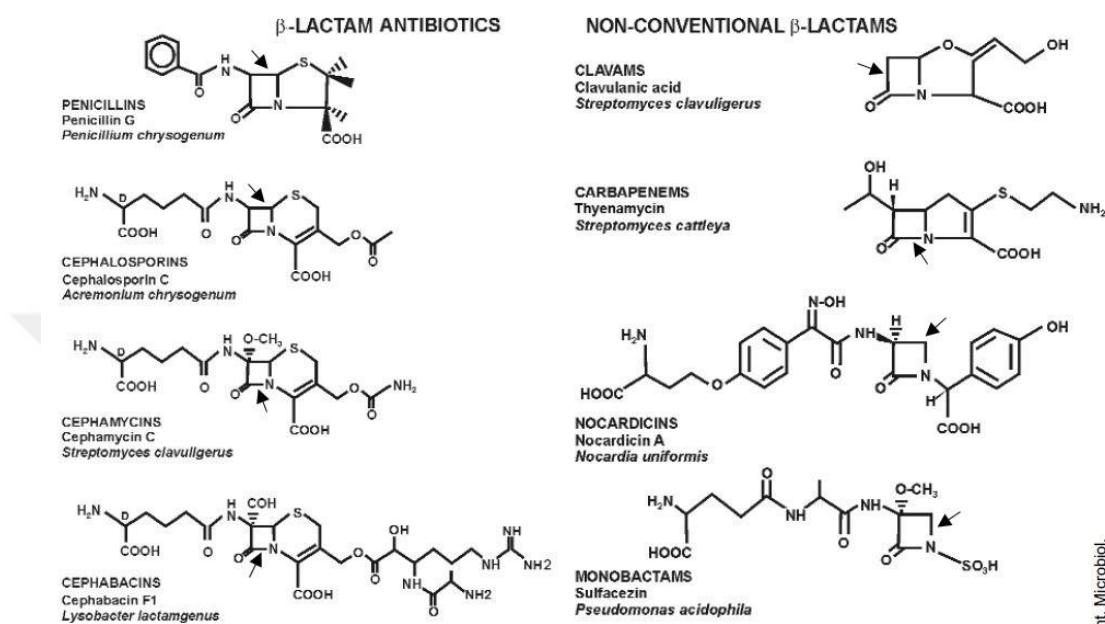


Figure 1.2 Derivatives of β -lactam antibiotics with their chemical structure and the producer microorganism. In the left column conventional β -lactam antibiotics, and in the right column non-conventional β -lactam antibiotics are shown. Black arrows point to the four-membered β -lactam ring that is found in the chemical structure of all β -lactam antibiotics (Liras and Martin, 2006).

The antibacterial action of β -lactam antibiotics involves the inhibition of cell wall formation, at the level of peptidoglycan. Peptidoglycan layer formation is a very complex biosynthesis process that has 3 different stages, with almost 30 different enzymes taking roles. The last step of the peptidoglycan formation is transglycosylation and transpeptidation catalyzed by penicillin-binding proteins (PBPs). In the last stage of peptidoglycan layer formation, β -lactam antibiotics act like PBPs' substrate. PBPs bind to β -lactam antibiotics rather than their substrate D-

alanyl-D-alanine moiety of the peptidoglycan chains to catalyze crosslinking of the adjacent peptidoglycan chains. PBPs become inactivated upon β -lactam binding, therefore, transpeptidation between two adjacent chains of peptidoglycan cannot be completed. The peptidoglycan layer is weakened, and bacteriolysis occurs with a rapid entry of water into the cell (Tipper and Strominger, 1965; Lima *et al.*, 2020).

1.2.1.1 Developing Resistance Against β -lactam Antibiotics

As a result of the increased usage and/or misuse of β -lactam antibiotics, microorganisms have developed β -lactam resistance in different ways, such as PBPs modifications, extensive increase in the efflux system expression, decreasing porin formation in cell wall and producing hydrolytic enzymes, namely β -lactamases that specifically degrade β -lactam ring structure, thereby inhibiting the binding of β -lactam antibiotics to PBPs (King *et al.*, 2017; Tooke *et al.*, 2019). The increasing number of β -lactamases (today over 4300) led to a grouping among them according to some specifications, such as their specific action mechanisms or common amino acid motifs in their chemical structure. According to the particular motifs in their amino acid sequences, there are four major groups: Class A, namely serine penicillinases, Class B namely metallo- β -lactamases, Class C namely cephalosporinases, and Class D namely oxacillinases. According to their action mechanisms, these four major groups are also subdivided (Drawz and Bonomo, 2010; Tooke *et al.*, 2019).

1.2.2 Clavulanic Acid as a β -lactamase Inhibitor

Clavulanic acid (CA) is produced by *S. clavuligerus* as a well-known pharmaceutical in the market with a very powerful inhibitory effect on Class A (and some of the

Class C) β -lactamases (Tehrani and Martin, 2018). Clavulanic acid's β -lactamase inhibitory effect was first studied in 1970s. Studies have shown that clavulanic acid does not have a strong β -lactam antibiotic activity. However, it had a very strong β -lactamase inhibitory effect, as shown by Brown *et al.*, (1976) and Reading and Cole (1977). Unlike other clavam metabolites like 5S-clavams, CA had a C₃ side chain, and different (3R, 5R) stereochemistry, its chemistry confers CA a β -lactamase inhibition capability in spite to its low antibacterial capacity (Baggaley *et al.*, 1997). Arms-race between β -lactam antibiotics and β -lactamases has opened a door for new drug formulations; β -lactam and β -lactamase inhibitor combinations. Augmentin™ is an antibiotic pill that formulates with amoxicillin and clavulanate, and Timentin™ is another antibiotic pill formulates with ticarcillin and clavulanate (Saudagar *et al.*, 2008). According to an online report written by Pandey and Sumant (2019), the worldwide market of β -lactam and β -lactamase inhibitor combination of pharmaceuticals' was over 27 million dollars in 2018. They also claimed that the market size is expected to grow by %2.3 to reach over 34 million dollars in 2028.

1.2.2.1 Clavulanic Acid Gene Cluster

Clavulanic acid (CA) gene cluster (Figure 1.3) and cephamycin C (CC) gene cluster are located adjacent to each other in the genome of *S. clavuligerus* and named as CC-CA Supercluster of nearly 60 kb. CC-CA Supercluster produces two products which are structurally similar. However, there are no common enzymes in any steps of their biosynthesis. (Ward and Hodgson, 1993; Özcengiz and Demain, 2013).

CA biosynthetic gene cluster is divided into two groups of genes; the early genes indicate those shared with clavulanic acid and 5S clavam biosynthetic pathways. The late genes are involved only in the clavulanic acid biosynthetic pathway. Five genes in the early group (*ceaS*, *bls*, *pah*, *oat* and *cas*) have homologs in the genome of *S. clavuligerus*. In the paralog gene cluster, homologs of *ceaS2*, *bls2*,

pah2, and *oat2* are located, but *cas2* homolog is located at a different part of the genome. The CC-CA Supercluster is located in the chromosomal DNA while the paralog gene cluster is located in the plasmid pLSC4, so-called the megaplasmid of *S. clavuligerus*. (MacKenzie, 2007; Medema *et al.*, 2010; Özcengiz and Demain, 2013).

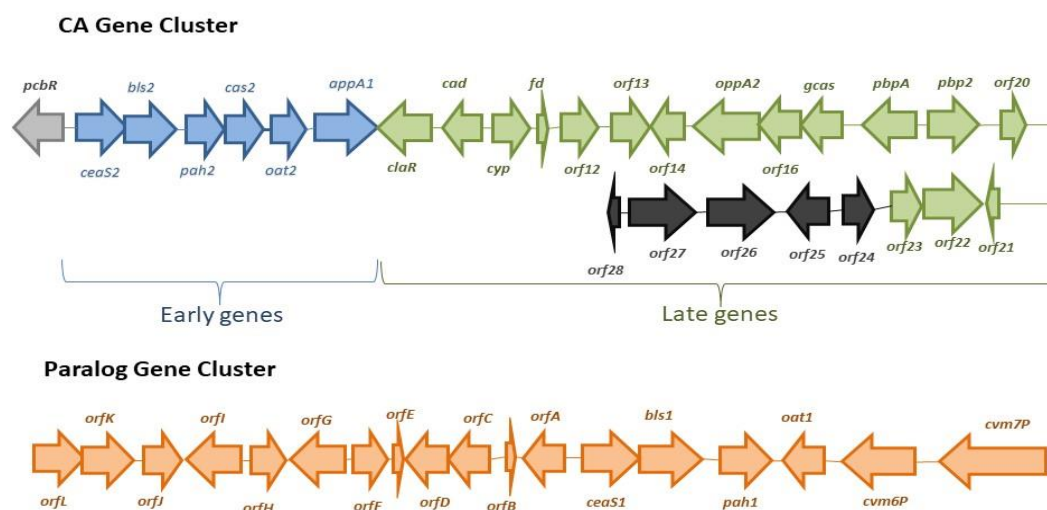


Figure 1.3 Clavulanic acid gene cluster and its paralog gene cluster. In CA Gene cluster, the gray arrow and blue, green and black arrows indicate Cephameycin C biosynthetic gene, early genes of CA biosynthesis, late genes of CA biosynthesis and the genes with possible functions in clavulanic acid biosynthesis.

The roles of some of the ORFs in CA biosynthesis are not entirely understood yet. In Table 1.1, the functions of the ORFs found in the CA biosynthetic gene cluster and functions of five additional ORFs (*orf24-orf28*) that are thought to have some roles in CA biosynthesis are listed.

Table 1.1 ORFs found in CA gene cluster and their functions

Gene		
<i>ceaS</i>	N2-(2-carboxyethyl) arginine synthase	(Khaleeli <i>et al.</i> , 1999)
<i>bls2</i>	β -lactam synthase	(Bachmann <i>et al.</i> , 1998)
<i>pah2</i>	Proclavamate amidinohydrolase	(Wu <i>et al.</i> , 1995)
<i>cas2</i>	Clavamate synthase	(Elson <i>et al.</i> , 1987)
<i>oat2</i>	Ornithine acetyltransferase	(Kershaw <i>et al.</i> , 2002)
<i>oppA1</i>	Oligopeptide binding protein	(Hodgson <i>et al.</i> , 1995)
<i>claR</i>	ClaR regulatory protein (DNA binding protein)	(Paradkar <i>et al.</i> , 1998; Pérez-Redondo <i>et al.</i> , 1998)
<i>cad</i>	Clavulanic acid dehydrogenase	(Nicholson <i>et al.</i> , 1994)
<i>cyp</i>	P450 mono-oxygenase	(Jensen <i>et al.</i> , 2000; Li <i>et al.</i> , 2000; Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>fd</i>	Ferredoxin	(Li <i>et al.</i> , 2000; Jensen <i>et al.</i> , 2004)
<i>orf12</i>	Acetyl transferase	(Li <i>et al.</i> , 2000; Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>orf13</i>	Efflux pump	(Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>orf14</i>	Acetyltransferase	(Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>oppA2</i>	Oligopeptide binding protein	(Hodgson <i>et al.</i> , 1995; Jensen <i>et al.</i> , 2000; Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004; Lorenzana <i>et al.</i> , 2004)
<i>orf16</i>	Hypothetical protein	(Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>gcas</i>	N-glycyl-clavaminic acid synthetase	(Arulanantham <i>et al.</i> , 2006)
<i>pbpA</i>	PBP	(Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>pbp2</i>	PBP	(Mellado <i>et al.</i> , 2002; Jensen <i>et al.</i> , 2004)
<i>orf20</i>	Putative cytochrome P450	(Jnawali <i>et al.</i> , 2008)
<i>orf21</i>	Sigma factor	(Song <i>et al.</i> , 2009; Jnawali <i>et al.</i> , 2011a)
<i>orf22/orf23</i>	Two component CA positive regulator	(Jnawali <i>et al.</i> , 2008; Song <i>et al.</i> , 2009)
<u>Genes with possible function in CA biosynthesis</u>		
<i>orf24</i>	Putative RNA polymerase sigma factor	(Jnawali <i>et al.</i> , 2008)
<i>orf25</i>	Hypothetical protein	(Jnawali <i>et al.</i> , 2008)
<i>orf26</i>	Putative FAD-dependent oxidoreductase	(Jnawali <i>et al.</i> , 2008)
<i>orf27</i>	Putative Ku70/Ku80 protein	(Jnawali <i>et al.</i> , 2008)
<i>orf28</i>	Putative DNA ligase	(Jnawali <i>et al.</i> , 2008)

1.2.2.2 Biosynthesis of Clavulanic Acid

The biosynthetic pathway of CA is shown in Figure 1.4. Clavulanic acid biosynthesis begins with the condensation of its precursors that are arginine and glyceraldehyde-3-phosphate (G3P) by N²-(2-carboxyethyl) arginine synthase (CEAS) expressed by *ceaS1/ceaS2*. L-N²-(2-carboxyethyl) arginine that is the product of this condensation is next acted on by β -lactam synthase (BLS) expressed by *bls1/bls2* to give deoxyguidinoproclaviminicacid, the first intermediate with a monocyclic β -lactam ring in its chemical structure. In the next step, deoxyguanidinoproclavaminicacid is converted into guinidinoproclavaminic acid by clavamate synthase (CAS) expressed by *cas1/cas2*. Arginine-derived guanidino group in the guinidinoproclavaminic acid is abolished to get proclavaminic acid as catalyzed by proclavamate amidinohydrolyase (PAH) expressed by *pah1/pah2*. After the proclavaminic acid formation, two-steps reaction catalyzed by CAS ends up with clavaminic acid formation, dihydroclavaminic acid being an intermediate. Clavaminic Acid is a common intermediate in the biosynthesis of 5S-Clavams and clavulanic acid (Egan *et al.*, 1997). After clavaminic acid formation, the products of the late start to be involved in CA biosynthesis. Using ATP, clavaminic acid is converted to N-glycyl-clavaminic acid by N-glycylclavaminicacid synthetase (GCAS) expressed by *gcas*. Subsequently, clavaldehyde is formed by the reactions possibly catalyzed, by the products of the remaining ORFs in the gene cluster. Clavaldehyde (3R,5R- clavulanate-9-aldehyde) is highly unstable and converted into the stable form of clavulanic acid in a reaction catalyzed by clavulanic acid dehydrogenase (CAD) expressed by *cad*.

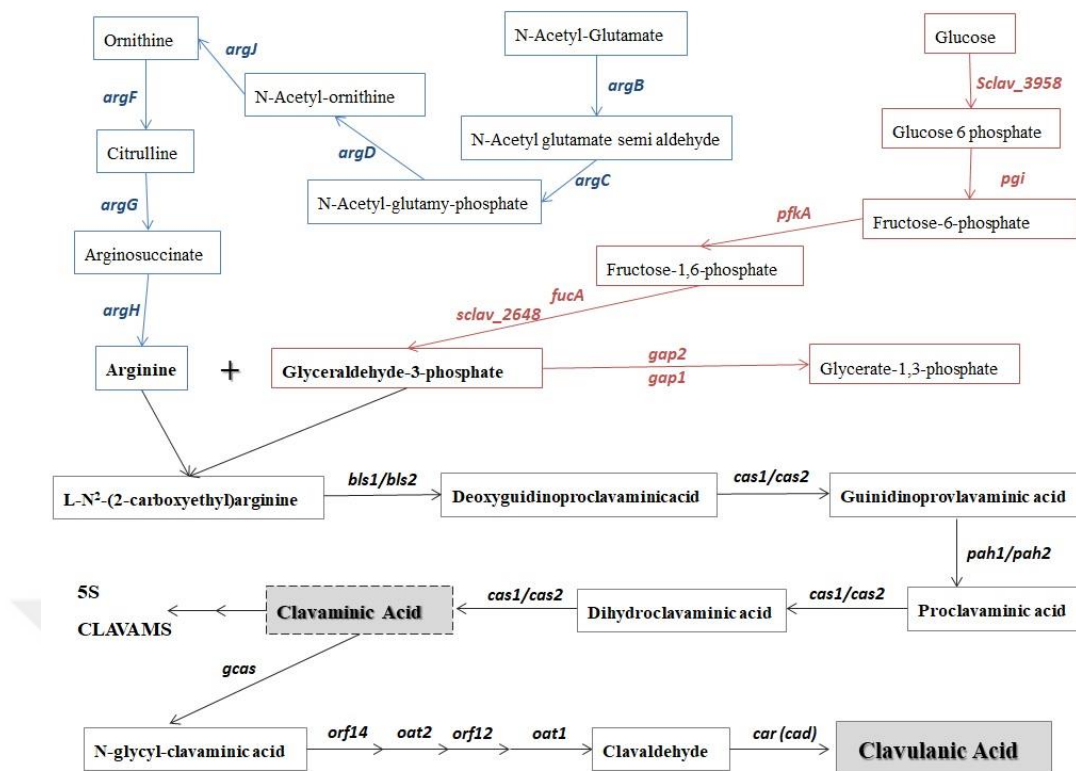


Figure 1.4 Biosynthetic pathway of clavulanic acid (adapted from Fu *et al.*, 2019a).

1.3 Regulation of Secondary Metabolism

In *Streptomyces* spp., secondary metabolite production rely on very complex regulatory systems that are still been studied. Transcriptional regulators that could be grouped as pleiotropic regulators and pathway-specific regulators are the key elements of regulation. Several sigma factors and small-noncoding RNAs have also important roles. The genes of pathway-specific regulators (PSR) are located in the biosynthetic gene cluster of the antibiotics and are directly attached to the promoter of the cluster. On the other hand, pleiotropic regulators that play role in regulation of the production of more than one secondary metabolite are usually located out of the biosynthetic gene cluster.

LysR family member *claR* and SARP (*Streptomyces* Antibiotic Regulatory Proteins) family member *ccaR* are two well-understood pathway-specific regulators found in the Cephamycin C-Clavulanic Acid Supercluster (Liras *et al.*, 2008; Lu *et al.*, 2017; López-Agudelo *et al.*, 2021). Although SARP family proteins commonly share helix-turn-helix DNA binding motifs in their N-termini, the *ccaR* has a binding domain resembling to OmpR-family proteins rather than helix-turn-helix motif (Wietzorrek and Bibb, 1997). The *ccaR* is involved in both CC biosynthesis via *lat*, *cefD-cmcl*, and *cefF* promoter regions, and CA biosynthesis via *ceaS2* promoter region which influences the expression of early CA biosynthetic genes that are *ceaS2*, *bls2*, *pah2*, and *cas2* (Liras *et al.*, 2008; Santamarta *et al.*, 2011; Tahlan *et al.*, 2004). The *ccaR* is also an autoregulatory protein, regulating its own expression. Additionally, CcaR affects *claR* by binding, thereby regulating *claR* related genes (late genes in the CA biosynthetic gene cluster) (Santamarta *et al.*, 2011; Kurt-Kızıldoğan *et al.*, 2013).

The other PSR, *claR* regulates the expression of late genes of the CA biosynthetic cluster which are *cyp*, *oppA1*, and *cad* because clavaminic acid, which is the final product of the early biosynthesis of CA accumulates when *claR* gene is disrupted (Paradkar *et al.*, 1998). As mentioned before, the *claR* is regulated by CcaR. While CA production is positively controlled by *claR*, the effect of this PSR on CC production is a negative control (Liras *et al.*, 2008; Pérez-Redondo *et al.*, 1998).

Pleiotropic (global) regulators constitute very important components of the regulation system of CA production in *S. clavuligerus*. Pleiotropic regulators control secondary metabolism both directly and indirectly, the latter being a manifestation of the control of morphological differentiation by them since morphological differentiation is very important for secondary metabolism in *S. clavuligerus*. *bld* gene products are the best-known pleiotropic regulators among *Streptomyces* spp. Anti-anti-sigma factor is expressed by *bldG* gene and is involved in regulation

of both morphological differentiation and antibiotic production in *S. clavuligerus*. *bldA* gene product is another pleiotropic regulator that regulates the sporulation activities in *S. clavuligerus* but is not directly involved in regulation of antibiotic production. *bld* gene products could have different functions in different *Streptomyces* spp. For instance, unlike *S. clavuligerus*, *S. coelicolor bldA* product has been reported to regulate both antibiotic production and morphological differentiation (Bignell *et al.*, 2005). BldG affects CcaR expression; and as a result, ClaR expression is also affected. In a hierarchical order, BldG resides at the top, CcaR is in the middle, and ClaR would be at the bottom of the order (Özcengiz and Demain, 2013).

γ -butyrolactone, which is part of the quorum-sensing system, is involved in the regulation of initiation of antibiotic production and morphological differentiation in *Streptomyces* spp. γ -butyrolactone is a small signaling molecule, and its production is induced by environmental or physiological signals (Horinouchi and Beppu, 1994). *brp* gene expresses the butyrolactone receptor protein which is known to repress CA and CC production in *S. clavuligerus* by binding ARE sequences upstream of the responsible pathway-specific regulator (CcaR) for antibiotic production. An increase (nearly 2-fold) in the production of CA and CC was reported in a *brp*-null mutant in *S. clavuligerus* with respect to its wild-type strain (Santamarta *et al.*, 2007). AreB protein expressed by *areB* gene is another protein that binds to ARE sequences upstream of CcaR with the help of a small molecule.

Stringent response is one of the protection systems developed by bacteria against conditions of nutrient limitation such as amino acid starvation. ppGpp and pppGpp, which are phosphorylated guanosine nucleotides have an important role in this regulation system (Cashel and Kabalcher, 1970; Haseltine *et al.*, 1972). *relA* and *spoT* genes are involved in (p)ppGpp synthesis and hydrolysis, respectively. In 2004, two different studies about the consequences of *relA* disruption in *S. clavuligerus* were reported that CA and CC production drastically diminished

(Ryu *et al.*, 2004; Jin *et al.*, 2004). However, later on, the study of Gomez-Escribano *et al.* (2008) showed that clavulanic acid production increases by 4-5-fold in a *relA*-disrupted mutant.

Two-component regulators act as global regulatory systems as affected by environmental changes. In *S. clavuligerus*, two-component system (TCSs) regulators are involved in the regulation of production of antibiotics and other secondary metabolites. Deletion of CagRS two-component regulator in *S. clavuligerus* F613-1 strain caused a decrease in CA production (Fu *et al.*, 2019a). Moreover, deletion of another two-component regulator, CepRS in *S. clavuligerus* F613-1 strain led to a diminished level of CC production while CA production was not affected (Fu *et al.*, 2019b).

1.4 Transcriptomics

RNA molecules carry genetic information and have many roles in cells to include catalytic activities in biological processes, RNA processing, gene regulation, and synthesis of proteins. Transcriptomic is the area that works with all RNA transcripts of the cells to reveal and understand their functions and abundance (Liang *et al.*, 2013; Lowe *et al.*, 2017).

Transcriptomic studies began in early 1990s, and over time, different methods were developed used in the relevant research (Figure 1.5). There were two main branches for transcriptomic approaches; sequencing-based approaches based on Sanger Sequencing; Expressed Sequence Tags (ESTs), Serial Analysis of Gene Expression (SAGE) , and Cap Analysis of Gene Expression (CAGE) and hybridization-based approaches (microarray) (Wang *et al.*, 2009).

Expressed Sequence Tag (EST) method was one of the first technologies for transcriptomic analysis, but it is very low-throughput. The procedure of EST includes RNA isolation, cDNA synthesis from RNA by reverse transcriptase, sequencing of 200-500 bp of nucleotides at the 5' and 3' ends of the cDNA by Sanger sequencing method, and then matching the sequences via databases that include the genome of the interested organism. Only 200-500 bp of the nucleotide sequence is acquired from a single mRNA. Because of its low-throughputness, Serial Analysis of Gene Expression (SAGE) and Cap Analysis of Gene Expression (CAGE) methods were improved so that more reliable results of quantitative expression could be obtained.

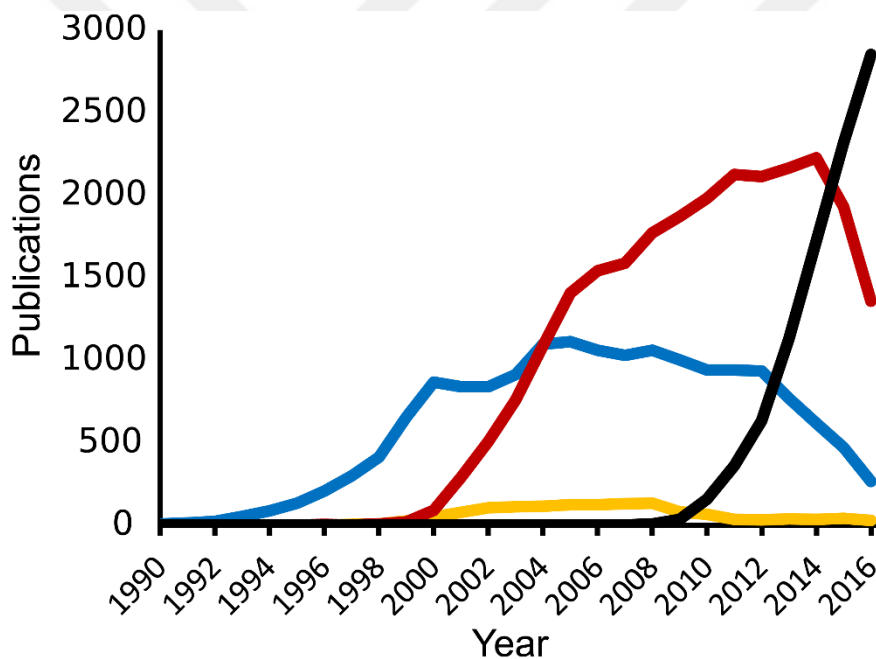


Figure 1.5 Transcriptomic methods used over time (Lowe *et al.*, 2017). The blue line represents EST, the red line represents Microarray, the yellow line represents SAGE/CAGE, the black line represents RNA-sequencing.

In the SAGE method, after cDNA synthesis, cDNA is cut into 11 bp small pieces of “3’ end tagged” fragments by restriction enzymes; after tagging, the fragments are conjugated into each other’s, and sequenced by the Sanger sequencing method. CAGE is a different version of the SAGE method. In the CAGE method, 5’cap of mRNA sequence is tagged. SAGE and CAGE were very expensive methods, and additionally, it was very laborious to align small pieces of tagged fragments with the reference genome. Many of the fragments could not be adequately mapped, thus finding a new gene was very difficult with these methods (Wang *et al.*, 2009; Lowe *et al.*, 2017).

RNA microarray technology has begun to be used for transcriptomic analysis in the mid-90s. Microarray technology logic was evolved from Southern blot hybridization. It has been used as a powerful technology to explore transcriptional profiles and genome differences. Microarrays consist of short nucleotide oligomers (20-100 or more base pairs), known as "probes," which are arrayed on a solid substrate. Transcript abundance is determined by hybridization of fluorescently labelled transcripts to these probes, and the fluorescence intensity at each probe location on the array indicates the transcript abundance for that probe. Microarrays are either spotted low-density arrays or high-density arrays. The former ones are prepared by using picolitre drops of a range of purified cDNAs arrayed on usually the surface of a glass slide. The probes are longer than those of high-density arrays in their size and do not possess the transcript resolution of high-density ones. High-density arrays use single channel detection, and each sample is hybridized and detected individually. Such arrays became very popular after the manufacture of Affymetrix GeneChip arrays, in which each transcript is quantified by several short 25-mer probes that together assay one gene. In microarray analysis, some prior knowledge of the organism of interest, e.g., in the form of an annotated genome sequence that can be used to generate the probes for the array is needed. Microarray systems have been used as a preferred technology for many years because of their

high sensitivity, high accuracy, low cost, labor-saving procedure, and reproducibility (Lowe *et al.*, 2017).

1.4.1 Next-Generation Sequencing (RNA-sequencing)

As introduced by Nagalakshmi *et al.* (2008), RNA-sequencing (RNA-seq) method used for transcriptomic analysis. This method has many additional advantages as compared to microarray analysis. Through RNA-sequencing, researchers could find novel (unannotated) transcripts, transcription boundaries at single-nucleotide resolution, and detect variations in the sequences, such as single nucleotide polymorphisms (SNPs) (Nagalakshmi *et al.*, 2008; Habesreiter, 2017). Because the RNA-sequencing method has a high discovery rate, high sensitivity, ease, accurate profiling, and quantifying transcripts, today it is accepted as the most convenient method (Wang *et al.*, 2009; Hölzer, 2017).

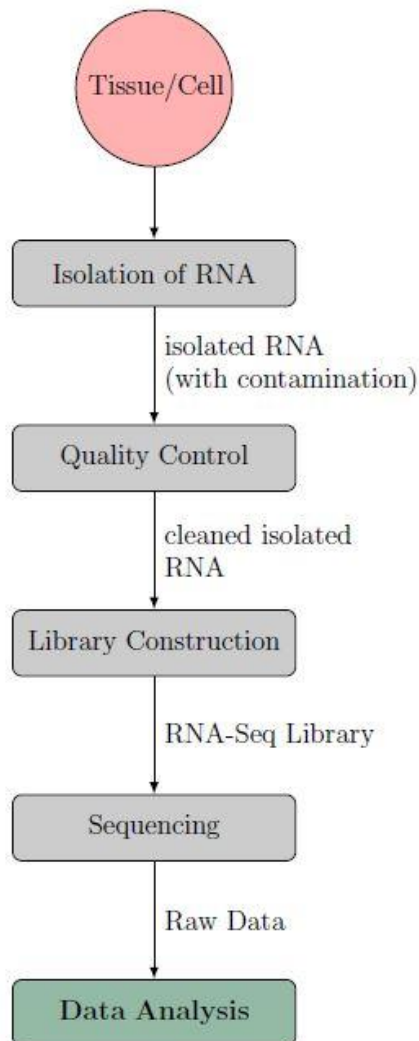


Figure 1.6 Workflow of RNA-sequencing experiment (Hablesreiter, 2017).

RNA-sequencing experiment workflow simply consists of 5 steps (Figure 1.6). After isolation of RNA from tissues or cells, quality control of RNA sample is needed to detect any degradation or contamination. RNA quantity is also to be determined to make sure that it is enough for analysis. Then, for library construction, high quality isolated RNA sample is converted into cDNA by reverse transcriptase. Synthesized cDNA is fragmented into pieces, and then adapters are attached to the end(s) of the fragments. If adapters are put into one end, it is called single-end sequencing; if they are put into both ends, it is called paired-end sequencing. PCR amplification of fragments is done for the enrichment of the library against the possibility of any bias. Protocol of library construction is to be chosen carefully, by taking into consideration the needs of the researchers. A properly prepared cDNA library is finally ready for

sequencing. Rather than the old Sanger method, a sequencing technology platform (manufacturer) such as Illumina HiSeq/MiSeq, Roche 454, or Ion Torrent is used for next-generation sequencing (Kukurba and Montgomery, 2015; Wang *et al.*, 2009; Hablesreiter, 2017; Hölzer, 2017). Of available platforms, Illumina provides the highest efficacy with a maximum throughput of 900 Gbp per run (Lowe *et al.*, 2017).

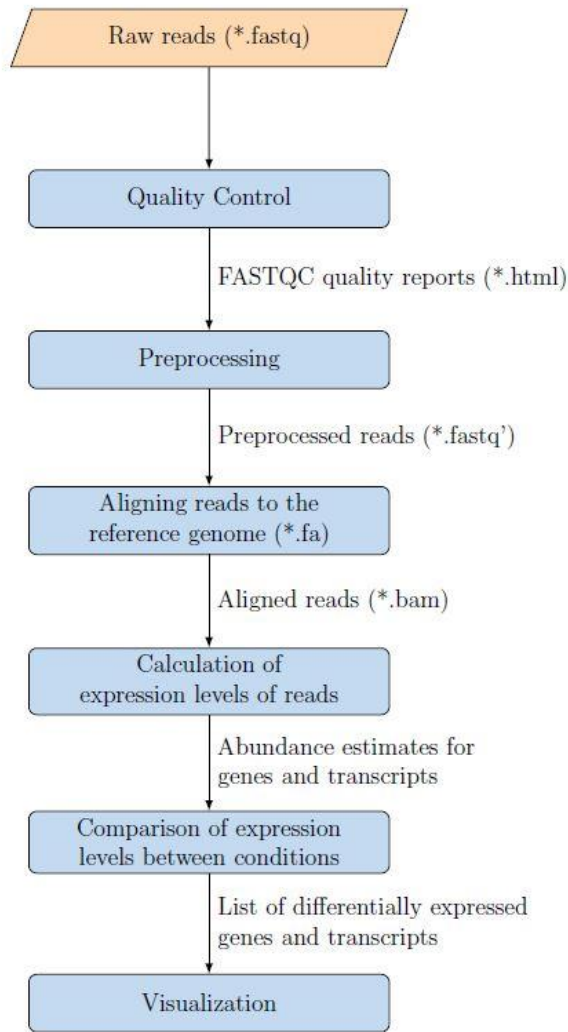


Figure 1.7 Data analysis workflow of RNA-seq analysis (Hablesreiter, 2017).

The data analysis workflow of RNA-seq technology is shown in Figure 1.7. When the sequencing step is done, the raw data must be analyzed bioinformatically, after which the results become meaningful. There are generally 6 steps of bioinformatic analysis (Hablesreiter, 2017). The quality of raw reads must be controlled at the beginning of the analysis. After quality control, adapter regions, low-quality reads, and linker sequences are trimmed from the raw data to get clean reads in the pre-processing step by tools such as SEECER (Le et al., 2013), Cutadapt (Martin, 2011), and rCorrector (Song and Florea, 2015). Then, clean reads are aligned and mapped with the reference genome by tools such

as STAR (Dobin *et al.*, 2013), TopHat (Trapnell *et al.*, 2009), HISAT (Kim *et al.*, 2015). Expression levels of the aligned reads could be calculated by tools such as RSEM (Li and Dewey, 2011) and afterward, expression levels are compared with each other in between diverse conditions. Differences in the expression levels are detected and listed as differentially expressed genes, accordingly. Lastly, deep understanding and exploring the data and its visualization is accomplished by using different tools, like IGV (Thorvaldsdóttir *et al.*, 2013).

1.5 Aim of the Present Study

As a very important pharmaceutical biotechnology product, the enhancement of CA production has always been a very big deal for the manufacturers. Owing to very strict random mutagenesis and screening programme of the R&D Department of DEPA Co., İzmit, spanning to the years, the industrial strain DEPA became a super producer with at least 100-fold higher CA titers as compared to its counterparts. Unfortunately, the factory closed down in early 2010s. In the present work, as an academic research group collaborated with DEPA scientists in the past (2006-2009) in frame of a TÜBİTAK-TEYDEB project, we took the advantage of still having that industrial overproducer in our laboratory and pursued comparative transcriptomics and integrated -omics work in order to understand the mechanisms behind the production of very high titers of CA produced by this strain of *S. clavuligerus*.

CHAPTER 2

MATERIALS AND METHODS

2.1 Bacterial Strains

In this study, *Streptomyces clavuligerus* NRRL3585 (Higgins and Kastner, 1971) and DEPA (DEPA Pharmaceuticals Co., İzmit, Turkey) strains were used. NRRL3585 is a wild-type strain that produces clavulanic acid, cephamycin C, and clavam metabolites whereas DEPA is an industrial overproducer obtained that produces at least 100 times more clavulanic acid.

2.2 Media and Culture Conditions

S. clavuligerus seed cultures were prepared in Tryptic Soy Broth (TSB) (APPENDIX A) in baffled flasks, and their growth conditions were incubation at 28°C by shaking at 220 rpm. 400-600 µL of stock culture was added into 50mL of TSB and incubated for 24-48 hours. For optical density measurement, the special procedure from Malmberg *et al.* (1993) was used. 1 mL of seed culture, 1 mL of 2.5 M HCl, and 6 mL of distilled water were mixed and then 30 seconds of sonication (Ultrasonic Processor, Cole Parmer) for 3 times at 50% amplitude was applied to this mixture. When the optical density of the seed culture, measured at 600 nm, reached 0.7 - 0.8, it indicated that the cell density has become suitable for transfer into the fermentation culture. Accordingly, 30 mL of seed culture was centrifuged at 4000 g for 10 minutes at 4°C. After discarding the supernatant, pellet was washed with Starch-Asparagine (SA) (APPENDIX A) medium. After consecutive washing steps, pellets were resuspended in 100mL of SA medium.

2.3 RNA Extraction and Purification

For RNA extraction, 2mL aliquots of 48 h SA fermentation culture samples of *S. clavuligerus* NRRL3585 and DEPA from were centrifuged. Nucleospin® RNA Kit (Macherey-Nagel GmbH & Co, Germany) was used for RNA isolation from 48 h pellets. For total RNA isolation, the manufacturer's protocol was mostly followed; some slight changes about suggested volumes of reagents were arranged for getting better results. The first arrangement was RA1 buffer volume increased to 450 µl from 200 µl and the second arrangement was 70% ethanol (prepared with DEPC treated water) volume increased to 450 µl from 200 µl.

The quality and quantity of extracted RNA were measured spectrophotometrically in NanoDrop (BioDrop, United Kingdom) at 260 nm and 280 nm. 1 µl of extracted RNA sample was enough to measure quality check and quantity measurement. A260/A280 ratio has been an important guiding role for quality control. A260/A280 ratio between 1.8 – 2.0 (± 0.1) has been accepted for good quality, while a higher or lower ratio referred to contaminated RNA.

RNA integrity was measured by running extracted RNA samples on the agarose gel electrophoresis. 0.8% agarose gel prepared with 1X TAE buffer (APPENDIX A). 3 µl extracted RNA samples mixed with 7 µl distilled water and 2 µl loading dye (6X), and loaded on the 0.8% agarose gel, and the sample was run at 100V for 45 minutes. RNA bands were imaged through Vilber Lourmat Gel Imaging System (Vilber Lourmat, France). In the gel image, 23S and 16S rRNA were seen as two sharp bands with no smear appearance.

2.4 Transcriptomic Analysis

2.4.1 Total RNA Isolation

Total RNA isolation was performed as mentioned in Section 2.3. The integrity of the RNA sample should be higher than 7.0 if the sample is used for RNA-sequencing analysis. All quality and quantity checks were successfully accomplished for the extracted RNA, which was then, stabilized by RNAsable® (Biomatrica, San Diego, CA, USA) against degradation at the room temperature to be used in the RNA-seq analysis.

2.4.2 RNA Sequencing

The RNA extracted from NRRL3585 and DEPA strains of *S. clavuligerus* were used for RNA-sequencing analysis. 2 x 150 bp paired-end libraries were constructed by using Illumina HiSeq 4000 system (BGI Genomics Co., Ltd., Hong Kong). Nearly 3.86 Gb data from each sample have been acquired at the end of the analysis. The data can be accessed in the Sequence Read Archive (SRA) of National Center for Biotechnology Information (NCBI) with the accession number SRP276705.

2.4.3 Bioinformatic Analyses

To get clean reads, low-quality reads, linker sequences, and the residual rRNA sequences were eliminated from the raw data. Obtained clean reads from that filtering process were aligned to reference genome by using *Bowtie2* software (Langmead and Salzberg, 2012) and *HISAT* software (Kim *et al.*, 2015). In this study, F613-1 strain of *S. clavuligerus* genome sequence was used as a reference genome. Accession numbers of chromosome and plasmid sequences of F613-1 strain

found in GenBank are CP016559 and CP016560, respectively. *Integrative Genomics Viewer (IGV)* 2.3.32 (Thorvaldsdóttir *et al.*, 2013) explores and understands the genomic data according to a reference genome visualizing. In order to further comprehend clean reads and alignments, *IGV* tool was used. Expression levels of the genes were computed by using *RSEM* software (Li and Dewey, 2011). Fragments Per Kilobase Million (FPKM) normalization model has been suggested to calculate for paired-end RNA-seq analysis; therefore, in this study, data normalization of the gene expression levels has been accomplished by using FPKM model. There have been three rules for deciding the transcripts that have tagged as significantly differentially expressed genes (DEGs) i) P-value < 0.05, ii) False Detection Rate (FDR) < 0.05, and iii) Log₂FoldChange ≥ 1 or ≤ -1. For categorization of DEGs and mapping their pathways, Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa *et al.*, 2008) database was used. In addition to KEGG, Gene Ontology (GO) knowledgebase for DEGs was used for the functional enrichment analysis.

2.5 Real-Time Quantitative Polymerase Chain Reaction

2.5.1 Total RNA Isolation

Total RNA isolation was performed as mentioned in Section 2.3.

2.5.2 cDNA Synthesis with Reverse Transcription PCR (RT-PCR)

Isolated RNA from 48 h pellets was used as a template for cDNA synthesis. cDNA synthesis was done by Bio-Rad cDNA Synthesis Kit. Bio-Rad cDNA Synthesis Kit includes all the materials needed for reverse transcription. The first reagent was 5X iScript[™] Reaction Mix that contains salts, oligo(dT), and random hexamer primers; the second reagent was iScript[™] reverse transcriptase that was modified from

Moloney murine leukemia virus (MMLV) for high yield of cDNA synthesis, and the third reagent was Nuclease Free Water. For a successful cDNA synthesis, the suggested reaction conditions and the amount of components are shown in Table 2.1.

Table 2.1 Reverse transcription PCR components and the protocol

COMPONENT	Volume per Reaction (μl)
5X iScript™ Reaction Mix	4
iScript™ reverse transcriptase	1
Nuclease-free Water	Variable (total volume 20 μl)
RNA template	Variable (100 ng to 1 μg RNA)
REACTION PROTOCOL	
Priming	5 minutes at 25 °C
Reverse transcription	20 minutes at 46 °C
RT inactivation	1 minute at 95 °C
Cooling	Hold at 4 °C

2.5.3 Real-Time Quantitative PCR (RT-qPCR)

cDNA, the product of reverse transcription PCR, was used as a template for Real-Time Quantitative PCR. For RT-qPCR experiments, SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, United States) was used. Essential ingredients for RT-qPCR experiments included in this Supermix were Sso7d fusion polymerase, dNTPs, MgCl₂, SYBR® Green I dye, enhancers, stabilizers, and a blend of passive reference dyes. LightCycler® 1.5 Real-Time PCR System (Roche Diagnostic, Sweden) was used for RT-qPCR experiments. Ingredients of RT-qPCR Master Mix and the reaction protocol are tabulated in Table 2.2 and Table 2.3, respectively. In the very first place, efficiency runs with 4 dilutions of cDNA were done for calculating primer efficiency. Sequences of the designed forward and

reverse primers with the help of the OligoAnalyzer Tool (idtdna.com) of each gene for RT-qPCR experiments are listed in Table 2.4. After successfully completing the primer efficiency runs, the runs for gene expression analysis were accomplished. For gene expression analysis by RT-qPCR, two technical replicates and three biological replicates for each gene was made available for each strain (NRRL3585 and DEPA) of *S. clavuligerus*, separately. Each run had one no-template-control (NTC). NTC has been contained dH₂O instead of the cDNA template of *S.clavuligerus* NRRL3585 or DEPA strains.

Melting curve analysis has been done for each gene expression, and primer efficiency runs. It was important to detect any non-specific amplification caused by primer-dimer formation or anything else.

Table 2.2 RT-qPCR master mix preparation

Components	Amount
SsoAdvanced™ Universal SYBR Green Supermix (2X)	10 µL
Forward primer	150 - 200 nM
Reverse primer	150 - 200 nM
cDNA template	Up to 100 ng
Nuclease-Free Water	4 µL

Table 2.3 Reaction protocol of RT-qPCR

Denaturation – Holding Stage	95°C	30 sec
Amplification – Cycling Stage	95°C	10 sec
	58-60°C	1 min
40 cycle of repeats amplification-cycling stage		
Melting Curve Stage	95°C	15 sec
	60°C	1 min
	95°C	15 sec
Cooling	40°C	30 sec

Table 2.4 Primer sequences

Primer Name	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
<i>hrdB</i>	CGCGGCATGCTCTTCCT	AGGTGGCGTACGTGGAGAAC
<i>SCLAV 2150</i>	ATCGGGCTCAGGAAC TACAA	AGCGACGAACCAAACCATTC
<i>SCLAV 3003</i>	GATATCCGAACTGGTCCAACC	CCGATCACATGCGAGACC
<i>Cas 2</i>	CGGAAGTATCTGCACTGGAC	TAGGTCCTGATGCTGTCGTA
<i>SCLAV 5080</i>	CTGGCGATCGTCTTCATCTTC	CGAGCAGGGCGAGATAGA
<i>SCLAV 5662</i>	GCCGGTCACTCTCATGTC	CTTCAGGACTTCGTGCTTCA
<i>SCLAV 1592</i>	GCTTTCCTGGCCTTCTT	GTCATCGACACCACTCCAC
<i>SCLAV 0155</i>	CCGCCATGAAATAGCTGTCTA	CACCAGGGTGCAGTTCTC
<i>SCLAV 2475</i>	TGAAGATCACCAAGGAGCAC	GACTTGGTCCTGGGCTTG
<i>SCLAV p0718</i>	TACTTCGACGGCTGGCA	GCATCTCATAGCGAATCCACTC
<i>SCLAV p0750</i>	GCCACGGACGTACTCATC	TAGTTCAGCTCGCTCATCAC
<i>SCLAV p0717</i>	CGGAAGCCAGGACATCAG	GATCAGGATGTCGGCGTAG
<i>SCLAV p0758</i>	CGCTCTTTGACAACGATCAGTA	CATGAGATTTCCGGCGAGTT
<i>SCLAV p0742</i>	GTCAGGGTGCTGCTCAC	GATGCTGTTGTCGATCTCCTC
<i>SCLAV p0747</i>	GCCAGAGCCACACCTTC	TAGCCGTCCTTGGTGATGTA
<i>SCLAV p0757</i>	CCCGCAACCACAGGAAG	GGTAGGTCAGCCCGGAA

2.5.4 Relative Quantification of qRT-PCR Data Based on Efficiency Correction

Relative Expression Ratio (R) that shows expression differences between the control gene and the target gene as based on their C_T value changes during RT-qPCR analysis was formulated as below:

$$R = 2^{-(\Delta C_{T\text{sample}} - \Delta C_{T\text{control}})}$$

$$R = 2^{-\Delta\Delta CT}$$

Given that R formulation can use when primers efficiencies of the control and target genes are equal to 2. In our case, because all the primer efficiencies were not equal to 2, efficiency correction-based formulations that are given below have been used for calculating the R values. *hrdB* gene that encodes sigma factor σ^{70} was used as a reference gene for calculations of the relative expression ratios.

$$R = \frac{(E_{\text{target}})^{\Delta C_{T\text{target}} (\text{MEAN control} - \text{MEAN sample})}}{(E_{\text{reference}})^{\Delta C_{T\text{reference}} (\text{MEAN control} - \text{MEAN sample})}}$$

2.5.5 Statistical Analysis

Statistical evaluation of the relative expression ratio of each gene was done using GraphPad Prism 8.0 (Graphpad Software, CA, USA). Statistical analysis was carried out by applying the student's t-test for collected C_T values. Statistical significance level was mentioned as ns (no significance, $p > 0.05$), * ($p < 0.05$), **($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.0001$).

2.6 Correlation Analysis

The data sets of transcriptomic and proteomic expressions were analyzed against each other by applying a correlation test. A correlation test was done by using non-parametric Spearman's rank-order method via R Studio. `cor.test()` and `cor()` functions were used for test analysis. The *ggplots2* package library was used to visualize results in a graph.



CHAPTER 3

RESULTS

3.1 Summary of Clean Data from RNA-sequencing Analysis of *S. clavuligerus* NRRL3585 and DEPA strains

40.73 Mb raw data were obtained from both DEPA and NRRL3585 strains. Data cleaning is an essential process to increase the accuracy of the quantitative analysis of the data. For data cleaning, adaptor, contaminants, and low-quality regions were discarded from the sequence, then checked the quality of clean data. 38.73 Mb (95.09%) of clean data was obtained from DEPA, and 38.50 Mb (94.54%) clean data was obtained from NRRL3585. Q20 and Q30 scores, which are known as Phred Quality Score, were used for quality check of the reads. Q20 score represents the chance of 1 incorrect base in 100; Q30 score represents the chance of 1 incorrect base in 1000. The average Q20 score of both strains is 98.04%. The average Q30 score of DEPA is 93.52%, and NRRL3585 is 93.55%. These scores revealed that clean data of each strain have good qualities. DEPA has 0.10%, and NRRL3585 has 0.11% discarded reads related to N. DEPA has 1.58%, and NRRL3585 has 1.79% discarded reads related to low quality. DEPA has 3.23%, and NRRL3585 has 3.56% discarded reads related to adapter. There are no other discarded reads for both DEPA and NRRL3585. After the cleaning processes, the ratio of total mapped reads is calculated as 59.68% for DEPA and 54.41% for NRRL3585. The statistical features of clean data are summarized in Table 3.1.

Table 3.1 Statistics of RNA-seq data

	Clean data (DEPA)	Clean data (NRRL3585)
Number of Reads	38,725,670	38,500,920
Data Size	3,872,567,000	3,850,092,000
GC (%)	67.76	67.8
Q20(%)	98.04	98.04
Q30(%)	93.52	93.55
Discard Reads related to N (%)	0.10	0.11
Discard Reads related to low quality (%)	1.58	1.79
Discard Reads related to Adapter (%)	3.23	3.56
Discard Reads related to Other (%)	0.00	0.00
Clean data/Raw data (%)	95.09	94.54
Total Mapped Reads (%)	59.68	54.41

According to RNA-sequencing analysis, NRRL3585 and DEPA strains share 6115 common genes expressed in both strains; on the other hand, DEPA has 60 genes that are not expressed in the NRRL3585 strain, and NRRL3585 has 188 genes that are not expressed in the DEPA strain. A total of 6363 genes were detected through the analysis of the whole data. When comparing these genes according to their expression levels, the results showed that DEPA has 372 significantly upregulated and 552 significantly downregulated genes. On the other hand, 5439 genes were accepted as significantly non-regulated. The differentially expressed genes (DEGs) have been shown on the volcano plot (Figure 3.1) by displaying their significance level i) False Detection Rate (FDR) ≤ 0.005 , and ii) $\text{Log}_2\text{FoldChange} \geq 1$ or ≤ -1 .

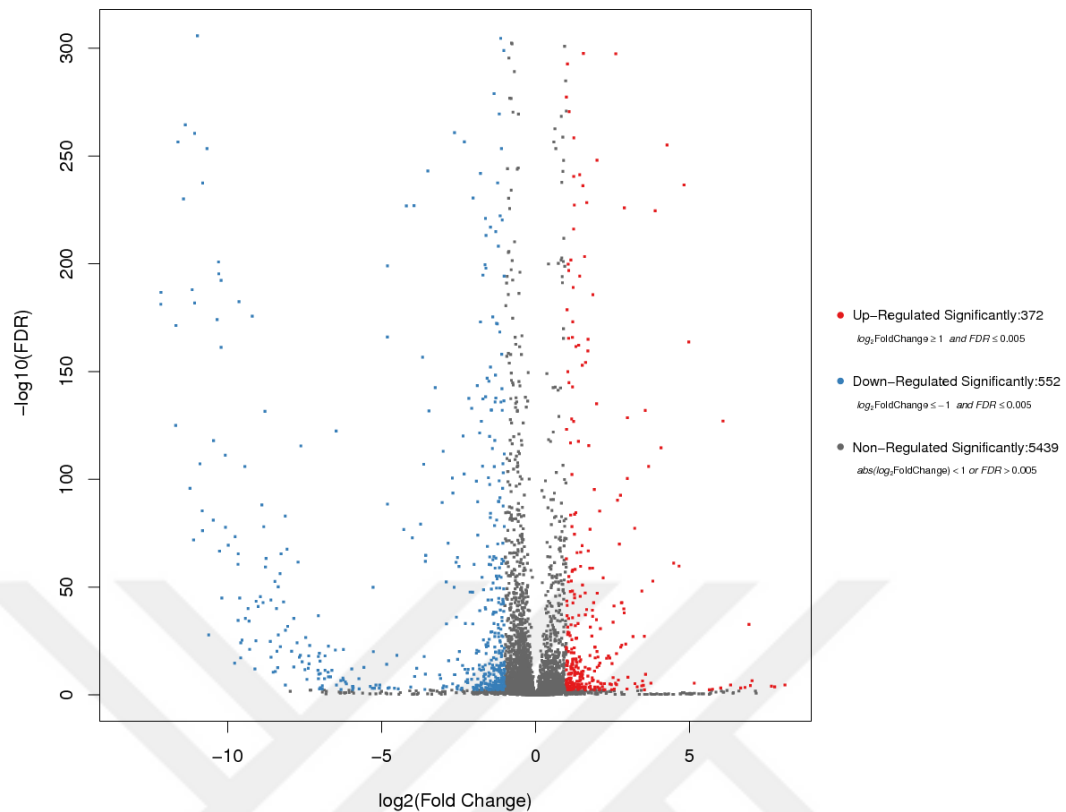


Figure 3.1 Volcano plot of differentially expressed genes.

3.1.1 Pathway Analysis of Differentially Expressed Genes

Pathway analysis is a very important step in functional genomics for viewing and comparing gene expression patterns in a wider perspective.

For pathway analysis of significantly differentially expressed genes (DEGs), the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used. The pathway classification led to the identification of 5 main categories:

Cellular Processes, Environmental Information Processing, Genetic Information Processing, Metabolism, and Organismal Systems (Figure 3.2). The “Metabolism” category dominated the other categories by having a total of 340 DEGs out of 924.

-Metabolism category had a total of 340 differentially expressed genes; 124 of them being related to “global and overview maps”, 52 of them were related to “amino acid metabolism”, 47 to “carbohydrate metabolism”, 29 to “metabolism of terpenoids and polyketides”, 20 to “energy metabolism”, 19 to “biosynthesis of other secondary metabolites”, 14 to “lipid metabolism”, 9 to “metabolism of cofactors and vitamins”, 9 to “other amino acids”, 5 to “nucleotide metabolism”, and 2 to “glycan biosynthesis and metabolism”.

-Environmental Information Processing category had a total of 47 DEGs; 41 of them being related to “membrane transport”, and 6 of them were related to “signal transduction”.

-Cellular Processes category had a total of 14 DEGs; 12 being related to “cellular community – prokaryotes”, 1 of them to “cell growth and death”, and 1 of them to “cell motility”.

-Genetic Information Processing category contained a total of 12 DEGs; 7 being related to “replication and repair”, and 5 of them being related to “folding, sorting and degradation”.

-Organismal Systems category had only 2 DEGs which were related to “immune system”.

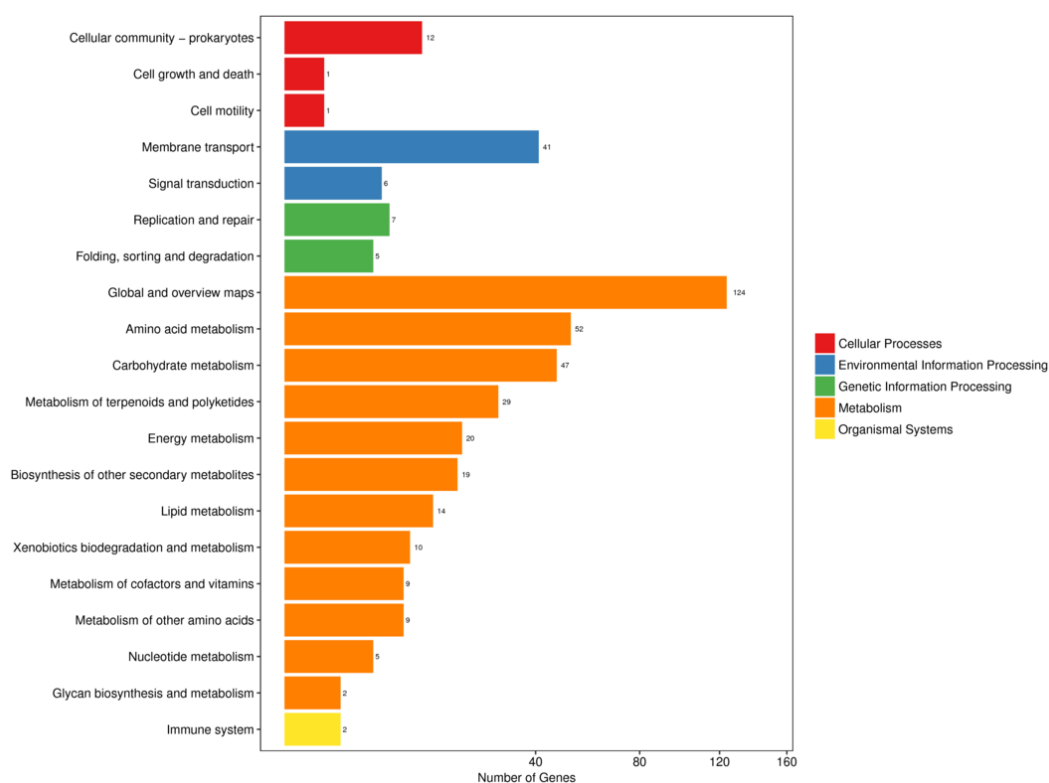


Figure 3.2 KEGG Pathway analysis of *S. clavuligerus* DEPA.

According to KEGG pathway analysis, the most enriched pathways were determined and represented in Figure 3.3. The most enriched pathway belonged to Global and Overview Maps, that is ‘Biosynthesis of Antibiotic’ by having 61 DEGs. ‘ABC Transporters’ (35) which belonged to Membrane Transport, ‘Biosynthesis of Amino Acids’ (25) and ‘Carbon Metabolism’ (24) which belonged to Global and Overview Maps were the other enriched pathways. Other enriched pathways included ‘2-Oxocarboxylic acid metabolism’ belonging to Global and Overview Maps had 12 DEGs; ‘Pentose phosphate pathway’ and ‘Butanoate metabolism’ belonging to Carbohydrate Metabolism had 11 and 10 DEGs, respectively; and ‘Arginine biosynthesis’ belonging to Amino Acid Metabolism had 9 DEGs.

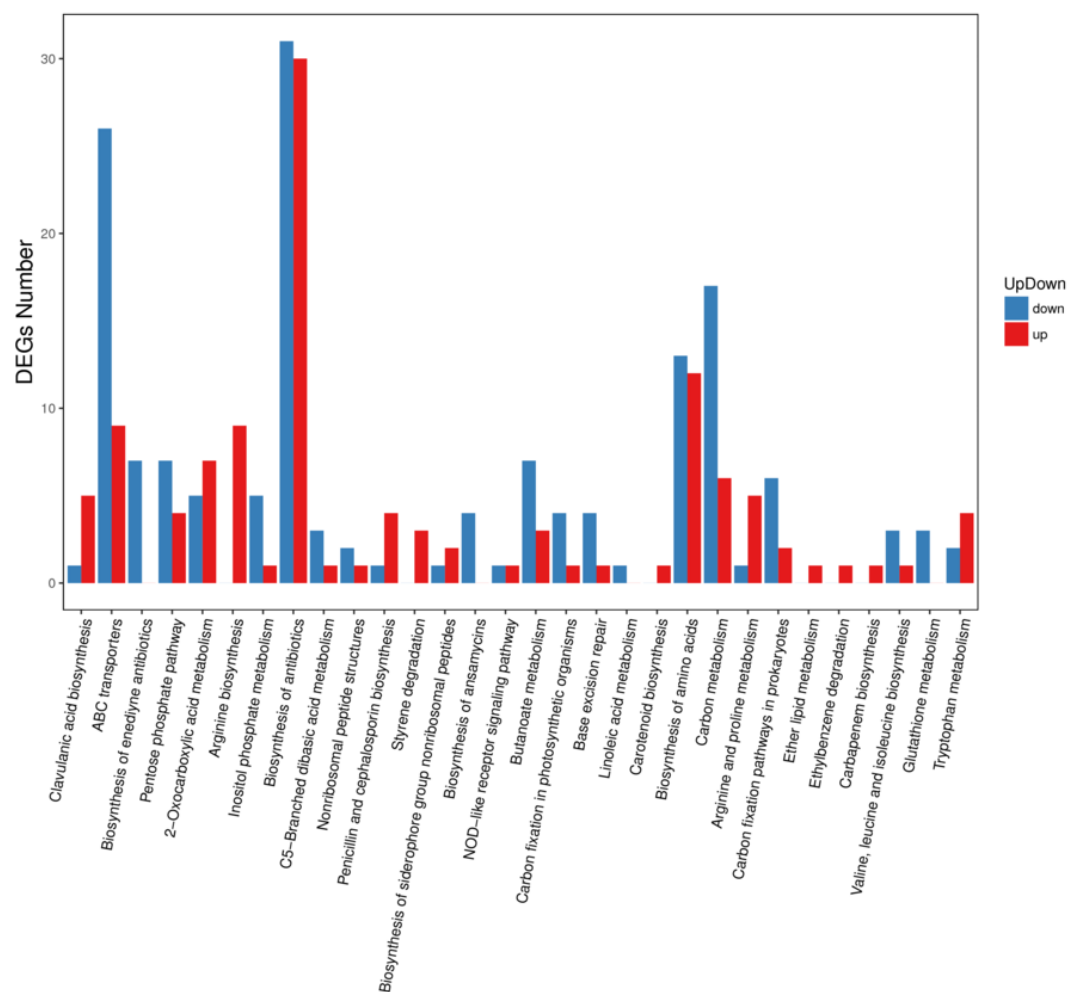


Figure 3.3 The most enriched pathways of *S. clavuligerus* DEPA.

3.1.2 Functional Classification of Differentially Expressed Genes

Functional classification of the differentially expressed genes was accomplished by using Gene Ontology (GO) knowledgebase. GO classification is divided into 3 main categories: Biological Process, Molecular Function, and Cellular Component.

The category “Molecular Function” had the highest number of DEGs (622) while the categories of “Biological Process” and “Cellular Component” had nearly equal numbers of DEGs (489 and 490, respectively). On the other hand, the DEGs seemed to affect 14 different functions in the category of Biological Process, 9 different functions in the category of Molecular Function, and 7 different functions in the category of Cellular Component.

-Molecular Function subcategories: Catalytic activity (318) and Binding (220) had the highest number of DEGs within all subcategories which were followed by Transporter activity (43) and Transcription regulator activity (20) subcategories.

-Biological Process subcategories: Metabolic process (166) and Cellular process (137) had the highest number of DEGs within this category which were followed by Localization (45), Biological regulation (42), Regulation of biological process (39), Response stimulation (32) and Signaling (15) subcategories.

-Cellular Component subcategories: Membrane (160) and Membrane part (158) had the highest number of DEGs within this category as followed by Cell (82) and Cell part (76) subcategories.

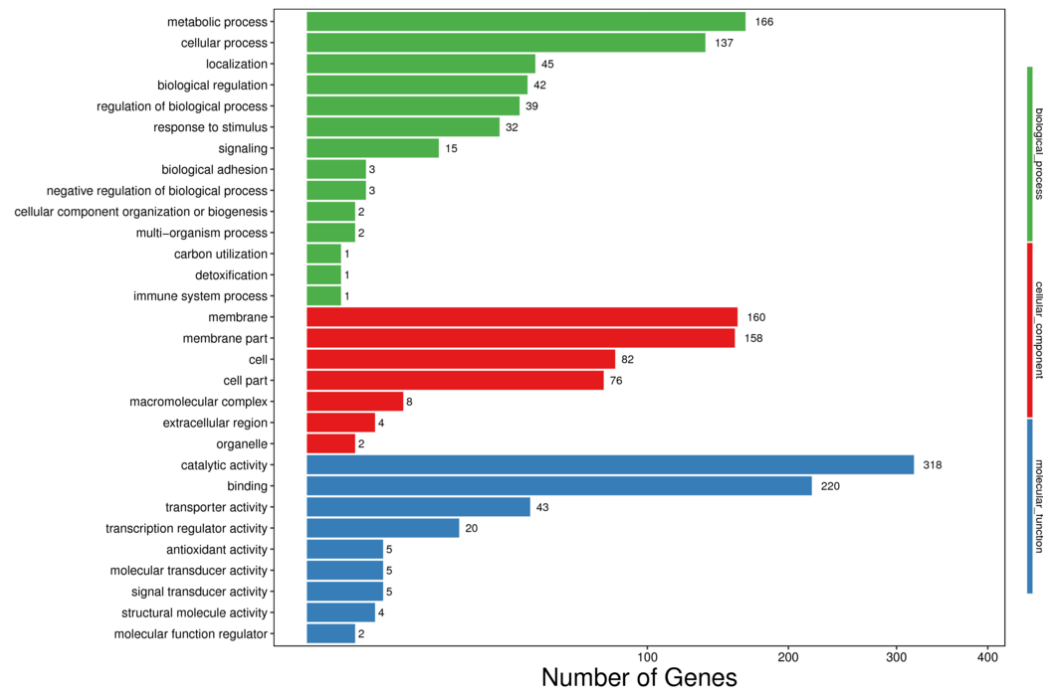


Figure 3.4 GO functional classification of *S. clavuligerus* DEPA.

In Figure 3.5, an enrichment analysis of GO terms are shown. The most enriched term is ‘catalytic activity’; with the following term ‘binding’ in the Molecular function category. In the Cellular component category, ‘membrane’ and ‘membrane part’ are found as the most enriched terms followed by ‘cell’ and ‘cell parts’. Finally, in the Biological process category, the most enriched terms are ‘metabolic process’ and ‘cellular process’.

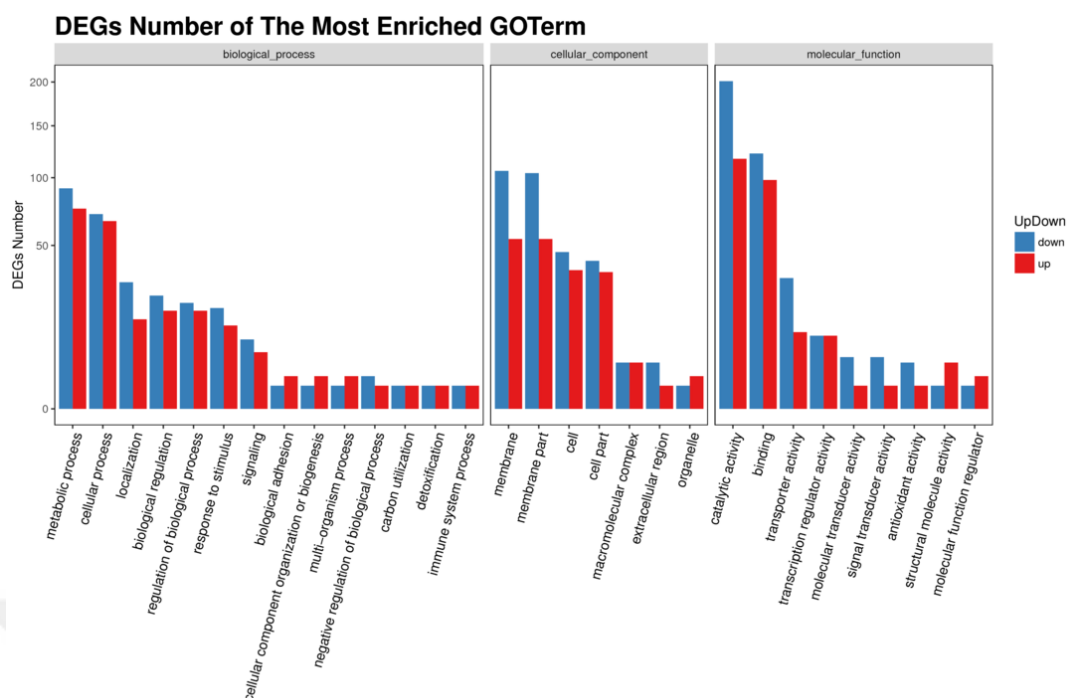


Figure 3.5 The most enriched GO terms of *S. clavuligerus* DEPA.

3.1.3 Differentially Expressed Genes Data Analysis

In this section, significantly differentially expressed genes (DEGs) are grouped according to their functional categories, and the results are discussed.

As it is known, an accession number (AC) is assigned to each sequence upon inclusion into UniProtKB. Such numbers are stable from release to release. Our raw transcriptome data, as in the case of all genome-wide studies, were initially based on such numbers. However, for an effective DEG pathway analysis, it is necessary to convert these numbers to species-specific loci. The first step in our data analysis therefore consisted of finding the specific SCLAV (*S. clavuligerus*) loci numbers that correspond to the UniProt numbers. After functional categories was determined by using COG database (<https://www.ncbi.nlm.nih.gov/research/cog>), the transcriptome data (DEGs) were reorganized to form Table 3.2.

Of a total of 372 DEGs listed in this manner, 158 were upregulated in a range of 1.5- to 7.8-fold and 214 of these DEGs were found as downregulated in a range of -1.5 to -13.3-fold. DEGs were grouped into 15 different functional categories.

Functionally unknown DEGs dominated the list, with 126 DEGs found in this category. This category was followed by Secondary metabolite biosynthesis, transport, and catabolism as the second most abundant category with 87 DEGs.



Table 3.2 Reorganized transcriptome data

Uniprot number	Protein name	Gene	Log ₂ FC	NRL3585-FPKM	DEPA-FPKM	FDR	P-value
Secondary metabolites biosynthesis, transport, and catabolism							
B5GZD1	Epimerase	SCLAV_5662	6.334	0.03	2.42	2.62x10 ⁻⁰⁵	1.01x10 ⁻⁰⁵
E2Q6Y4	Putative phosphotransferase	SCLAV_0155	5.819	27.14	1532.58	0	0
B5GPH9	Transcriptional regulator WhiB	SCLAV_2475 (whiB)	5.746	0.03	1.61	2.37x10 ⁻⁰³	1.16x10 ⁻⁰³
E2PW36	AmfT protein (amfT)	SCLAV_4953	4.27	1.81	34.91	7.72x10 ⁻²⁵⁶	2.14x10 ⁻²⁵⁷
E2PW37	Membrane translocator (amfB)	SCLAV_4954	3.671	1.34	17.07	1.01x10 ⁻¹⁰⁶	6.15x10 ⁻¹⁰⁸
E2PY81	Putative Mce family protein	SCLAV_5185	3.575	0.35	4.17	2.87x10 ⁻¹⁰	7.82x10 ⁻¹¹
E2Q6Y3	Regulatory protein	SCLAV_0154	3.52	61.98	711.02	0	0
B5H0E0	ABC-transporter integral membrane protein	SCLAV_5183	3.475	0.34	3.78	3.33x10 ⁻⁰⁷	1.10x10 ⁻⁰⁷
B5GMR1	Response regulator receiver protein	SCLAV_p1081	3.448	0.48	5.24	6.70x10 ⁻⁰⁵	2.70x10 ⁻⁰⁵
E2Q6Y8	Terpene synthase (EC 4.2.3.-)	SCLAV_0159	2.909	20.97	157.47	0	0
E2PZ65	GAF domain-containing protein	SCLAV_5253	2.483	3.12	17.44	2.92x10 ⁻¹⁵	6.37x10 ⁻¹⁶
D5SL22	HPC2 multi-domain protein	SCLAV_p1129	2.464	0.06	0.68	3.82x10 ⁻⁰⁵	1.50x10 ⁻⁰⁵
E2PW38	Membrane translocator (amfA)	SCLAV_4955	2.411	1.2	6.38	7.78x10 ⁻¹⁸	1.56x10 ⁻¹⁸
E2PY82	Secreted protein	SCLAV_5186	2.209	0.32	1.48	3.93x10 ⁻⁰³	1.99x10 ⁻⁰³
D5SLB8	Valine decarboxylase (EC 4.1.1.22)	SCLAV_p1225	2.195	5.14	23.53	5.19x10 ⁻⁵⁵	5.22x10 ⁻⁵⁶
B5GW98	Putative ABC transporter solute-binding protein	SCLAV_4727	2.17	0.6	2.7	1.51x10 ⁻⁰⁴	6.39x10 ⁻⁰⁵
B5GMI9	Beta-lactamase domain containing protein	SCLAV_p1007	2.082	8.3	35.13	5.53x10 ⁻⁸⁶	3.86x10 ⁻⁸⁷
E2QAC1	Putative multidrug resistance protein	SCLAV_4748	2.018	0.61	2.47	2.41x10 ⁻⁰⁴	1.04x10 ⁻⁰⁴
E2Q808	Monooxygenase	SCLAV_4469	1.997	44.58	177.93	9.09x10 ⁻²⁴⁹	2.57x10 ⁻²⁵⁰
E2PWF0	Multicopper oxidase type 2	SCLAV_0906	1.968	95.99	375.63	0	0
P0DIQ5	Glutamate N-acetyltransferase 2	SCLAV_4193 (oat2)	1.936	595.02	2277.56	0	0
B5H3S9	Putative secreted protein (Secreted protein)	SCLAV_1071	1.916	2.6	9.81	9.65x10 ⁻¹²	2.44x10 ⁻¹²
Q6TMN1	O-methyltransferase (Putative transferase)	pSCL2.8.25.7c SSCG_05674	1.907	42.6	159.74	5.05x10 ⁻⁹⁶	3.30x10 ⁻⁹⁷

Table 3.2 Reorganized transcriptome data (continued)

B5GYQ2	Transcriptional regulator	SCLAV_p1213	1.888	24.08	89.1	1.73x10 ⁻⁴³	2.05x10 ⁻⁴⁴
E2Q805	3-hydroxyacyl-[acyl-carrier-protein] dehydratase	SCLAV_4466	1.88	53.71	197.73	0	0
E2PZ64	ATP/GTP-binding protein	SCLAV_5252	1.874	2.7	9.9	1.40x10 ⁻⁰⁷	4.53x10 ⁻⁰⁸
E2PUT1	Polyprenyl synthetase	SCLAV_4756	1.854	7.14	25.81	3.72x10 ⁻³⁷	4.87x10 ⁻³⁸
E2PZ62	LC7 family protein	SCLAV_5250	1.839	4.26	15.24	3.35x10 ⁻⁰⁷	1.11x10 ⁻⁰⁷
E2PZ61	Membrane protein	SCLAV_5249	1.829	3.55	12.61	5.17x10 ⁻²⁸	7.89x10 ⁻²⁹
E2Q809	Putative secreted lipase	SCLAV_4470	1.745	112.83	378.07	0	0
E2PWH3	Homogentisate 1,2-dioxygenase	SCLAV_0929	1.727	26.65	88.23	2.12x10 ⁻¹¹⁶	1.21x10 ⁻¹¹⁷
E2Q806	Short-chain dehydrogenase/reductase SDR	SCLAV_4467	1.705	33.03	107.7	1.01x10 ⁻¹⁶⁵	4.35x10 ⁻¹⁶⁷
E2Q810	Putative membrane protein	SCLAV_4471	1.627	57.03	176.12	6.13x10 ⁻¹⁵⁵	2.77x10 ⁻¹⁵⁶
E2Q9H2	UPF0089 domain-containing protein	SCLAV_0516	1.623	0.87	2.68	2.47x10 ⁻⁰⁴	1.07x10 ⁻⁰⁴
D5SLB3	Pentapeptide repeat protein	SCLAV_p1220	1.541	4.93	14.35	2.49x10 ⁻⁰⁸	7.70x10 ⁻⁰⁹
E2Q811	Amidase	SCLAV_4472	1.538	69.37	201.51	6.24x10 ⁻²³⁷	1.90x10 ⁻²³⁸
E2Q5Q3	ABC-type dipeptide transport system, periplasmic component	SCLAV_4192 (oppA1)	1.522	239.53	687.85	0	0
Q8KRB5	Acetyltransferase GNAT family protein (Orf14)	SCLAV_4185 (orf14)	1.518	66.65	190.9	1.17x10 ⁻¹⁵³	5.30x10 ⁻¹⁵⁵
B5GYS5	Enediynes biosynthesis protein UnbV	SCLAV_p1231	-1.506	16.3	5.74	1.64x10 ⁻²⁶	2.60x10 ⁻²⁷
E2Q8V8	3-isopropylmalate dehydratase large subunit	SCLAV_4463 (leuC)	-1.512	124.2	43.55	3.22x10 ⁻¹⁴⁶	1.51x10 ⁻¹⁴⁷
E2Q2S1	Putative hydrolase	SCLAV_5715	-1.523	9.08	3.16	1.80x10 ⁻⁰⁹	5.11x10 ⁻¹⁰
E2PY54	Regulatory protein	SCLAV_5156	-1.612	357.62	116.97	1.18x10 ⁻¹⁹⁸	4.29x10 ⁻²⁰⁰
E2Q2J6	Rieske 2Fe-2S domain-containing protein	SCLAV_5640	-1.635	7.05	2.27	3.43x10 ⁻⁰⁸	1.07x10 ⁻⁰⁸
B5H481	Methyltransferase type 11	SCLAV_5345	-1.679	11.08	3.46	3.18x10 ⁻⁰⁹	9.20x10 ⁻¹⁰
E2Q2Q8	Putative siderophore synthetase component	SCLAV_5702	-1.686	10.1	3.14	1.72x10 ⁻¹²	4.25x10 ⁻¹³
B5GPX4	TetR-family transcriptional regulator	SCLAV_2302	-1.709	255.58	78.19	4.83x10 ⁻¹³⁸	2.41x10 ⁻¹³⁹
E2PZE8	Putative methyltransferase	SCLAV_5342	-1.747	1.88	0.56	4.86x10 ⁻⁰³	2.51x10 ⁻⁰³
E2Q8R4	Putative relaxases/mobilisation protein	SCLAV_2479	-1.766	1.19	0.35	4.86x10 ⁻⁰³	2.51x10 ⁻⁰³
E2Q2Q9	Putative siderophore synthetase component	SCLAV_5703	-1.823	5.98	1.69	1.01x10 ⁻¹²	2.46x10 ⁻¹³
E2Q1N2	ABC transporter ATP-binding protein	SCLAV_5591	-1.875	10.09	2.75	3.96x10 ⁻⁰⁸	1.24x10 ⁻⁰⁸
B5H439	Cyclopropane-fatty-acyl-phospholipid synthase	SCLAV_p1265	-1.877	4.26	1.16	3.18x10 ⁻⁰⁵	1.24x10 ⁻⁰⁵
B5GVE1	L-isoaspartyl protein carboxyl methyltransferase	SCLAV_4388	-1.88	65.95	17.92	2.46x10 ⁻⁹⁰	1.66x10 ⁻⁹¹
E2Q2M7	Terpene synthase metal-binding protein	SCLAV_5671	-1.908	3.19	0.85	2.60x10 ⁻⁰⁴	1.13x10 ⁻⁰⁴
B5GVE3	Lantibiotic dehydratase domain-containing protein	SCLAV_4390	-1.918	5.33	1.41	4.64x10 ⁻²⁰	8.65x10 ⁻²¹

Table 3.2 Reorganized transcriptome data (continued)

B5GNL7	ChiF (Chitinase ChiF)	SCLAV_3515	-1.998	55.48	13.89	3.12x10 ⁻⁶¹	2.88x10 ⁻⁶²
E2QIN6	DUF779 domain-containing protein	SCLAV_5595	-2.028	565	138.56	3.07x10 ⁻²³¹	9.47x10 ⁻²³³
B5GN67	Beta-lactamase domain-containing protein	SCLAV_p0761	-2.033	11.05	2.7	7.78x10 ⁻¹³	1.88x10 ⁻¹³
D5SLC2	Major facilitator superfamily MFS_1	SCLAV_p1229	-2.279	14.66	3.02	8.22x10 ⁻³⁴	1.14x10 ⁻³⁴
E2PXA1	Putative membrane protein	SCLAV_0946	-2.555	1.88	0.32	2.98x10 ⁻⁰⁴	1.30x10 ⁻⁰⁴
D5SLC5	Eneidyne biosynthesis protein UnbU	SCLAV_p1232	-2.644	28.51	4.56	1.25x10 ⁻²⁰	1.31x10 ⁻²¹
B5GWT0	Cupin family protein	SCLAV_5486	-2.693	8.28	1.28	7.43x10 ⁻⁰⁶	2.71x10 ⁻⁰⁶
E2PWJ6	Alpha/beta hydrolase (EC 2.3.1.31)	SCLAV_2924 (cvm4)	-2.999	59.96	7.5	9.75x10 ⁻¹¹⁴	5.65x10 ⁻¹¹⁵
B5GWT9	Daurorubicin resistance ABC transporter ATPase subunit	SCLAV_5496	-3.11	4.75	0.55	5.46x10 ⁻¹⁰	1.51x10 ⁻¹⁰
E2Q7S6	Magnesium or manganese-dependent protein phosphatase	SCLAV_4387	-3.577	61.56	5.16	1.04x10 ⁻⁶²	9.31x10 ⁻⁶⁴
B5GN13	AfsA domain-containing protein	SCLAV_p0812	-3.633	53.09	4.28	1.03x10 ⁻¹⁰⁷	6.17x10 ⁻¹⁰⁹
Q9X5G9	Flavin-dependent oxidoreductase, F420-dependent methylene-tetrahydromethanopterin (Putative luciferase)	SCLAV_2923 (cvm5)	-3.668	57.07	4.49	2.16x10 ⁻¹⁵⁷	9.69x10 ⁻¹⁵⁹
B5GN11	Phosphoenolpyruvate synthase	SCLAV_p0814	-3.948	31.63	2.05	1.12x10 ⁻²²⁷	3.54x10 ⁻²²⁹
B5H0Q8	Alkaline serine protease (Secreted serine protease)	SCLAV_0093	-4.077	3129.5	185.44	0	0
E2PWJ4	Putative pyridoxal phosphate-dependent aminotransferase	SCLAV_2922 (cvm6)	-4.193	132.8	7.26	0	0
E2PWJ7	Clavamate synthase 1 (EC 1.14.11.21)	SCLAV_2925 (cas1)	-4.812	61.53	2.19	1.01x10 ⁻¹⁶⁶	4.26x10 ⁻¹⁶⁸
B5GN12	Guanylate kinase (Isoprenylcysteine carboxyl methyltransferase)	SCLAV_p0813	-5.28	27.58	0.71	1.17x10 ⁻⁵⁰	1.23x10 ⁻⁵¹
B5GN73	Methylamine utilization protein MauE	SCLAV_p0755	-6.989	3.81	0.03	1.26x10 ⁻¹⁰	3.34x10 ⁻¹¹
B5GNB6	Putative transketolase (Transketolase B subunit)	SCLAV_p0714	-7.355	4.91	0.03	3.66x10 ⁻¹⁵	8.03x10 ⁻¹⁶
B5GN68	Transcriptional regulator	SCLAV_p0760	-7.639	5.98	0.03	4.17x10 ⁻¹⁹	7.97x10 ⁻²⁰
D5SIU9	Putative transport protein	SCLAV_p0705	-7.672	6.12	0.03	3.40x10 ⁻¹⁸	6.69x10 ⁻¹⁹
B5GNC1	Phytanoyl-CoA dioxygenase	SCLAV_p0709	-7.744	6.43	0.03	6.89x10 ⁻²⁵	1.12x10 ⁻²⁵
D5SIV5	Condensation domain protein	SCLAV_p0711	-8.265	9.23	0.03	3.51x10 ⁻⁶⁶	3.02x10 ⁻⁶⁷
D5SIV2	tRNA synthetase	SCLAV_p0708	-8.356	9.83	0.03	1.50x10 ⁻²⁸	2.27x10 ⁻²⁹
D5SIV0	ACPS domain-containing protein	SCLAV_p0706	-8.399	10.13	0.03	6.31x10 ⁻²¹	1.13x10 ⁻²¹
B5GNB8	Permease (Putative permease of the major facilitator superfamily)	SCLAV_p0712	-8.775	13.14	0.03	4.65x10 ⁻⁶⁰	4.35x10 ⁻⁶¹

Table 3.2 Reorganized transcriptome data (continued)

B5GNB7	Transketolase A subunit (EC 2.2.1.1)	SCLAV_p0713	-9.076	16.19	0.03	3.84x10 ⁻⁴⁴	4.48x10 ⁻⁴⁵
D5SJX6	Cytochrome P450	SCLAV_p0732	-9.201	35.32	0.06	2.18x10 ⁻¹⁷⁶	8.78x10 ⁻¹⁷⁸
B5GNC8	Carbonic anhydrase (EC 4.2.1.1) (Carbonate dehydratase)	SCLAV_p0703	-9.609	23.42	0.03	1.13x10 ⁻⁴⁵	1.29x10 ⁻⁴⁶
D5SIJX0	Cytochrome P450 hydroxylase	SCLAV_p0726	-10.812	53.92	0.03	3.63x10 ⁻²³⁸	1.10x10 ⁻²³⁹
D5SLH1	NAD-dependent epimerase/dehydratase	SCLAV_p1278	-11.575	91.5	0.03	0	0
(MoeE5)						0	0
B5GN70	Putative DNA-binding protein	SCLAV_p0758	-11.864	111.85	0.03	2.62x10 ⁻⁰⁵	1.01x10 ⁻⁰⁵
Energy production and conversion							
B5GZX5	Divalent metal ions/citrate complex secondary transporter	SCLAV_0928	-1.609	158.76	52.05	6.53x10 ⁻²¹⁴	2.18x10 ⁻²¹⁵
E2Q204	Aldehyde dehydrogenase	SCLAV_1555	-1.633	28.82	9.29	2.93x10 ⁻⁴¹	3.60x10 ⁻⁴²
E2PXA6	Radical SAM domain-containing protein	SCLAV_0951	-4.836	2.57	0.09	6.06x10 ⁻¹⁵	1.34x10 ⁻¹⁵
D5SIW6	Alkylglycerone-phosphate synthase	SCLAV_p0722	-7.022	3.9	0.03	5.08x10 ⁻²⁰	9.50x10 ⁻²¹
D5SIW2	Putative monooxygenase	SCLAV_p0718	-13.298	302.16	0.03	0	0
Amino acid transport and metabolism							
E2Q1Y6	PAT1 domain-containing protein	SCLAV_1537	2.8	1.47	10.24	8.55x10 ⁻²⁴	1.43x10 ⁻²⁴
E2Q9H9	Urease subunit beta	SCLAV_0523	2.109	2.87	12.38	3.88x10 ⁻⁰⁶	1.38x10 ⁻⁰⁶
E2PVC6	Argininosuccinate synthase,	SCLAV_0796 (argY)	2	2912.1	11646.79	0	0
B5H257	Argininosuccinate lyase	SCLAV_0795 (argH)	1.784	557.74	1920.19	0	0
B5H252	Arginine biosynthesis bifunctional protein ArgJ	SCLAV_0800 (argJ)	1.734	338.34	1125.75	0	0
E2PUJ1	Putative amino acid decarboxylase	SCLAV_2698	1.699	14.84	48.18	2.57x10 ⁻¹⁶⁰	1.13x10 ⁻¹⁶¹
E2PVD1	N-acetyl-gamma-glutamyl-phosphate reductase, AGPR	SCLAV_0801 (argC)	1.631	945.47	2929.14	0	0
E2PVC9	Acetylglutamate kinase	SCLAV_0799 (argB)	1.502	300.57	851.06	0	0
B5H4B9	ABC transporter ATP-binding protein	SCLAV_5426	-1.766	9.93	2.92	3.49x10 ⁻¹²	8.69x10 ⁻¹³
E2PWH8	Secreted hydrolase	SCLAV_0934	-1.788	210.79	61.05	9.79x10 ⁻¹⁷⁴	4.01x10 ⁻¹⁷⁵
B5H1V7	Carboxypeptidase (EC 3.4.17.18) (Zinc-carboxypeptidase)	SCLAV_3908	-2.044	26.56	6.44	2.35x10 ⁻⁴⁸	2.57x10 ⁻⁴⁹
B5GXU2	Putative glyoxalase family protein	SCLAV_2158	-2.211	15.05	3.25	9.58x10 ⁻⁰⁸	3.05x10 ⁻⁰⁸
E2Q7Q0	Neutral metalloproteinase	SCLAV_4359	-2.569	108.31	18.25	0	0

Table 3.2 Reorganized transcriptome data (continued)

Carbohydrate transport and metabolism									
E2Q0G5	Pyruvate phosphate dikinase (EC 2.7.9.1)	SCLAV_5441 (pddK)	4.559	84.02	1980.47	0	0	0	0
B5GKZ5	ATP-dependent 6-phosphofructokinase, EC 2.7.1.11 (Phosphofructokinase, Phosphohexokinase)	SCLAV_4332 (pfkA)	2.998	0.8	6.39	6.87x10 ⁻⁰⁹	2.03x10 ⁻⁰⁹		
E2Q3Q7	Putative phosphoglycerate mutase (EC 5.4.2.1)	SCLAV_1803	2.45	0.28	1.53	2.58x10 ⁻⁰²	1.52x10 ⁻⁰²		
E2Q445	Chitinase I	SCLAV_3909	-1.55	16.43	5.61	7.03x10 ⁻²⁵	1.15x10 ⁻²⁵		
E2P XK2	Putative glycosyl hydrolase	SCLAV_1046	-1.597	13.76	4.55	5.41x10 ⁻²³	9.24x10 ⁻²⁴		
E2PY25	Putative abasic site repairing enzyme	SCLAV_5128	-1.762	2.95	0.87	1.01x10 ⁻⁰³	4.73x10 ⁻⁰⁴		
E2Q8M8	Beta-mannanase	SCLAV_2443	-1.814	34.24	9.74	8.55x10 ⁻⁴⁰	1.08x10 ⁻⁴⁰		
E2PY19	Putative ABC transporter substrate-binding protein	SCLAV_5122	-1.845	17.67	4.92	6.11x10 ⁻²¹	1.09x10 ⁻²¹		
E2Q3P3	Acyltransferase 3	SCLAV_1789	-2.852	436.85	60.5	0	0		
E2PXA0	Chitin-binding domain-containing protein	SCLAV_0945	-2.901	28.98	3.88	3.95x10 ⁻⁵³	4.04x10 ⁻⁵⁴		
E2Q0G4	Putative secreted chitin-binding protein	SCLAV_5440	-4.193	182.76	9.99	1.29x10 ⁻²²⁷	4.08x10 ⁻²²⁹		
B5GNA7	Carbohydrate kinase (Carbohydrate kinase FGGY)	SCLAV_p0723	-5.737	1.6	0.03	3.13x10 ⁻⁰⁸	9.70x10 ⁻⁰⁹		
B5GN81	Chitin-binding protein (Putative secreted chitin-binding protein)	SCLAV_p0747	-10.466	42.44	0.03	8.08x10 ⁻⁸²	5.97x10 ⁻⁸³		
B5GN86	Uncharacterized protein	SCLAV_p0742	-11.682	98.54	0.03	4.23x10 ⁻¹⁷²	1.76x10 ⁻¹⁷³		
Lipid transport and metabolism									
D5SL20	Short-chain dehydrogenase/reductase SDR	SCLAV_p1127	2.137	1.03	4.53	6.06x10 ⁻⁰⁶	2.20x10 ⁻⁰⁶		
E2PZJ4	Short chain dehydrogenase	SCLAV_1225	1.93	6.68	25.46	7.03x10 ⁻²³	1.21x10 ⁻²³		
E2Q807	Short-chain dehydrogenase/reductase SDR	SCLAV_4468	1.862	52.58	191.07	2.43x10 ⁻¹⁸⁶	9.54x10 ⁻¹⁸⁸		
D5SLC6	Eneidine Polyketide synthase	SCLAV_p1233	-1.53	19.73	6.83	1.79x10 ⁻¹⁰¹	1.12x10 ⁻¹⁰²		
B5GRJ8	Probable short-chain dehydrogenase	SCLAV_4964	-1.619	3.84	1.25	3.88x10 ⁻⁰⁴	1.72x10 ⁻⁰⁴		
D5SLF7	AMP-dependent synthetase and ligase (fatty-acyl-CoA synthase)	SCLAV_p1264	-1.662	5.79	1.83	3.04x10 ⁻¹⁰	8.30x10 ⁻¹¹		
E2Q045	Enoyl-CoA hydratase/isomerase	SCLAV_3393	-1.732	3.09	0.93	2.77x10 ⁻⁰³	1.37x10 ⁻⁰³		
E2Q9B0	3-oxoacyl-acyl-carrier-protein synthase III (EC 2.3.1.180)	SCLAV_0454	-1.931	2.25	0.59	1.25x10 ⁻⁰³	5.91x10 ⁻⁰⁴		
B5GZ70	Dehydrogenase (Putative dehydrogenase)	SCLAV_1511	-2.076	7.38	1.75	5.48x10 ⁻⁰⁸	1.73x10 ⁻⁰⁸		
B5H0W5	Dehydrogenase	SCLAV_1015	-2.517	3.72	0.65	6.49x10 ⁻⁰⁵	2.62x10 ⁻⁰⁵		

Table 3.2 Reorganized transcriptome data (continued)

E2QIH2	Putative 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30)	SCLAV_5531	-2.534	50.68	8.75	1.98x10 ⁻⁶⁴	1.74x10 ⁻⁶⁵
D5SIW5	Short-chain dehydrogenase/reductase SD	SCLAV_p0721	-6.143	2.12	0.03	7.72x10 ⁻⁰⁶	2.82x10 ⁻⁰⁶
B5GNC3	Holo-acyl synthase	SCLAV_p0707	-9.116	16.65	0.03	9.57x10 ⁻¹³	2.33x10 ⁻¹³
Coenzyme transport and metabolism							
E2Q5G9	Putative polyprenyl diphosphate synthase	SCLAV_4108	1.994	4.19	16.69	7.35x10 ⁻²¹	1.33x10 ⁻²¹
E2Q161	5-amino-6-(D-ribitylamino)uracil--L-tyrosine hydroxyphenyl transferase	SCLAV_3494	-1.54	14.89	5.12	1.82x10 ⁻⁰³	8.75x10 ⁻⁰⁴
B5GNZ2	ThiamineS protein	SCLAV_3249	-1.604	89.56	29.46	6.20x10 ⁻²⁰	1.16x10 ⁻²⁰
E2PY24	Putative transferase	SCLAV_5127	-1.785	4.24	1.23	2.60x10 ⁻⁰⁵	1.0x10 ⁻⁰⁵
E2PX07	Bifunctional deaminase-reductase domain-containing protein	SCLAV_5011	-1.927	6.2	1.63	3.47x10 ⁻⁰⁵	1.36x10 ⁻⁰⁵
B5GN94	Polyprenyl synthetase domain-containing protein	SCLAV_p0735	-6.254	2.29	0.03	1.57x10 ⁻⁰⁸	4.78x10 ⁻⁰⁹
B5GN79	Biotin carboxylase	SCLAV_p0749	-6.837	3.43	0.03	8.41x10 ⁻¹⁹	1.62x10 ⁻¹⁹
Nucleotide transport and metabolism							
E2Q1I0	Cytosine deaminase-like metal-dependent hydrolase	SCLAV_5539	-1.516	10.27	3.59	4.84x10 ⁻¹¹	1.26x10 ⁻¹¹
E2Q793	Putative MutT-family protein	SCLAV_0264	-10.824	54.4	0.03	4.18x10 ⁻⁸⁶	2.92x10 ⁻⁸⁷
Inorganic/Organic molecule transport and metabolism							
D5SKD7	Putative integral membrane protein	SCLAV_p0893	4.801	4.2	117.08	0	0
E2Q8Y8	Signal recognition particle receptor FtsY	SCLAV_4531 (ftsY)	2.981	4.75	37.5	4.19x10 ⁻¹⁰¹	2.64x10 ⁻¹⁰²
B5GTZ5	ABC-type cobalamin/Fe3+-siderophore transport system	SCLAV_0392	1.85	5.45	19.65	1.59x10 ⁻⁵⁹	1.50x10 ⁻⁶⁰
E2PVC3	DHA2-subfamily multidrug transporter	SCLAV_0793	1.663	50.97	161.46	4.08x10 ⁻²²⁹	1.28x10 ⁻²³⁰
D5SKS5	Multidrug transporter, MFS superfamily protein	SCLAV_p1032	1.522	3.74	10.74	2.70x10 ⁻¹⁰	7.35x10 ⁻¹¹
E2Q8I0	ABC transporter permease	SCLAV_2405	-1.505	1088.22	383.38	0	0
E2Q3G0	Putative permease of the major facilitator superfamily	SCLAV_3803	-1.566	56.83	19.19	1.36x10 ⁻⁶⁹	1.13x10 ⁻⁷⁰
E2PV17	Putative integral membrane protein	SCLAV_2838	-1.571	79.52	26.77	1.25x10 ⁻¹⁴⁷	5.79x10 ⁻¹⁴⁹
B5GZ53	Polyamine ABC transporter integral membrane protein	SCLAV_4580	-1.581	3.92	1.31	1.03x10 ⁻⁰³	4.82x10 ⁻⁰⁴
B5H4C0	Choline transport system permease protein opuBB	SCLAV_5427	-1.612	9.05	2.96	4.54x10 ⁻⁰⁶	1.62x10 ⁻⁰⁶

Table 3.2 Reorganized transcriptome data (continued)

E2Q1F7	Potassium-transporting ATPase subunit	SCLAV_5516	-1.723	3.07	0.93	4.39x10 ⁻⁰⁶	1.57x10 ⁻⁰⁶
E2PVJ8	Deferrioxalate/oxidase	SCLAV_2839	-1.74	35.71	10.69	2.84x10 ⁻⁵⁵	2.83x10 ⁻⁵⁶
B5H1J8	Putative ABC transporter permease protein	SCLAV_4884	-1.824	13.28	3.75	1.69x10 ⁻¹⁴	3.79x10 ⁻¹⁵
E2Q1J1	Sheath polysaccharide-degrading enzyme	SCLAV_5550	-1.91	19.02	5.06	2.14x10 ⁻²¹	3.77x10 ⁻²²
E2Q0C2	Solute-binding protein	SCLAV_5398	-2.002	7.25	1.81	3.19x10 ⁻¹⁰	8.73x10 ⁻¹¹
B5GPN3	Sugar phosphate permease	SCLAV_2530	-2.215	8.73	1.88	5.03x10 ⁻¹¹	1.31x10 ⁻¹¹
B5GP69	1-phosphatidylinositol phosphodiesterase	SCLAV_3179	-2.37	10.6	2.05	2.34x10 ⁻¹⁴	5.27x10 ⁻¹⁵
E2Q1H3	Major facilitator superfamily permease	SCLAV_5532	-2.632	107.07	17.27	1.62x10 ⁻²⁶¹	4.29x10 ⁻²⁶³
E2PVW8	Transport system integral membrane protein	SCLAV_4885	-2.822	10.61	1.5	1.35x10 ⁻¹⁶	2.81x10 ⁻¹⁷
D5SKS2	Putative transmembrane transport protein	SCLAV_p1029	-3.571	19.01	1.6	1.96x10 ⁻⁶⁵	1.70x10 ⁻⁶⁶
B5GN76	Signal peptidase I (EC 3.4.21.89)	SCLAV_p0752	-5.728	1.59	0.03	1.71x10 ⁻⁰³	8.20x10 ⁻⁰⁴
E2Q208	Putative integral membrane protein	SCLAV_1559	-8.05	7.95	0.03	1.59x10 ⁻³²	2.25x10 ⁻³³
B5GNA3	Alcohol dehydrogenase (Putative alcohol dehydrogenase) (EC 1.1.1.1)	SCLAV_p0727 (adhA)	-12.551	180	0.03	0	0
Transcription & replication, recombination, and repair							
B5GV89	CRISPR-associated endoribonuclease	SCLAV_1861 (cas2)	7.661	0.03	6.07	9.53x10 ⁻⁰⁵	3.92x10 ⁻⁰⁵
D5SKZ2	Acetyltransferase, ribosomal protein N-acetylase	SCLAV_p1099	7.163	0.03	4.3	1.84x10 ⁻⁰¹	1.33x10 ⁻⁰¹
D5SKD6	Transcriptional regulator, TetR family protein	SCLAV_p0892	5.428	90.37	3889.66	0	0
E2Q0G7	TetR/AcrR family transcriptional regulator	SCLAV_5443	4.86	155.9	4526.96	0	0
D5SKD5	Transcriptional regulator	SCLAV_p0891	3.562	8.96	105.8	1.10x10 ⁻¹³²	5.78x10 ⁻¹³⁴
E2Q7L6	RNA polymerase ECF-subfamily sigma factor	SCLAV_2349	3.221	4.92	45.89	5.36x10 ⁻⁷⁸	4.08x10 ⁻⁷⁹
E2Q7N8	Putative alanine-rich protein	SCLAV_2371	2.629	0.27	1.67	1.74x10 ⁻⁰⁵	6.62x10 ⁻⁰⁶
B5GQK1	Helix-turn-helix domain-containing protein (Putative DNA-binding protein)	SCLAV_1642	2.606	36.79	223.96	3.71x10 ⁻²⁹⁸	8.86x10 ⁻³⁰⁰
B5GQJ0	Substrate binding protein	SCLAV_1652	2.518	2.39	13.69	9.92x10 ⁻⁰⁹	2.97x10 ⁻⁰⁹
E2Q096	Transcriptional regulator	SCLAV_5372	2.091	2.83	12.06	1.65x10 ⁻¹⁵	3.56x10 ⁻¹⁶
E2Q783	Putative acetyltransferase (GNAT family)	SCLAV_0254	2.001	3.14	12.57	1.65x10 ⁻¹³	3.87x10 ⁻¹⁴
E2Q742	LuxR family transcriptional regulator	SCLAV_0213	1.98	42.03	165.85	8.14x10 ⁻¹³⁶	4.16x10 ⁻¹³⁷
E2Q0G1	Regulatory protein	SCLAV_5437	1.978	10.92	43.02	1.37x10 ⁻³⁵	1.84x10 ⁻³⁶
E2Q3P6	Putative acetyltransferase	SCLAV_1792	1.759	6.02	20.37	9.98x10 ⁻¹³	2.44x10 ⁻¹³
E2Q310	Regulatory protein	SCLAV_1688	1.567	125.32	371.22	0	0

Table 3.2 Reorganized transcriptome data (continued)

E2Q8M3	Putative DNA polymerase I	SCLAV_2438	-1.641	125.17	40.12	2.60x10 ⁻²⁰⁰	9.33x10 ⁻²⁰²
B5H146	HATPase c domain-containing protein	SCLAV_p1158	-1.678	14.94	4.67	2.37x10 ⁻⁰⁷	7.77x10 ⁻⁰⁸
E2Q2P0	Putative SNF2/RAD54 family helicase	SCLAV_5684	-1.788	73	21.14	1.11x10 ⁻²⁴²	3.28x10 ⁻²⁴⁴
E2Q1X8	Ribonuclease R	SCLAV_1529	-2.294	5.54	1.13	1.42x10 ⁻¹²	3.48x10 ⁻¹³
E2Q6M0	Integrase	SCLAV_4245	-2.353	54.32	10.63	7.40x10 ⁻¹²¹	4.09x10 ⁻¹²²
B5GQR9	Secreted protein	SCLAV_1573	-2.584	58.15	9.7	3.22x10 ⁻⁶²	2.90x10 ⁻⁶³
B5GPJ3	HTH_1cIR domain-containing protein	SCLAV_2489	-3.386	1.15	0.11	9.93x10 ⁻⁰³	5.40x10 ⁻⁰³
B5GL78	ATP-binding protein	SCLAV_4243	-3.498	73.31	6.49	8.91x10 ⁻²⁴⁴	2.59x10 ⁻²⁴⁵
B5GN08	NUDIX hydrolase	SCLAV_p0817	-3.859	9.43	0.65	4.57x10 ⁻¹³	1.10x10 ⁻¹³
B5GNB1	Transcriptional regulator (Transcriptional regulator, TetR family)	SCLAV_p0719	-7.052	3.98	0.03	3.13x10 ⁻⁰⁸	9.70x10 ⁻⁰⁹
D5SJW0	ArsR family transcriptional regulator	SCLAV_p0716	-7.055	10.64	0.08	1.93x10 ⁻³⁷	2.52x10 ⁻³⁸
D5SJW4	Glycerol-3-phosphate responsive antiterminator	SCLAV_p0720 (glpP)	-9.305	18.97	0.03	5.69x10 ⁻³⁵	7.75x10 ⁻³⁶
B5GN83	MerR-family transcriptional regulator (Putative MerR-family transcriptional regulator)	SCLAV_p0745	-11.072	64.58	0.03	1.50x10 ⁻¹⁸²	5.95x10 ⁻¹⁸⁴
Translation, ribosomal structure and biogenesis							
E2Q097	Endoribonuclease L-PSP	SCLAV_5373	2.236	2.21	10.41	5.86x10 ⁻⁰⁶	2.12x10 ⁻⁰⁶
B5GNA5	Putative endoribonuclease (YjgF)	SCLAV_p0725	-11.687	98.91	0.03	1.01x10 ⁻¹²⁵	5.52x10 ⁻¹²⁷
Post-translational modification, protein turnover, and chaperones							
E2Q9H7	Urease accessory protein UreF	SCLAV_0521 (ureF)	3.228	0.38	3.56	5.92x10 ⁻⁰⁶	2.14x10 ⁻⁰⁶
B5GTC0	Chaperone protein ClpB	SCLAV_2659	1.906	228.33	855.48	0	0
E2Q0F5	Subtilisin-like serine protease	SCLAV_5431	1.753	211.59	8	0	0
B5GTB1	Chaperone protein DnaK (HSP70)	SCLAV_2666 (dnaK)	1.643	3949.82	12335.7	0	0
E2PUF8	Protein GrpE (HSP-70 cofactor)	SCLAV_2665	1.584	1607.16	4819.87	0	0
E2Q5I7	Lon protease	SCLAV_4136 (lonA)	1.53	160.06	462.25	0	0
E2PZ12	Trypsin-like protease	SCLAV_1213	-1.762	153.37	45.21	1.26x10 ⁻¹²⁷	6.76x10 ⁻¹²⁹
B5GQE3	Extracellular small neutral protease (EC 3.4.24.77)	SCLAV_4112	-3.459	95.01	8.64	1.92x10 ⁻¹³²	1.01x10 ⁻¹³³
E2Q0F5	Subtilisin-like serine protease	SCLAV_5431	-4.725	2.13	7.18	3.22x10 ⁻⁰⁶	1.14x10 ⁻⁰⁶
E2PXJ5	Putative secreted subtilisin-like serine protease	SCLAV_1039	-7.744	312.99	1.46	0	0
D5SJZ4	Subtilisin-like protease	SCLAV_p0750	-12.707	267.39	0.04	0	0

Table 3.2 Reorganized transcriptome data (continued)

Cell wall/membrane/envelope biogenesis									
E2Q859	UDP-N-acetylglucosamine 2-epimerase,	SCLAV_0315	2.309	1.86	9.22	1.08x10 ⁻¹⁷	2.16x10 ⁻¹⁸		
E2Q0N6	Putative NLP/Pe0-family secreted protein	SCLAV_1350	1.909	0.53	1.99	3.78x10 ⁻⁰³	1.91x10 ⁻⁰³		
E2Q1I3	Dolichol-phosphate mannosyltransferase (EC 2.4.1.83)	SCLAV_5542	1.612	3.82	11.68	1.56x10 ⁻¹³	3.65x10 ⁻¹⁴		
D5SL37	Peptidoglycan-binding domain 1 protein	SCLAV_p1144	1.61	17.33	52.9	5.36x10 ⁻⁴⁸	5.88x10 ⁻⁴⁹		
B5GPL6	Lipoprotein (Putative lipoprotein)	SCLAV_2512	-2.039	23.71	5.77	1.14x10 ⁻³³	1.58x10 ⁻³⁴		
B5GNM7	Peptidase S1 and S6 (Peptidase S1 and S6, chymotrypsin/Hap)	SCLAV_3506	-2.169	84.31	18.75	2.83x10 ⁻¹³⁸	1.40x10 ⁻¹³⁹		
E2Q172	Peptidase S1 and S6, chymotrypsin/Hap	SCLAV_3505	-2.312	137.33	27.65	2.80x10 ⁻²⁵⁷	7.62x10 ⁻²⁵⁹		
E2PVJ5	Secreted protein (SCO family protein)	SCLAV_2836	-2.321	101.87	20.39	3.59x10 ⁻¹⁰³	2.24x10 ⁻¹⁰⁴		
B5GNA2	Uncharacterized protein	SCLAV_p0728	-6.723	3.17	0.03	3.98x10 ⁻⁰⁹	1.16x10 ⁻⁰⁹		
Cell cycle control, cell division, chromosome partitioning, chromatin structure and dynamics									
E2PX00	NTP transf_3 domain-containing protein	SCLAV_5004	6.015	0.03	1.94	6.56x10 ⁻⁰⁴	2.99x10 ⁻⁰⁴		
E2PYX0	Integral membrane protein (DoxX family)	SCLAV_3230	2.001	6.19	24.78	6.59x10 ⁻⁴⁸	7.26x10 ⁻⁴⁹		
E2PVR1	Partitioning or sporulation protein	SCLAV_2902	1.964	301.66	1177.11	0	0		
E2Q131	Secreted protein	SCLAV_3465	1.923	1.06	4.02	2.35x10 ⁻⁰⁴	1.01x10 ⁻⁰⁴		
B5GWW9	Aminopeptidase	SCLAV_3585	-1.543	27.78	9.53	1.95x10 ⁻²³	3.30x10 ⁻²⁴		
E2PWG7	Penicillin acylase	SCLAV_0923	-1.624	14.33	4.65	1.58x10 ⁻⁴⁰	1.96x10 ⁻⁴¹		
B5H2P7	Uncharacterized protein	SCLAV_0297	-1.657	248.97	78.94	0	0		
E2Q6L1	Sporulation-related protein	SCLAV_4236	-3.017	78.95	9.75	0	0		
Cell motility									
B5GNA0	Integrin-like protein	SCLAV_p0730	-10.213	35.6	0.03	5.31x10 ⁻¹⁹³	2.02x10 ⁻¹⁹⁴		
B5GN99	Integrin-like protein	SCLAV_p0731	-10.288	37.51	0.03	4.55x10 ⁻¹⁹⁶	1.68x10 ⁻¹⁹⁷		
Signal transduction mechanisms									
B5GQQ0	Anti-sigma regulatory factor Ser/Thr protein kinase	SCLAV_1592	6.088	2.05	139.45	9.82x10 ⁻¹²⁸	5.26x10 ⁻¹²⁹		
E2Q5W5	Uncharacterized protein	SCLAV_0049	2.519	2.95	16.91	1.82x10 ⁻³⁴	2.49x10 ⁻³⁵		
B5H4A7	Rhs element Vgr protein	SCLAV_0044	2.364	3.54	18.23	9.03x10 ⁻¹⁹	1.74x10 ⁻¹⁹		
B5GXA5	Pyridoxamine-phosphate oxidase	SCLAV_1471	1.614	1.65	5.05	4.46x10 ⁻⁰³	2.29x10 ⁻⁰³		

Table 3.2 Reorganized transcriptome data (continued)

E2Q3T7	Putative secreted protein	SCLAV_1832	1.592	62.24	187.63	4.72x10 ⁻²⁰⁴	1.62x10 ⁻²⁰⁵
B5GQ52	Uncharacterized protein	SCLAV_4023	1.567	3.19	9.45	8.92x10 ⁻⁰⁴	4.14x10 ⁻⁰⁴
E2Q501	Lipoprotein	SCLAV_4085	1.547	2.86	8.36	1.07x10 ⁻¹¹	2.71x10 ⁻¹²
E2PVF3	Tetratricopeptide TPR_4	SCLAV_2795	-1.602	230.79	76.03	0	0
B5GMX3	Putative two-component system sensor kinase	SCLAV_p0852	-1.607	194.02	63.68	5.55x10 ⁻²⁴	9.26x10 ⁻²⁵
B5GPA5	Magnesium or manganese-dependent protein phosphatase	SCLAV_3148	-1.691	22.18	6.87	2.98x10 ⁻²⁶	4.75x10 ⁻²⁷
E2Q733	Regulator protein	SCLAV_0204	-2.072	254.65	60.57	1.19x10 ⁻¹³³	6.14x10 ⁻¹³⁵
B5GYG2	Histidine kinase	SCLAV_5390	-2.188	31.08	6.82	1.46x10 ⁻¹²	3.59x10 ⁻¹³
B5GL80	Uncharacterized protein	SCLAV_4241	-2.703	80.03	12.29	2.72x10 ⁻¹⁰¹	1.71x10 ⁻¹⁰²
E2Q7X6	Integral membrane phosphodiesterase	SCLAV_4437	-3.632	580.1	46.78	0	0
E2Q9Q9	Crp/Fnr family transcriptional regulator	SCLAV_2564	-5.931	1.83	0.03	1.16x10 ⁻⁰⁴	4.81x10 ⁻⁰⁵
Defense mechanisms							
E2Q8D9	ABC-type multidrug transport system, ATPase and permease component	SCLAV_0395	4.823	1.82	51.52	2.69x10 ⁻²³⁷	8.15x10 ⁻²³⁹
D5SK74	Transport permease protein	SCLAV_p0831	3.452	2.77	30.31	6.59x10 ⁻⁴⁹	7.12x10 ⁻⁵⁰
B5GMZ5	Antibiotic resistance ATP-binding protein	SCLAV_p0830	2.985	6.91	54.69	2.53x10 ⁻¹²⁹	1.35x10 ⁻¹³⁰
E2Q610	ABC transporter ATP-binding protein	SCLAV_2231	1.564	319.08	943.26	0	0
E2Q8M5	Putative ATP-binding component of an ABC transport protein	SCLAV_2440	-1.71	85.28	26.06	1.93x10 ⁻¹⁹⁵	7.16x10 ⁻¹⁹⁷
E2Q8M1	Beta-lactamase (Penicillinase)	SCLAV_2436	-2.541	1.63	0.28	2.38x10 ⁻⁰³	1.17x10 ⁻⁰³
D5SJZ7	ABC-type bacteriocin/lantibiotic exporter	SCLAV_p0753	-5.97	1.88	0.03	9.58x10 ⁻¹³	2.33x10 ⁻¹³
D5SIV9	Restriction endonuclease	SCLAV_p0715	-10.069	32.22	0.03	1.92x10 ⁻⁷⁸	1.46x10 ⁻⁷⁹
Functions Unknown							
B5GU72	Uncharacterized protein	SCLAV_3003	7.764	0.03	6.52	1.82x10 ⁻⁰⁴	7.72x10 ⁻⁰⁵
B5H2L2	Uncharacterized protein	SCLAV_0499	7.14	0.03	4.23	1.56x10 ⁻⁰²	8.85x10 ⁻⁰³
E2Q4K6	Prim-Pol domain-containing protein	SCLAV_1971	6.93	0.13	15.85	2.17x10 ⁻³³	3.02x10 ⁻³⁴
E2PXC6	Uncharacterized protein	SCLAV_0970	6.803	0.03	3.35	3.45x10 ⁻⁰⁴	1.52x10 ⁻⁰⁴
E2Q699	Uncharacterized protein	SCLAV_2150	6.663	0.03	3.04	6.56x10 ⁻⁰⁴	2.99x10 ⁻⁰⁴
E2PY86	Uncharacterized protein	SCLAV_5190	5.644	0.03	1.5	4.46x10 ⁻⁰³	2.28x10 ⁻⁰³
E2Q5V4	Phage tail region protein	SCLAV_0038	4.659	2.12	53.55	1.92x10 ⁻⁶⁰	1.78x10 ⁻⁶¹

Table 3.2 Reorganized transcriptome data (continued)

E2Q0G6	Putative transcriptional regulator	SCLAV_5442	4.568	145.19	3443.29	0	0
B5H0W2	DUF320 domain-containing protein	SCLAV_1012	4.485	5.41	121.17	6.96×10^{-62}	6.33×10^{-63}
E2Q5V5	Putative phage tail sheath protein	SCLAV_0039	4.075	1.82	30.68	2.47×10^{-115}	1.42×10^{-116}
D5SK67	Secreted protein	SCLAV_p0824	4.031	62.77	1026.09	0	0
E2Q8Y9	Bifunctional DNA primase/polymerase	SCLAV_4532	3.887	10.09	149.28	2.50×10^{-225}	8.06×10^{-227}
E2Q5W2	Baseplate J domain-containing protein	SCLAV_0046	3.81	0.81	11.36	1.68×10^{-53}	1.70×10^{-54}
E2Q756	Hydrolase domain-containing protein	SCLAV_0227	3.747	0.28	3.76	3.68×10^{-06}	1.31×10^{-06}
E2Q5W9	Uncharacterized protein	SCLAV_0053	3.533	0.52	6.02	6.28×10^{-28}	9.61×10^{-29}
B5GN00	Uncharacterized protein	SCLAV_p0825	3.528	1713.38	19759.68	0	0
B5GWE4	Uncharacterized protein	SCLAV_3562	3.345	57.89	588.25	0	0
E2Q9X0	Subtilisin inhibitor-like protein	SCLAV_2625	2.97	0.49	3.84	1.91×10^{-03}	9.24×10^{-04}
B5GTZ7	Inhibitor I36 domain-containing protein	SCLAV_0394	2.931	4.19	31.96	2.69×10^{-24}	4.45×10^{-25}
E2Q5V3	Phage tail sheath protein, putative	SCLAV_0037	2.883	7.28	53.72	1.14×10^{-226}	3.65×10^{-228}
B5GNM2	Uncharacterized protein	SCLAV_3510	2.88	3.17	23.33	9.28×10^{-39}	1.19×10^{-39}
B5H4A8	Rhs element Vgr protein	SCLAV_0043	2.866	2.57	18.74	1.24×10^{-40}	1.54×10^{-41}
B5H484	Uncharacterized protein	SCLAV_2624	2.804	2.84	19.84	1.53×10^{-43}	1.81×10^{-44}
E2Q5V1	Uncharacterized protein	SCLAV_0035	2.786	6.5	44.84	2.02×10^{-43}	2.41×10^{-44}
B5H4A9	Peptidoglycan-binding LysM	SCLAV_0042	2.768	2.9	19.76	7.42×10^{-23}	1.28×10^{-23}
E2Q0G2	DUF4166 domain-containing protein	SCLAV_5438	2.723	9.53	62.94	1.17×10^{-70}	9.57×10^{-72}
E2PXX4	Uncharacterized protein	SCLAV_3123	2.69	1.02	6.58	1.33×10^{-07}	4.30×10^{-08}
E2Q0G3	Uncharacterized protein	SCLAV_5439	2.661	12.77	80.74	4.77×10^{-91}	3.20×10^{-92}
E2PXC5	Uncharacterized protein	SCLAV_0969	2.629	2.29	14.17	7.60×10^{-20}	1.43×10^{-20}
E2Q2T7	Uncharacterized protein	SCLAV_1615	2.56	0.78	4.6	2.26×10^{-03}	1.10×10^{-03}
E2Q5V2	Uncharacterized protein	SCLAV_0036	2.549	6.41	37.51	5.64×10^{-42}	6.88×10^{-43}
B5GZD6	GlcNAc-PI de-N-acetylase family protein	SCLAV_5656	2.475	0.43	2.39	3.74×10^{-03}	1.89×10^{-03}
E2Q5V7	Uncharacterized protein	SCLAV_0041	2.251	2.14	10.19	3.93×10^{-03}	1.99×10^{-03}
E2PZG3	Uncharacterized protein	SCLAV_5357	2.104	8.51	36.59	1.87×10^{-31}	2.69×10^{-32}
E2Q749	Uncharacterized protein	SCLAV_0220	2.083	0.8	3.39	9.71×10^{-04}	4.53×10^{-04}
B5GME7	Uncharacterized protein	SCLAV_p0966	2.076	2.83	11.93	3.92×10^{-03}	1.99×10^{-03}
B5H4B1	Phage tail region protein	SCLAV_0040	2.056	5.51	22.92	2.63×10^{-14}	5.97×10^{-15}
E2PV72	Peroxidase	SCLAV_0743	2.039	187.35	770.1	0	0
E2Q5W7	Uncharacterized protein	SCLAV_0051	2.029	3.11	12.69	2.80×10^{-18}	5.50×10^{-19}

Table 3.2 Reorganized transcriptome data (continued)

E2Q753	Uncharacterized protein	SCLAV_0224	2.024	1.97	8.01	6.12x10 ⁻⁰⁷	2.06x10 ⁻⁰⁷
E2Q5W3	Baseplate J domain-containing protein	SCLAV_0047	1.939	1.87	7.17	6.72x10 ⁻¹⁸	1.34x10 ⁻¹⁸
B5H4A6	Putative GPW/gp25 family protein	SCLAV_0045	1.932	1.95	7.44	5.89x10 ⁻⁰⁴	2.67x10 ⁻⁰⁴
E2PZG1	Uncharacterized protein	SCLAV_5355	1.848	12.4	44.64	5.51x10 ⁻⁴⁰	6.92x10 ⁻⁴¹
E2Q6S9	Uncharacterized protein	SCLAV_0143	1.839	2.96	10.59	1.33x10 ⁻⁰⁸	4.03x10 ⁻⁰⁹
B5H403	Uncharacterized protein	SCLAV_3534	1.781	2.41	8.28	1.29x10 ⁻⁰⁴	5.41x10 ⁻⁰⁵
E2Q7X2	Uncharacterized protein	SCLAV_4433	1.779	54.68	187.69	3.60x10 ⁻³²	5.11x10 ⁻³³
E2Q154	Uncharacterized protein	SCLAV_3487	1.773	75.81	259.04	1.50x10 ⁻⁷⁷	1.15x10 ⁻⁷⁸
D5SK76	Uncharacterized protein	SCLAV_p0833	1.766	11.42	38.84	5.68x10 ⁻¹⁹	1.09x10 ⁻¹⁹
E2PZG2	Uncharacterized protein	SCLAV_5356	1.712	23.64	77.44	1.55x10 ⁻⁶⁷	1.31x10 ⁻⁶⁸
B5GRP2	Uncharacterized protein	SCLAV_5005	1.7	9.59	31.16	2.09x10 ⁻⁰⁹	5.96x10 ⁻¹⁰
E2Q5W6	Uncharacterized protein	SCLAV_0050	1.659	6.28	19.83	2.63x10 ⁻⁴⁰	3.28x10 ⁻⁴¹
E2PVT7	Putative secreted protease	SCLAV_4854	1.647	1.21	3.79	9.50x10 ⁻⁰⁶	3.50x10 ⁻⁰⁶
E2PXA4	TIR domain-containing protein	SCLAV_0949	1.619	11.11	34.13	7.56x10 ⁻³⁸	9.76x10 ⁻³⁹
E2Q5W8	Uncharacterized protein	SCLAV_0052	1.559	2.81	8.28	2.02x10 ⁻⁰⁴	8.60x10 ⁻⁰⁵
B5GYZ8	Secreted protein	SCLAV_0607	1.553	223.16	654.61	2.74x10 ⁻²⁹⁸	6.49x10 ⁻³⁰⁰
E2Q861	Uncharacterized protein	SCLAV_0317	1.528	82.93	239.16	0	0
E2PVF0	Putative integral membrane protein	SCLAV_2792	1.525	28.78	82.8	1.16x10 ⁻⁵¹	1.21x10 ⁻⁵²
E2Q243	Uncharacterized protein	SCLAV_1594	1.513	36.93	105.4	5.64x10 ⁻⁷⁰	4.65x10 ⁻⁷¹
E2Q4C4	Uncharacterized protein	SCLAV_1889	1.503	5.01	14.2	7.49x10 ⁻⁰⁶	2.74x10 ⁻⁰⁶
B5GTS9	Alkyl hydroperoxide reductase AhpD	SCLAV_3934	-1.506	145.99	51.4	3.13x10 ⁻⁶¹	2.89x10 ⁻⁶²
E2Q3B7	Short-chain dehydrogenase	SCLAV_3760	-1.515	3.4	1.19	4.77x10 ⁻⁰³	2.46x10 ⁻⁰³
E2Q191	Uncharacterized protein	SCLAV_3524	-1.517	14.37	5.02	8.19x10 ⁻⁰⁵	3.34x10 ⁻⁰⁵
B5H0M1	Permease (Putative permease)	SCLAV_3316	-1.543	35.75	12.27	1.03x10 ⁻⁴⁴	1.19x10 ⁻⁴⁵
B5GMG9	Uncharacterized protein	SCLAV_p0989	-1.549	19.96	6.82	1.04x10 ⁻⁰⁵	3.84x10 ⁻⁰⁶
E2Q4Y3	Sell-like repeat-containing serine/threonine protein kinase	SCLAV_4068	-1.569	19.2	6.47	7.85x10 ⁻⁰⁴	3.60x10 ⁻⁰⁴
B5GMR9	Uncharacterized protein	SCLAV_p1087	-1.622	227.95	74.04	5.50x10 ⁻¹³⁹	2.70x10 ⁻¹⁴⁰
B5GNM5	Uncharacterized protein	SCLAV_3507	-1.625	36.17	11.73	5.93x10 ⁻⁰⁶	2.15x10 ⁻⁰⁶
B5GPX2	Cell division protein	SCLAV_2300	-1.626	723.23	234.38	8.54x10 ⁻²²²	2.78x10 ⁻²²³
E2Q1I1	Uncharacterized protein	SCLAV_5540	-1.632	7.19	2.32	2.35x10 ⁻¹⁰	6.34x10 ⁻¹¹
E2PZF0	Putative membrane protein	SCLAV_5344	-1.688	11.31	3.51	1.76x10 ⁻⁴⁹	1.90x10 ⁻⁵⁰

Table 3.2 Reorganized transcriptome data (continued)

D5SL18	Uncharacterized protein	SCLAV_p1125	-1.711	29.69	9.07	1.01x10 ⁻¹³	2.36x10 ⁻¹⁴
E2Q3C1	Putative membrane protein, ribonuclease family	SCLAV_3764	-1.721	110.42	33.49	8.26x10 ⁻¹⁰⁷	5.00x10 ⁻¹⁰⁸
E2Q781	Putative membrane protein	SCLAV_0252	-1.756	2.6	0.77	5.77x10 ⁻⁰⁴	2.62x10 ⁻⁰⁴
B5H440	Putative acyl-ACP desaturase	SCLAV_p1266	-1.764	5.13	1.51	1.49x10 ⁻⁰⁵	5.61x10 ⁻⁰⁶
E2PVJ4	DUF1775 domain-containing protein	SCLAV_2835	-1.814	134.93	38.37	2.99x10 ⁻¹²²	1.64x10 ⁻¹²³
B5GMA3	Uncharacterized protein	SCLAV_p0925	-1.815	24.95	7.09	8.67x10 ⁻⁰⁵	3.55x10 ⁻⁰⁵
B5H3Y6	Putative membrane protein	SCLAV_5697	-1.878	160	43.54	7.81x10 ⁻⁷⁵	6.06x10 ⁻⁷⁶
B5GZT5	Uncharacterized protein	SCLAV_2956	-1.893	275.88	74.26	3.30x10 ⁻¹⁴⁴	1.57x10 ⁻¹⁴⁵
E2PY53	Uncharacterized protein	SCLAV_5155	-1.92	224.69	59.36	7.35x10 ⁻¹⁰⁰	4.70x10 ⁻¹⁰¹
E2Q722	Uncharacterized protein	SCLAV_0193	-2.002	8.61	2.15	3.78x10 ⁻⁰³	1.92x10 ⁻⁰³
E2Q6B9	RNA polymerase sigma factor	SCLAV_2170	-2.024	496.7	122.11	0	0
B5GVE2	Putative lantibiotic	SCLAV_4389	-2.123	239.57	54.98	2.06x10 ⁻⁴⁸	2.24x10 ⁻⁴⁹
E2Q8U4	Putative secreted protein	SCLAV_2510	-2.203	6.31	1.37	2.00x10 ⁻⁰⁶	6.96x10 ⁻⁰⁷
E2PY56	Putative DNA-binding protein	SCLAV_5158	-2.217	36.77	7.91	8.31x10 ⁻¹¹	2.18x10 ⁻¹¹
E2PZE7	Uncharacterized protein	SCLAV_5341	-2.264	4.37	0.91	7.24x10 ⁻⁰⁷	2.45x10 ⁻⁰⁷
D5SLF4	Uncharacterized protein	SCLAV_p1261	-2.392	11.02	2.1	8.63x10 ⁻⁰⁴	3.98x10 ⁻⁰⁴
B5GWJ7	Uncharacterized protein	SCLAV_2822	-2.427	5	0.93	4.04x10 ⁻⁰³	2.05x10 ⁻⁰³
B5GL84	Uncharacterized protein	SCLAV_4237	-2.473	26.76	4.82	7.61x10 ⁻²¹	1.37x10 ⁻²¹
E2Q1S7	CBS domain-containing protein	SCLAV_1476	-2.486	98	17.49	2.72x10 ⁻⁶⁰	2.54x10 ⁻⁶¹
E2Q6L0	Putative sporulation-related protein	SCLAV_4235	-2.57	44.41	7.48	8.57x10 ⁻³⁷	1.13x10 ⁻³⁷
B5GPL1	Integral membrane protein	SCLAV_2508	-2.637	3.67	0.59	4.24x10 ⁻¹⁰	1.17x10 ⁻¹⁰
E2Q6K6	Putative secreted protein	SCLAV_4231	-2.682	81.08	12.63	2.20x10 ⁻⁹⁴	1.45x10 ⁻⁹⁵
E2Q6K3	Uncharacterized protein	SCLAV_4228	-2.84	89.99	12.57	4.04x10 ⁻⁷¹	3.29x10 ⁻⁷²
B5GL81	Uncharacterized protein	SCLAV_4240	-2.893	35.94	4.84	1.18x10 ⁻³³	1.63x10 ⁻³⁴
B5GMD4	Uncharacterized protein	SCLAV_p0956	-2.895	4.09	0.55	1.51x10 ⁻⁰⁴	6.37x10 ⁻⁰⁵
E2Q6L7	Prim-Pol domain-containing protein	SCLAV_4242	-3.032	55.36	6.77	5.78x10 ⁻⁹⁰	3.92x10 ⁻⁹¹
B5GL83	Uncharacterized protein	SCLAV_4238	-3.084	14.58	1.72	2.41x10 ⁻⁰⁸	7.44x10 ⁻⁰⁹
E2Q8R5	Uncharacterized protein	SCLAV_2480	-3.577	10.62	0.89	2.40x10 ⁻¹⁰	6.47x10 ⁻¹¹
B5GY82	Uncharacterized protein	SCLAV_2719	-3.595	5.68	0.47	3.04x10 ⁻⁰³	1.51x10 ⁻⁰³
B5GL87	Uncharacterized protein	SCLAV_4234	-3.618	40.03	3.26	1.61x10 ⁻¹⁸	3.13x10 ⁻¹⁹
E2PYL0	Uncharacterized protein	SCLAV_1154	-3.732	38.01	2.86	6.59x10 ⁻⁸⁰	4.92x10 ⁻⁸¹

Table 3.2 Reorganized transcriptome data (continued)

D5SK59	Putative dehydrogenase	SCLAV_p0816	-4.002	24.04	1.5	1.30x10 ⁻⁷³	1.02x10 ⁻⁷⁴
E2Q8S2	SPDY domain-containing protein	SCLAV_2487	-4.053	2.49	0.15	2.59x10 ⁻⁰⁴	1.12x10 ⁻⁰⁴
B5GL91	Uncharacterized protein	SCLAV_4230	-4.28	129.95	6.69	1.91x10 ⁻⁷⁷	1.46x10 ⁻⁷⁸
B5GN80	Uncharacterized protein	SCLAV_p0748	-4.459	0.66	0.03	1.71x10 ⁻⁰³	8.20x10 ⁻⁰⁴
E2PXF7	MSP1_C domain-containing protein	SCLAV_1001	-4.503	0.68	0.03	4.17x10 ⁻¹⁹	7.97x10 ⁻²⁰
E2PYL1	Uncharacterized protein	SCLAV_1155	-4.81	112.23	4	8.81x10 ⁻²⁰⁰	3.17x10 ⁻²⁰¹
D5SK58	Uncharacterized protein	SCLAV_p0815	-4.81	32.55	1.16	3.30x10 ⁻⁸⁹	2.25x10 ⁻⁹⁰
D5SIX8	Uncharacterized protein	SCLAV_p0734	-5.073	1.01	0.03	8.73x10 ⁻⁰⁴	4.04x10 ⁻⁰⁴
B5GNC0	Uncharacterized protein	SCLAV_p0710	-5.183	1.09	0.03	3.00x10 ⁻⁰⁵	1.16x10 ⁻⁰⁵
E2Q6K8	Uncharacterized protein	SCLAV_4233	-5.267	30.41	0.79	8.84x10 ⁻²¹	1.60x10 ⁻²¹
B5GL82	Uncharacterized protein	SCLAV_4239	-5.578	20.06	0.42	1.79x10 ⁻¹³	4.22x10 ⁻¹⁴
D5SIJ5	Uncharacterized protein	SCLAV_p0751	-6.242	2.27	0.03	1.25x10 ⁻⁰⁷	4.01x10 ⁻⁰⁸
D5SID9	Uncharacterized protein	SCLAV_p0543	-6.615	2.94	0.03	3.86x10 ⁻¹²	9.63x10 ⁻¹³
B5GNA1	Uncharacterized protein	SCLAV_p0729	-7.426	5.16	0.03	6.25x10 ⁻⁰⁸	1.97x10 ⁻⁰⁸
B5GN71	Uncharacterized protein	SCLAV_p0757	-7.85	6.92	0.03	1.19x10 ⁻¹³	2.78x10 ⁻¹⁴
B5GN69	Uncharacterized protein	SCLAV_p0759	-8.128	8.39	0.03	3.00x10 ⁻⁰⁵	1.16x10 ⁻⁰⁵
E2Q785	Uncharacterized protein	SCLAV_0256	-8.786	39.74	0.09	2.76x10 ⁻¹³²	1.46x10 ⁻¹³³
D5SIW8	Uncharacterized protein	SCLAV_p0724	-9.285	18.71	0.03	7.73x10 ⁻²²	1.35x10 ⁻²²
D5SIX7	HAD-superfamily hydrolase subfamily 1A	SCLAV_p0733	-9.337	19.4	0.03	8.83x10 ⁻⁴¹	1.09x10 ⁻⁴¹
D5SIY5	Uncharacterized protein	SCLAV_p0741	-9.423	20.6	0.03	3.43x10 ⁻³⁶	4.54x10 ⁻³⁷
B5GNC7	Uncharacterized protein	SCLAV_p0704	-9.572	22.83	0.03	6.88x10 ⁻²⁵	1.12x10 ⁻²⁵
D5SIJ0	Erythromycin esterase	SCLAV_p0746	-10.211	35.55	0.03	5.81x10 ⁻¹⁶²	2.55x10 ⁻¹⁶³
D5SIY8	Uncharacterized protein	SCLAV_p0744	-10.898	57.23	0.03	6.68x10 ⁻¹⁰⁸	4.00x10 ⁻¹⁰⁹
B5GN85	Uncharacterized protein	SCLAV_p0743	-11.106	66.14	0.03	1.29x10 ⁻⁷²	1.03x10 ⁻⁷³
B5GNB3	Snoal-like domain-containing protein	SCLAV_p0717	-12.168	138.03	0.03	1.82x10 ⁻¹⁸⁷	7.05x10 ⁻¹⁸⁹

3.1.3.1 The Most Significantly Upregulated Genes in DEPA Strain

The most highly upregulated genes with 7.66 to 2.99-fold increment in transcript abundance are tabulated in Table 3.2 by excluding the genes encoding yet uncharacterized proteins.

The most upregulated gene in DEPA was found as *SCLAV_1861 (cas2)* that expresses CRISPR-associated endoribonuclease protein at a 7.7-fold elevated level. CRISPR-associated system is known as bacterial defense system against invading phages and plasmids in prokaryotes (Sorek *et al.*, 2013), however, its relation with clavulanic overproduction is yet unknown.

SCLAV_5662 encodes a protein that shows dTDP-4-dehydrorhamnose 3,5-epimerase activity was 6.3-fold upregulated in DEPA. *SCLAV_5662* is involved in polyketide sugar unit biosynthesis, subsequently streptomycin biosynthesis by converting dTDP-4-oxo-6-deoxy-D-glucose into dDTP-4-oxo-6-deoxy-L-mannose.

SCLAV_p0892, *SCLAV_5443* and *SCLAV_p0891* genes, which code for TetR/AcrR family transcriptional regulators, were detected as 5.4, 4.9 and 3.6-fold upregulated, respectively, in DEPA. TetR family transcriptional regulator with AcrR domain play an important role in regulating multidrug resistance efflux pumps and self-resistance in antibiotic producers (Cuthbertson and Nodwell, 2013; Deng *et al.*, 2013). On the other hand, *SCLAV_0395*, *SCLAV_5185*, *SCLAV_5183*, *SCLAV_p0831* and *SCLAV_p0830* genes, which produce ABC-type multidrug transport system related proteins, were detected as respectively 4.8, 3.6, 3.5, 3.5 and 3.0-fold upregulated in DEPA. ABC-type transporter family is one of the largest and oldest transport families. Exporters are responsible for exporting toxins, drugs, and toxic metabolites. ABC transporters' essential mission is secreting the metabolites such as antibiotics out of the cell through the cell membrane and wall, to prevent self-toxicity. In the antibiotic biosynthesis gene clusters of *Actinomycetes*, many ABC transporters have

been found so far (Méndez and Salas, 2001). The increasing expression level of ABC-type multidrug transport system genes is thought to be a manifestation of upregulated TetR/AcrR transcriptional regulators.

SCLAV_0038 and *SCLAV_0043* which produce phage tail proteins and *SCLAV_0039* which produce putative phage tail sheet protein have been detected as 4.7, 3.2 and 4.1-fold upregulated in DEPA. In our previous study of comparative 2DE-based proteomic analysis between DEPA and NRRL3585, Rhs element Vgr (*SCLAV_0043*) protein had been detected as 3.8-fold upregulated in DEPA. When the association of this protein with other proteins was analyzed by the STRING database, *SCLAV_0037*, *SCLAV_0038*, *SCLAV_0039*, *SCLAV_0040* (all are phage tail protein) and *SCLAV_0043*, are predicted as the functional partners. We claimed for the first time that *S. clavuligerus* very likely possesses Type 6-secretion system (T6SS) and its overexpression helps DEPA strain to gain extra advantages in stress sensing, bacteria-bacteria interactions, and competitions (Ünsaldı *et al*, 2017). According to our transcriptomics results, all the related genes are significantly upregulated, supporting our former claim.

SCLAV_2475 (*whiB* transcriptional regulator) *SCLAV_1012* (DUF320 domain-containing protein also known as Chaplin protein), *SCLAV_4953* (AmfT protein), *SCLAV_4954* (membrane translocator) and *SCLAV_3699* (*bldM*; LuxR family two-component regulator) genes have been detected as upregulated in DEPA. All these genes are involved in the morphological differentiation of *S. clavuligerus*. Of these, *whiB* gene was about 5.8-fold upregulated in DEPA. *whi* genes in *Streptomyces* species are needed for the formation of mature spores from the aerial mycelium. *whiB* is responsible for the septation of aerial mycelium to the formation of spores (Davis and Chater, 1991). Additionally, *whiB* and *whiA* together play role in the initiation of cell division (Bush *et al.*, 2016). *SCLAV_3699* (*bldM*) expresses the LuxR-family two-component regulator which is responsible for aerial mycelium formation. *bld* and *whi* regulatory cascades are together responsible for morphological changes

from aerial mycelium to spore formation in *Streptomyces* (Chater, 2000). *SCLAV_4953* (*amfT*) and *SCLAV_4954* (*amfB*) genes were found to be 4.3 and 3.7 upregulated in DEPA, respectively. In *S. griseus amfTSBA* gene cluster, which is an orthologous of *ramCSAB* in *S. coelicolor*, is responsible for aerial mycelium formation and is regulated by *amfR* (*ramR* in *S. coelicolor*), *bldD*, and *adpA* (Yamazaki *et al.*, 2003; Ueda *et al.*, 2005; O'Connor *et al.*, 2005). In addition to these two *amf* genes, *SCLAV_4955* (*amfS*) was also detected as 2.4-fold upregulated in DEPA. In a *claR*-deleted mutant of *S. clavuligerus*, *amfT*, *amfB*, and *amfA* genes were reported to be downregulated -6.0, -2.0, and -2.5-fold, respectively, while the expression of *bldD* and *adpA* genes did not significantly change in the mutant strain (Martínez-Burgo *et al.*, 2015). Consequently, the authors postulated that *claR* rather than the *bldD* and *adpA*, might have a regulatory effect on *amfTSAB* operon in *S. clavuligerus*. Lastly, *SCLAV_1012* expresses one of the chaplin proteins which are responsible for generating a hydrophobic layer surrounding aerial mycelium to reduce surface tension (Capstick *et al.*, 2007). The expression of chaplin family of cell surface-associated proteins with gene designations *chpA–H* was found to be very poor in *bldM*, *bldN*, *bldG*, *bldH*, *bldB* and *bldC* mutant backgrounds (Elliot *et al.*, 2003). Overall, all these genes are involved in aerial mycelium formation, septation and sporulation. Multinuclear aerial mycelium formation due to nutrient depletion and stress factors is a very crucial developmental stage of *S. clavuligerus* which triggers secondary metabolite production (Elliot *et al.*, 2007; Manteca *et al.*, 2008). To conclude, it is highly possible that clavulanic acid overproduction is related to the increased positive regulation of aerial mycelium formation in DEPA.

Lastly, *SCLAV_5441* (*pddK*; pyruvate phosphate dikinase), and *SCLAV_4332* (*pfkA*; ATP-dependent 6-phosphofructokinase) genes were detected as 4.6- and 3-fold upregulated, respectively. In addition, *SCLAV_1803* (putative phosphoglycerate mutase) gene was also detected as 2.5-fold upregulated in DEPA. All these genes are part of the glycolysis pathway that produces the C₃ precursor glyceraldehyde-3-

phosphate (G3P) which is a rate-limiting substrate for CA production (Saudagar and Singhal, 2007).

Table 3.3 The top highly expressed genes in *S. clavuligerus* DEPA

Uniprot Nr.	Protein Name	Gene	log ₂ FC	p-value
B5GV89	CRISPR-associated endoribonuclease	<i>SCLAV_1861 (cas2)</i>	7.661	7.72x10 ⁻⁰⁵
B5GZD1	Epimerase	<i>SCLAV_5662</i>	6.334	1.01x10 ⁻⁰⁵
B5GPH9	Transcriptional regulator WhiB	<i>SCLAV_2475 (whiB)</i>	5.746	1.16x10 ⁻⁰³
D5SKD6	TetR/AcrR family transcriptional regulator	<i>SCLAV_p0892</i>	5.428	0
E2Q0G7	TetR/AcrR family transcriptional regulator	<i>SCLAV_5443</i>	4.86	0
E2Q8D9	ABC-type multidrug transport system, ATPase and permease component	<i>SCLAV_0395</i>	4.823	8.15x10 ⁻²³⁹
E2Q5V4	Phage tail region protein	<i>SCLAV_0038</i>	4.659	1.78x10 ⁻⁶¹
E2Q0G5	Pyruvate phosphate dikinase (EC 2.7.9.1)	<i>SCLAV_5441 (pdkK)</i>	4.559	0
B5H0W2	DUF320 domain-containing protein (Chaplin protein)	<i>SCLAV_1012</i>	4.485	6.33x10 ⁻⁶³
E2PW36	AmfT protein (amfT)	<i>SCLAV_4953</i>	4.270	2.14x10 ⁻²⁵⁷
E2Q5V5	Putative phage tail sheath protein	<i>SCLAV_0039</i>	4.075	1.42x10 ⁻¹¹⁶
E2PW37	Membrane translocator (amfB)	<i>SCLAV_4954</i>	3.671	6.15x10 ⁻¹⁰⁸
E2PY81	Putative Mce family protein	<i>SCLAV_5185</i>	3.575	7.82x10 ⁻¹¹
D5SKD5	TetR/AcrR family transcriptional regulator	<i>SCLAV_p0891</i>	3.562	5.78x10 ⁻¹³⁴
B5H0E0	ABC-transporter integral membrane protein	<i>SCLAV_5183</i>	3.475	1.10x10 ⁻⁰⁷
D5SK74	Transport permease protein (ABC type)	<i>SCLAV_p0831</i>	3.452	7.12x10 ⁻⁵⁰
B5GYV1	Two component transcriptional regulator, LuxR family	<i>SCLAV_3699 (bldM)</i>	3.310	0
E2Q9H7	Urease accessory protein UreF	<i>SCLAV_0521 (ureF)</i>	3.228	2.14x10 ⁻⁰⁶
E2Q5W4	Phage tail protein	<i>SCLAV_0048</i>	3.166	1.29x10 ⁻²⁸
B5GKZ5	ATP-dependent 6-phosphofructokinase	<i>SCLAV_4332 (pfkA)</i>	2.998	2.03x10 ⁻⁰⁹
B5GMZ5	Daunorubicin resistance ABC transporter ATP-binding subunit	<i>SCLAV_p0830</i>	2.985	1.35x10 ⁻¹³⁰

3.1.3.2 The Most Significantly Downregulated Genes in DEPA Strain

The most significantly downregulated genes with 13.3 to 7.0.-fold decrease in transcript abundance are tabulated in Table 3.2, by excluding the genes encoding yet uncharacterized proteins.

Overall, the most strikingly downregulated genes are related with the biosynthesis of secondary metabolites (terpenes, macrolides, moenomycin, etc.) other than CA biosynthesis. In DEPA strain, the random mutations that reduce the production of other secondary metabolites should have led to a boost in CA production. For instance, *SCLAV_p0718* (putative monooxygenase) had the most noteworthy decrease in its expression within the DEPA transcriptome (13.3-fold downregulation). Monooxygenases are known to be involved in many secondary metabolite biosynthesis pathways in *Streptomyces* spp. (Valton *et al.*, 2004; Zhang *et al.*, 2015) but not in CA biosynthesis. *SCLAV_p1278* (*moeE5*) was detected -11.6-fold downregulated in DEPA. *moe* genes are involved in moenomycin biosynthesis which is produced in the late stages of *Streptomyces* life cycle (Ostash *et al.*, 2007). *SCLAV_p0726* and *SCLAV_p0732* genes with -10.8 and -9.2-fold downregulation in DEPA encode cytochrome P450 proteins which take role in production of secondary metabolites such as macrolides in *Streptomyces* spp. (Cho *et al.*, 2019). *SCLAV_p0760* (transcriptional regulator) was detected -7.6-fold downregulated in DEPA. In addition, *SCLAV_p0761* (beta-lactamase domain-containing protein) gene was -2.0-fold downregulated. Both genes are located in terpene biosynthetic gene cluster (Hwang *et al.*, 2019). *SCLAV_p0703-p0714* genes (except for *SCLAV_p0704* and *SCLAV_p0710*) which are located in the “other secondary metabolites biosynthetic gene cluster” (Hwang *et al.*, 2019) were downregulated in a range of -7.4 to -9.6-fold. *SCLAV_p0706* expresses an ACPS domain-containing protein that has holo-[acyl-carrier-protein] synthase activity according to the GO molecular function categorization, and *SCLAV_p0707* encodes a holo-acp synthase involved in fatty acid biosynthesis, according to the EggNog database. Active holo-form is

acquired from inactive apo-proteins by transporting 4'-phosphopantetheinyl moiety of CoA to the side chain hydroxy group of a conserved serine residue in each ACP domain. All polyketide synthases, fatty-acid synthases, and non-ribosomal peptide synthases need that activation process (Lambalot *et al.*, 1998).

SCLAV_p0727 (alcohol dehydrogenase) was detected as much as -12.6-fold downregulated in DEPA. ADHs catalyze a large variety of reactions. Their substrates also differ considerably ranging from normal and branched chain alcohols to aldehydes and ketones. Furthermore, organisms usually contain more than one ADHs (Reid and Fewson, 1994). For these reasons, it is difficult to predict the exact physiological function of the enzyme encoded by *SCLAV_p0727*.

SCLAV_p0750 (subtilisin-like protease) and *SCLAV_1039* (putative secreted subtilisin-like serine protease) genes were detected -12.7 and -7.7-fold downregulated levels, respectively in DEPA. For many years, production of serine proteases, sporulation, and antibiotic production have been believed to be coordinately regulated in *Streptomyces* spp, as consistently shown by physiological studies, subtilisin-like proteases being involved in mycelial fragmentation. For instance, *S. lactamdurans* strains which produce superior levels of cephamycin C form equally superior levels of the serine protease, and the mutants which fail to produce aerial hyphae (bald mutants) also fail to synthesize the antibiotic and serine proteases (Ginther, 1979). It was therefore surprising to find significant decreases in the transcript levels of such proteases in our present work. Nonetheless, as recently demonstrated by comparative transcriptomic analysis with the actinomycete *Actinosynnema pretiosum*, which is the producer of the Ansamitocin P-3 (AP-3), an antitumor agent, the deletion of a subtilisin-like serine peptidase encoding gene *APASM_4178* resulted in a substantially reduced the mycelial fragmentation, but led to an increased biomass and improved AP-3 yield by 43.65% (Wu et al., 2020).

SCLAV_p0720 (*glpP*) coding for glycerol-3-phosphate responsive anti-terminator GlpP was detected as -9.3-fold downregulated in DEPA. In *B. subtilis*, GlpP is responsible for the regulation of *glpD* gene and the operon activity as an anti-

terminator in conjunction with glycerol-3-phosphate and, consequently, GlpP mutants fail to grow on glycerol as a sole carbon and energy source. (Glatz *et al.*, 1996). Till the present study, there has been no record on GlpP antiterminator in *Streptomyces* spp and its relation with secondary metabolism. It is solely known that GylR protein mediates induction and catabolite repression of the *S. coelicolor* glycerol operon (Hindle and Smith, 1994).

SCLAV_p0747 (chitin-binding protein) was found to be -10.5-fold downregulated in DEPA. *SCLAV_5440* (putative secreted chitin-binding protein; -4.2-fold), *SCLAV_0945* (chitin-binding domain-containing protein; -3.0-fold), and *SCLAV_3909* (chitinase I; -1.6-fold) genes were also downregulated. In *Streptomyces* spp, chitin-derived substrates are known to affect antibiotic production positively. A study with *S. coelicolor* showed that an induction of antibiotic production by chitin substances occurs only in either soil or agar plate, but not in liquid cultures (Nazari *et al.*, 2013).

SCLAV_p0716 (ArsR-family; -7.1-fold), and *SCLAV_p0745* (MerR-family; -11.1-fold) are the other transcriptional factors that were also significantly downregulated in DEPA. Generally, the ArsR transcriptional regulators in many bacterial species or archaea act as metal sensors that repress gene expression during peacetime, and release from the promoters to derepress gene expression when metal ions become abundant. However, some of the ArsR-type regulators have also been found to be involved in bacterial pathogenesis MerR family members are responsive to environmental effectors, such as oxidative stress, heavy metals or antibiotics (Hayashi *et al.*, 2013).

Table 3.4 The most significantly downregulated genes in *S. clavuligerus* DEPA

Uniprot Number	Protein Name	Gene	log ₂ FC	p-value
D5SJW2	Putative monooxygenase	<i>SCLAV_p0718</i>	-13.298	0
D5SJZ4	Subtilisin-like protease	<i>SCLAV_p0750</i>	-12.707	0
B5GNA3	Alcohol dehydrogenase	<i>SCLAV_p0727 (adhA)</i>	-12.551	0
B5GNA5	Putative endoribonuclease (YjgF)	<i>SCLAV_p0725</i>	-11.687	5.52x10 ⁻¹²⁷
D5SLH1	NAD-dependent epimerase/dehydratase	<i>SCLAV_p1278 (moeE5)</i>	-11.575	0
B5GN83	MerR-family transcriptional regulator	<i>SCLAV_p0745</i>	-11.072	5.95x10 ⁻¹⁸⁴
E2Q793	Putative MutT-family protein	<i>SCLAV_0264</i>	-10.824	2.92x10 ⁻⁸⁷
D5SJX0	Cytochrome P450 hydroxylase	<i>SCLAV_p0726</i>	-10.812	1.10x10 ⁻²³⁹
B5GN81	Chitin-binding protein	<i>SCLAV_p0747</i>	-10.466	5.97x10 ⁻⁸³
B5GNC8	Carbonic anhydrase (EC 4.2.1.1)	<i>SCLAV_p0703</i>	-9.609	1.2 x10 ⁻⁴⁶
D5SJW4	Glycerol-3-phosphate responsive antiterminator	<i>SCLAV_p0720 (glpP)</i>	-9.305	7.75x10 ⁻³⁶
D5SJX6	Cytochrome P450	<i>SCLAV_p0732</i>	-9.201	8.78x10 ⁻¹⁷⁸
B5GNC3	Holo-acp synthase	<i>SCLAV_p0707</i>	-9.116	2.33x10 ⁻¹³
B5GNB7	Transketolase A subunit (EC 2.2.1.1)	<i>SCLAV_p0713</i>	-9.076	4.48x10 ⁻⁴⁵
B5GNB8	Permease	<i>SCLAV_p0712</i>	-8.775	4.35x10 ⁻⁶¹
D5SJV0	ACPS domain-containing protein	<i>SCLAV_p0706</i>	-8.399	1.13x10 ⁻²¹
D5SJV2	tRNA synthetase	<i>SCLAV_p0708</i>	-8.356	2.27x10 ⁻²⁹
D5SJV5	Condensation domain protein	<i>SCLAV_p0711</i>	-8.265	3.02x10 ⁻⁶⁷
B5GNC1	Phytanoyl-CoA dioxygenase	<i>SCLAV_p0709</i>	-7.744	1.12x10 ⁻²⁵
E2PXJ5	Putative secreted subtilisin-like serine protease	<i>SCLAV_1039</i>	-7.744	0
D5SJU9	Putative transport protein	<i>SCLAV_p0705</i>	-7.672	6.69x10 ⁻¹⁹
B5GN68	Transcriptional regulator	<i>SCLAV_p0760</i>	-7.639	7.97x10 ⁻²⁰
B5GNB6	Putative transketolase (Transketolase B subunit)	<i>SCLAV_p0714</i>	-7.355	8.03x10 ⁻¹⁶
D5SJW0	ArsR family transcriptional regulator	<i>SCLAV_p0716</i>	-7.055	2.52x10 ⁻³⁸

3.1.3.3 Secondary Metabolites Biosynthesis, Transport, and Catabolism

In this category, a total of 87 DEGs were found, 38 of them upregulated in an expression range of 6.3- to 1.5-fold, and 49 of them downregulated between a range of -11.9- to -1.5-fold.

SCLAV_5662, *SCLAV_2475* (*whiB*), *SCLAV_4953* (*AmfT*), *SCLAV_4954* (*AmfB*), and *SCLAV_4955* (*AmfS*), have already been discussed in 3.1.3.1.

SCLAV_0154 (Regulatory Protein), *SCLAV_0155* (Putative phosphotransferase), and *SCLAV_0159* (Terpene synthase) genes which are located in the terpene biosynthetic gene cluster (Hwang *et al.*, 2019), were detected as upregulated ones by 3.5, 5.8, and 2.9-fold increments in DEPA, respectively.

SCLAV_p1220 (Pentapeptide repeat protein) and *SCLAV_p1225* (Valine decarboxylase) genes, which are located in enediynes polyketide synthase biosynthetic gene cluster (Hwang *et al.*, 2019), were detected as upregulated 1.5 and 2.2-fold, respectively. Valine decarboxylase catalyzes reversibly the reaction of 2-methylpropanamine formation from L-Valine that is needed for the formation of all the β -lactam antibiotics (Liras and Martín, 2006). However, in the same gene cluster, *SCLAV_p1229* (major facilitator superfamily protein), *SCLAV_p1231* (enediynes biosynthesis protein UnbV), *SCLAV_p1232* (enediynes biosynthesis protein UnbU), and *SCLAV_p1233* (enediynes polyketide synthase) were detected as downregulated -2.3, -1.5, -2.6 and -1.5-fold, respectively. Enediynes polyketides are the secondary metabolites that have antitumor and antibiotic features (Wang and Hong, 1999). While the functions of UnbV and UnbU are still unknown, they might relate to the stabilization, formation, or/and transport of the warhead (reactive chromophore ring structure) in the enediynes (Zazopoulos *et al.*, 2003).

SCLAV_5249 (membrane protein), *SCLAV_5250* (LC7- family protein), *SCLAV_5252* (ATG/GTP-binding protein), and *SCLAV_5253* (GAF domain-

containing protein) genes which are located in terpene biosynthetic gene cluster (Hwang *et al.*, 2019) were detected as upregulated 1.8, 1.8, 1.9 and 2.5-fold, respectively. However, in 2010 these genes were reported to be found in phytoene/squalene biosynthetic gene cluster (SMC17) (Medeme *et al.*, 2010). Phytoene is known to be the intermediate of the biosynthesis of carotenoid, which is synthesized by *Streptomyces* species after photo-induction (Kato *et al.*, 1995); Squalene is known to be the intermediate of the biosynthesis of hopanoid, which is one of the bacterial membrane elements like sterol found in the eukaryotic membrane (Kannenberg and Poralla, 1999).

The early gene *SCLAV_4193* (*oat2*) (glutamate N-acetyl transferase 2), and the late genes *SCLAV_4185* (*orf14*) (acetyl-transferase GNAT family protein) and *SCLAV_4192* (*oppA1*) (ABC-type dipeptide transport system, periplasmic component) of clavulanic acid biosynthetic gene cluster were detected as upregulated respectively 1.9, 1.5 and 1.5-fold in DEPA. Lack of *orf14* leads to a decrease in the production of CA but not completely blocked the production, consequently, reported that *orf14* is influential but not vital for CA production (Mellado *et al.*, 2002). On the other hand, *oppA1* is essential for CA production, it is in charge of the transportation of molecules essential for CA biosynthesis. Lack of *oppA1* leads to stop the production of CA in *S. clavuligerus* (Lorenzena *et al.*, 2004). *oat2* that homolog to *argJ* was reported that might be involved in acetylation of arginine to produce N-acetylarginine, or of glutamic acid to produce N-acetylglutamate, when the arginine is low in the environment. N-acetylglutamate is a key intermediate in arginine biosynthesis, N-acetylarginine is a substrate for the clavamate synthase (*cas1/cas2*). (de la Fuente *et al.*, 2004; Kershaw *et al.*, 2002).

SCLAV_4466-4472 genes located in polyketide synthase-nonribosomal peptide-synthase (PKS-NRPS) gene cluster (Hwang *et al.*, 2019) were detected upregulated between a range of 1.5 – 2.0-fold in DEPA. Only *SCLAV_4463* (*leuC*) gene in the same cluster was detected as downregulated, -1.5-fold. As mentioned in the section

1.3, *leuC* is regulated by *areB*, their interaction is essential for the production of leucine and catabolism of fatty acid (Santamarta *et al.*, 2007). From our RNA-seq analysis, there is no significant change detected for *areB* in DEPA.

SCLAV_5183 (ABC-transporter integral membrane protein), *SCLAV_5185* (putative Mce family protein), and *SCLAV_5186* (secreted protein) genes were detected as upregulated respectively 3.5, 3.6, and 2.2-fold in DEPA. While these genes are not located in any secondary metabolite gene cluster, but they are involved in secondary metabolite biosynthesis, transport, and catabolism. In another study, *SCLAV_5183* and *SCLAV_5186* have been detected to be downregulated in the pSCL4-absent mutant in *S. clavuligerus* (Álvarez-Álvarez *et al.*, 2017).

SCLAV_2922 (*cvm6*) (putative pyridoxal phosphate-dependent aminotransferase), *SCLAV_2923* (*cvm5*) (flavin-dependent oxidoreductase), *SCLAV_2924* (*cvm4*) (alpha/beta hydrolase) and *SCLAV_2925* (*cas1*) (clavimate synthase1) genes which located in clavam gene cluster (Medema *et al.*, 2010) were detected downregulated respectively -4.2, -3.7, -3.0 and -4.8-fold in DEPA. In the *cvm3*, *cvm4*, and *cvm6*-deleted mutant of *S. clavuligerus*, it has been observed that 5S clavam and clavulanic acid production were the same levels as its wild-type strain. However, the *cvm5*-disrupted mutant was shown no production in 5S clavam metabolites, but in the *cvm5*-deleted mutant new clavam metabolite production was observed (Tahlan *et al.*, 2007). On the other hand, in 2011, another study about comparative gene expression between CA overproducer and its wild-type strain of *S. clavuligerus* was stated that the clavam gene cluster expression increased in overproducer strain significantly (Medema *et al.*, 2011). *cas1*, which is regulated nutritionally, is the paralogue gene of *cas2*. In the starch-asparagine medium growth, the expression of *cas1* has not been observed. Whereas, it has been reported that *cas2* expression is essential for clavulanic acid production in SA medium growth. (Paradkar and Jensen, 1995).

SCLAV_5702 and *SCLAV_5703* genes express the putative siderophore synthetase component were detected as downregulated respectively -1.7 and -1.8-fold in DEPA.

These genes locate in the IucA/IucC family siderophore biosynthetic gene cluster (Medema *et al.*, 2010), generally siderophore products depend on the modular non-ribosomal peptide synthetase (Lamb, 2015); however, IucA/IucC family products are in the class of NRPS-independent siderophores (NIS) (Challis, 2005).

SCLAV_p0812 (AfsA domain-containing protein), *SCLAV_p0813* (guanylate kinase), and *SCLAV_p0814* (phosphoenolpyruvate (PEP) synthase) genes, which locate in butyrolactone biosynthetic gene cluster (Hwang *et al.*, 2019), were detected downregulated respectively -3.6, -5.3, and -4.0-fold in DEPA. According to a study about industrial CA overproducer DS48802 strain of *S. clavuligerus*, *SCLAV_p0812*, *SCLAV_p0813*, and *SCLAV_p0814* genes were reported as upregulated in over-producer strain compared with the wild-type strain (Medema *et al.*, 2011). The gene cluster had not been characterized yet when they did the research.

Saudagar *et al.* (2008) reported that genetic engineering in the industrial strain used eliminating undesirable pathways such as antipodal clavam metabolites or cephamycin C could be a way to increase the production of clavulanic acid production.

3.1.3.4 Energy Production and Conversion

In this category, 5 genes detected and all downregulated in DEPA. *SCLAV_p0718* (putative monooxygenase) and *SCLAV_p0722* (Alkylglycerone-phosphate synthase) were shown to be the most noteworthy changes, respectively -13.3 and -7.0-fold downregulated. Then, *SCLAV_0951* (radical SAM domain-containing protein) was detected as -4.8-fold downregulated. Lastly, *SCLAV_1555* (aldehyde dehydrogenase) and *SCLAV_0928* (divalent metal ions/citrate complex secondary transporter) were detected as downregulated, -1.6-fold. Radical SAM proteins are involved in a broad range of reactions such as isomerization, ring formation like

thiazole ring, antibiotic production pathways, post-translational modifications like methylation, epimerization, etc. (Sofia *et al.*, 2001; Benjdia *et al.*, 2017). According to the KEGG database, *SCLAV_p0722* has a role in ether lipid metabolism and catalyzes 1-Alkyl-glycerone-3-phosphate from 1-Acyl-glycerone-3-phosphate. Monooxygenases have been discussed in 3.1.3.2.

3.1.3.5 Amino Acid Transport and Metabolism

Arginine biosynthetic gene cluster, *argB*, *argC*, *argD*, *argJ*, *argR*, *argG*, *argH*, *argF*, and *argY* genes were significantly upregulated between the range of 1.0-fold to 2.0-fold in DEPA. Arginine is the C₅ precursor of clavulanic acid. However, excessive arginine consumption leads to failing to increase the level of CA because the C₃ precursor of CA has been acting as the rate-limiting factor in CA production (Ives and Bushell, 1997).

SCLAV_0523 (ureB) expresses urease subunit beta was found as upregulated (2.1-fold) in DEPA. *ureB* together with *ureA* and *ureC* are roled in the urea cycle that is part of the nitrogen metabolism. Ornithine and arginine, both used as precursors of CA, are in the urea cycle (Saudagar *et al.*, 2008). Consequently, the increasing transcriptional level of the arginine biosynthetic gene and upregulation of *ureB* might correlate with each other.

SCLAV_3908 (zinc-carboxypeptidase) that has metallo-carboxypeptidase activity and *SCLAV_4359* (neutral metalloproteinase) genes were detected -2.0 and -2.6-fold downregulated in DEPA. Both genes are responsible for the degradation of proteins into small pieces, and both require Zinc.

3.1.3.6 Carbohydrate Transport and Metabolism

In this section, *SCLAV_5441* (*pddK*) (pyruvate phosphate dikinase), *SCLAV_4332* (*pfkA*) (ATP-dependent 6-phosphofructokinase), and *SCLAV_1803* (putative phosphoglycerate mutase) genes were detected as upregulated respectively 4.6, 3.0, and 2.5-fold in DEPA. These genes have been discussed in section 3.1.3.1.

The most remarkable downregulated genes in this category are *SCLAV_p0747* (chitin-binding protein; -10.5-fold), *SCLAV_5440* (putative secreted chitin-binding protein; -4.2-fold), *SCLAV_0945* (chitin-binding domain-containing protein; -3.0-fold), and *SCLAV_3909* (chitinase I; -1.6-fold) discussed in section 3.1.3.2.

3.1.3.7 Lipid Transport and Metabolism

SCLAV_p1127 and *SCLAV_4468* (short-chain dehydrogenase/reductase SDR) and *SCLAV_1225* (short-chain dehydrogenase) were found to be upregulated 2.1, 1.9, and 1.9-fold in DEPA. However, *SCLAV_4964* (probable short-chain dehydrogenase) and *SCLAV_p0721* (short-chain dehydrogenase/reductase SDR) were found to be downregulated -1.6, and -6.1-fold, respectively. Belong is one of the most prominent protein families; short-chain dehydrogenases have roles in many biological processes such as lipid metabolism, carbohydrate, and mechanisms like redox sensor (Kavanagha *et al.*, 2008; Oppermann *et al.*, 2003). According to KEGG, *SCLAV_1225* has 3-oxoacyl-[acyl-carrier-protein] reductase (NADPH) activity, and it catalyzes the type II fatty acid synthase system (FASII) like FabG protein which is one of the FASII members. FASII is responsible for producing many important compounds that are essential for cells like cell envelope, phospholipids, lipoproteins, lipopolysaccharides, etc. *fab* operon includes the genes involved in the FASII (Heath *et al.*, 2001).

SCLAV_0454 (3-oxoacyl-acyl-carrier-protein synthase III) was found as downregulated -1.9-fold in DEPA. Enzymatic activity of the corresponding protein is in charge of the initiate the straight-chain fatty acid biosynthesis, and it belongs to FASII.

3.1.3.8 Coenzyme Transport and Metabolism

In this category, *SCLAV_4108* (putative polyprenyl diphosphate synthase) was the only upregulated gene (2.0-fold) detected in DEPA. It is involved in terpenoid backbone biosynthesis and isoprenoid biosynthetic process. However, *SCLAV_p0735* (polyprenyl-synthase domain-containing protein), also involved in the isoprenoid biosynthetic process, was downregulated (-6.3-fold).

SCLAV_p0749 (biotin carboxylase) is another gene that was detected as downregulated (-6.8-fold) in DEPA. Acetyl-CoA carboxylase is known to be part of malonyl-CoA production (precursor of fatty acid and polyketide biosynthesis) is composed of 3 components; one of them is biotin carboxylase (Rodríguez and Gramajo, 1999; Rodríguez *et al.*, 2001).

SCLAV_3494 (*FbiC*) (5-amino-6-(D-ribitylamino)uracil--L-tyrosine 4-hydroxyphenyl transferase) and *SCLAV_5011* (bifunctional deaminase-reductase domain-containing protein) were detected respectively -1.5 and -2.0-fold downregulated. According to KEGG pathways, *SCLAV_5011* which is involved in riboflavin metabolism, catalyzes the reaction to produce 5-Amino-6-(5-phospho-D-ribosylamino)uracil. After that, 5-Amino-6-(5-phospho-D-ribosylamino)uracil is catalyzed by another enzyme to produce 5-Amino-6-ribitylaminouracil. Lastly, 5-Amino-6-ribitylaminouracil is catalyzed by *SCLAV_3494*; this reaction is related to coenzyme F₄₂₀ biosynthesis. Riboflavin is also known as vitamin B, has been used as a precursor of coenzymes such as flavine adenine dinucleotide (FAD) and flavin

mononucleotide (FMN); F₄₂₀ is also derived from flavin (Vitreschak *et al.*, 2002). In *Streptomyces* spp., F₄₂₀ is used for the biosynthesis of antibiotics/antitumor agents such as lincomycin, tetracycline, and mitomycin C (Coats *et al.*, 1989; Kuo *et al.*, 1989; Mao *et al.*, 1999).

3.1.3.9 Nucleotide Transport and Metabolism

SCLAV_5539 (cytosine deaminase-like metal-dependent hydrolase; -1.5-fold) and *SCLAV_0264* (putative MuTt-family protein; -10.8-fold) are involved in nucleotide metabolism and transport. MuTt protein specifically hydrolyses a mutagenic substrate such as oxidized guanine (8-oxo-7,8-dihydroguanine; 8-oxo-G) for DNA synthesis (Maki and Sekiguchi, 1992; Taddei *et al.*, 1997).

3.1.3.10 Inorganic and Organic Molecule Transport

SCLAV_4531 (*ftsY*) (signal recognition particle receptor), *SCLAV_3179* (1-phosphatidylinositol phosphodiesterase), and *SCLAV_p0752* (signal peptidase I) have been functioned in intracellular trafficking, secretion, and vesicular transport category. *SCLAV_4531* (*ftsY*) (signal recognition particle receptor) was found 3.0-fold upregulated in DEPA. *ftsY* influences cell differentiation, cell growth, sporulation, spore morphology, and antibiotic production (Gill and Salmond, 1987; Kakeshita *et al.*, 2001; Maeda *et al.*, 2003; Shen *et al.*, 2008). In *S. coelicolor*, *ftsY* affect sporulation via *whiH* gene; subsequently, antibiotic production (actinorhodin) is affected. *SCLAV_3179* and *SCLAV_p0752* were detected respectively -2.4 and -5.7-fold downregulated in DEPA. According to the EggNog database, *SCLAV_3179* has a role in protein uptaking independently in TonB complex. TonB complex is

responsible for energy-dependent substance transportation across the membrane (Noinaj *et al.*, 2010).

ABC-type transporters detected in transcriptomic analysis such as *SCLAV_0392* (ABC-type cobalamin/Fe³⁺ siderophore transport system; 1.9-fold) was found upregulated, however, *SCLAV_2405* (ABC transporter permease; -1.5-fold), *SCLAV_4580* (polyamine ABC transporter integral membrane protein; -1.6-fold), *SCLAV_4884* (putative ABC transporter permease protein; -1.8-fold), and *SCLAV_5398* (solute binding protein; -2.0-fold) were found downregulated in DEPA. ABC-type transporters have discussed in section 3.1.3.1.

Another large transporter family Major Facilitator Superfamily (MFS) members have also been detected in transcriptomic analysis, such as *SCLAV_p1032* (multidrug transporter, MFS protein; 1.5-fold) was detected upregulated. However, *SCLAV_3803* (putative permease of MFS; -1.6-fold), *SCLAV_2530* (sugar phosphate permease; -2.2-fold), and *SCLAV_5532* (MFS permease; -2.6-fold) were detected downregulated in DEPA. MFS is another largest and oldest transport system responsible for the transportation of sugars, drugs, amino acids, vitamins, organic/inorganic anions/cations into/out of the cell. Some MFSs are capable of the transport macromolecules such as proteins, carbohydrates, and phospholipids (Pao *et al.*, 1998).

SCLAV_0793 (DHA2-subfamily multidrug transporter) was detected 1.7-fold upregulated in DEPA. Comparative transcriptomic analysis of *S. clavuligerus ccaR*-deleted mutant and wild type showed that *SCLAV_0793* downregulated in *ccaR*-deleted strain (Álvarez- Álvarez *et al.*, 2014).

SCLAV_p0727 (*adhA*) (alcohol dehydrogenase) has already discussed in section 3.1.3.2.

3.1.3.11 Transcription/ DNA Replication, Recombination and Repair/ Translation, Ribosomal Structure, and Biogenesis

SCLAV_1861 (*cas2*) (CRISPR-associated endoribonuclease) was the most significant upregulated gene in this category (7.7-fold) (discussed in section 3.1.3.1), then *SCLAV_p1099* (acetyl-transferase, ribosomal protein N-acetylase) is followed the *SCLAV_1861* with 7.2-fold upregulation in DEPA. *SCLAV_0254* (2.0-fold) and *SCLAV_1792* (1.8-fold) are the other putative acetyltransferases detected as upregulated in DEPA. *SCLAV_0254* is GNAT-family acetyltransferase that has been also detected 3.0-fold overrepresented in our previous study 2DE gel analysis (Ünsaldı et al., 2017).

SCLAV_p0892 (5.4-fold), *SCLAV_5443* (4.9-fold), *SCLAV_p0891* (3.6-fold), and *SCLAV_p0719* (-7.0-fold) are the TetR-family transcriptional regulators have been already discussed in section 3.1.3.1.

SCLAV_5372 (2.1-fold), *SCLAV_0213* (LuxR-family; 2.0-fold), and *SCLAV_5437* (2.0-fold) are the transcriptional regulators detected as upregulated, however, *SCLAV_p0716* (ArsR-family; -7.1-fold), and *SCLAV_p0745* (MerR-family; -11.1-fold) are the other transcriptional factors that were detected downregulated in DEPA. MerR family members are responsive to environmental effectors (Hayashi *et al.*, 2013), increasing level of CA may be led to drastic downregulation of its transcription. *SCLAV_0213* expresses a protein that was found 43% identical to PimM regulator, which is a powerful effect on the secondary metabolite regulation in *S. clavuligerus*; importantly, their DNA-binding domains were found to be share 63% identity (Martínez-Burgo *et al.*, 2019). On the other hand, *SCLAV_0213* was reported as upregulated, but non-significantly, in CA overproducer industrial strain of *S. clavuligerus* (Medema *et al.*, 2011).

SCLAV_p0720 (*glpP*) has been discussed in section 3.1.3.2.

Two endoribonucleases *SCLAV_5373* (endoribonuclease L-PSP) and *SCLAV_p0725* (putative endoribonuclease, YjgF) were showed different expression trends, 2.2-fold upregulation and -11.7-fold downregulation, respectively, in DEPA. Endoribonucleases are responsible for cleaving dsRNA or ssRNA. In the STRING database, *SCLAV_5373* and *SCLAV_p0725* are described as predicted functional partners.

3.1.3.12 Posttranslational Modification, Protein Turnover, and Chaperones

SCLAV_0521 (*ureF*) (urease accessory protein UreF) was detected 3.2-fold upregulated in DEPA. UreF with UreD and UreG proteins together forms a complex act as GTP-hydrolysis-dependent molecular chaperone for urease activation (Farrugia *et al.*, 2013).

SCLAV_2659 (*clpB*) and *SCLAV_2666* (*dnaK*) chaperon proteins, *SCLAV_2665* (*grpE*) and *SCLAV_4136* (*lonA*) (lon protease) were detected 1.9, 1.6, 1.6 and 1.5-fold upregulated in DEPA. *clpB*, *dnaK*, *grpE*, and *lonA* are involved in the heat-shock response process and known to be they are positively controlled by alternative sigma factor σ^{32} in *E. coli* (Georgopoulos *et al.*, 1994; Gross, 1996; Yura and Nakahigashi, 1999).

SCLAV_1213 (trypsin-like protease), *SCLAV_1039* (putative secreted subtilisin-like serine protease) and *SCLAV_p0720* (subtilisin-like protease) were detected -1.8, -7.7, and -12.7-fold downregulated in DEPA.

3.1.3.13 Cell Wall/ Membrane/ Envelope Biogenesis

SCLAV_0315 (UDP-N-acetyl glucosamine 2-epimerase) gene was detected to be upregulated 2.3-fold in DEPA. *SCLAV_0315* is involved in the reversible reaction between UDP-N-acetyl-alpha-D-glucosamine (UDP-GlcNAc), which is one of the components of the peptidoglycan layer, and UDP-N-acetyl-alpha-D-mannosamine (UDP-ManNAc), which is one of the cell surface polysaccharides especially involved in capsule formation in pathogenic bacteria (Campbell *et al.*, 2000). *SCLAV_3505* and *SCLAV_3506* both express the Peptidase S1 and S6 (chymotrypsin/hap) were detected as downregulated -2.3 and -2.2-fold, respectively. In the EggNog database, these genes identified as their cysteine-type peptidase activity. *SCLAV_3505* was reported as upregulated in *oppA2*-deleted mutant in *S. clavuligerus* (Álvarez-Álvarez *et al.*, 2018). In our transcriptomic analysis, 1.14-fold upregulation was detected in *oppA2* gene in DEPA.

SCLAV_p1144 expresses the peptidoglycan-binding domain I protein that peptidoglycan degradative enzymes bind to, was detected -2.0-fold downregulated in DEPA.

3.1.3.14 Cell Cycle Control, Cell Division, Chromosome Partitioning & Cell Motility & Chromatin Structure and Dynamics

SCLAV_p0730 and *SCLAV_p0731* which express integrin-like proteins were found to be downregulated -10.2-fold in DEPA. These proteins belong in cell motility in functional categorization. However, their exact functions in the cell are not available neither in the EggNog database nor the STRING database.

SCLAV_5004 (NTP_transf_3 domain-containing protein), *SCLAV_3230* (doXx family integral membrane protein), *SCLAV_2902* (partitioning or sporulation), and

SCLAV_3465 (secreted protein) were detected as upregulated respectively, 6.0, 2.0, 2.0, and 1.9-fold. In UniProtKB, *SCLAV_3465* is defined as it is a part of the peptidoglycan layer biosynthetic process. In contrast to *SCLAV_2902*, *SCLAV_4236* expresses another sporulation-related protein that was detected -3.0-fold downregulated in DEPA. According to the EggNog database, *SCLAV_2902* has a role in plasmid maintenance, and *SCLAV_4239* has functioned as *ftsK/spoIII*. *ftsK*-like protein is responsible for controlling chromosomal DNA segregation in the sporulation stage of *S. coelicolor* (Wang *et al.*, 2007). *SCLAV_3585* (aminopeptidase; -1.5-fold) and *SCLAV_0923* (penicillin acylase; -1.6-fold) were the other detected downregulated genes in DEPA.

3.1.3.15 Signal Transduction Mechanism

SCLAV_1592 (anti-sigma regulatory factor Ser/Thr protein kinase) was detected 6.1-fold upregulated, *SCLAV_5390* (histidine kinase) was detected -2.1-fold downregulated, and *SCLAV_p0852* (putative two-component system sensor kinase) was detected -1.6-fold downregulated in DEPA. Ser/Thr kinase, such as *AfsK*, has been known to affect the secondary metabolite production via morphological changes in *Streptomyces* spp. (Atsushi *et al.*, 1994). Two-component systems typically comprise a membrane-bound histidine kinase (HK) that are able to sense the environmental stimuli and respond to them at the level of transcriptional regulation of the responsible genes (Rodríguez *et al.*, 2013). Two-component system regulation in *S. clavuligerus* such as *CagRS* and *CepRS* has been an effect on antibiotic biosynthesis. (Fu *et al.*, 2019a, b).

SCLAV_0044 express *Rhs* element *Vgr* protein was detected 2.4-fold upregulated in DEPA. In the previous research of our group, *Rhs* element *Vgr* protein encoded by *SCLAV_0043*, has been reported 3.8-fold overrepresented in DEPA by 2DE-gel

analysis. *SCLAV_0043* and their functional partner have been discussed in section 3.1.3.1.

3.1.3.16 Defense Mechanism

SCLAV_p0715 (restriction endonuclease) was detected as -10-fold downregulated, *SCLAV_p0753* (ABC-type bacteriocidin/lantibiotic export) was detected as -6.0-fold downregulated, and *SCLAV_2436* (beta-lactamase) was detected as -2.5-fold downregulated in DEPA. The reason for the downregulation of the genes that responsible for defense mechanisms might be related to no needed for that defense systems because of the overproduction of clavulanic acid that might also defend the cell from environmental threat. In addition, the genes that are express ABC-type transport systems proteins (*SCLAV_0395*; 4.8-fold; *SCLAV_2231* 1.6-fold), transport permease protein (*SCLAV_p0831*; 3.5-fold) and daunorubicin resistance ABC transporter ATP-binding subunit protein (*SCLAV_p0830*; 3.0-fold) were found to be upregulated in DEPA. However, *SCLAV_2440* expresses that the putative-ATP binding component of an ABC transport protein was reported to be -1.7-fold downregulated. Differences in the transport systems might be correlated with substances that are transported by them. There are many downregulated genes (or vice versa) in the secondary metabolite gene clusters that could be related to the defense mechanism detected in the transcriptomic analysis.

3.1.3.17 Functions Unknown

The genes between *SCLAV_0035-SCLAV_0053* (except *SCLAV_0048* and *SCLAV_0049*) were detected as upregulated between the range of 1.6 to 4.6-fold. These genes have been discussed in section 3.1.3.1.

SCLAV_0224 (uncharacterized protein) and *SCLAV_0227* (hydrolase domain-containing protein) were detected as 2.0 and 3.8-fold upregulated in DEPA. According to the STRING database, functional partners with the *SCLAV_0225* encode ABC transporter protein that has a role in defense mechanism.

SCLAV_0256 (uncharacterized protein) was detected -8.8-fold downregulated. In the STRING data, its predicted functional partners are responsible for amino acid transport and histone acetylation.

SCLAV_0317 (uncharacterized protein), which is probably involved in cell wall/membrane/envelope biogenesis according to functional partners in the STRING data, was detected 1.5-fold upregulated in DEPA.

SCLAV_0394 (inhibitor_I36 domain-containing protein) was detected 2.9-fold upregulated in DEPA. In the STRING data, all the predicted functional partners are ABC-transporter, some of which have a role in defense mechanism and others in cobalamin/Fe³⁺ siderophore transport system.

According to the STRING data, *SCLAV_0607* (secreted protein), *SCLAV_4235* (putative-sporulation protein), and *SCLAV_3230* (doxX-family integral membrane protein) were mentioned in section 3.1.3.14, are predicted functional partners, and all are found upregulated in DEPA. These genes might be involved in the sporulation process.

SCLAV_0949 express TIR-domain containing protein was detected as 1.6-fold upregulated. Toll/Interleukin-1 Receptor (TIR) domain in pathogenic bacteria is

responsible for interfering with the innate immunity of the host (Rana *et al.*, 2013). According to the STRING data, *SCLAV_0950*, which is involved in the defense mechanism by catalyzing an N3'-acetyl-2-deoxystreptamine antibiotic production reaction, is a predicted functional partner of *SCLAV_0949*. *SCLAV_0949* is possibly involved in the defense mechanism of *S. clavuligerus*.

SCLAV_1154 and *SCLAV_1155* (uncharacterized protein) were detected as -3.7 and -4.8-fold downregulated in DEPA. According to the STRING data, they relate with putative sporulation transcription regulator *whiA*, which is required for sporulation, and putative gluconeogenesis factor which is required for morphogenesis in glucogenic growth conditions, so they might be involved in sporulation and morphological differentiation process in *S. clavuligerus*.

SCLAV_1476 (CBS domain-containing protein) was detected as -2.5-fold downregulated in DEPA. CBS domain is found in a wide range of species. It has been reported that CBS might have a regulatory role to make protein sensitive to adenosyl carrying ligands (Janošík *et al.*, 2001). In the STRING data, *SCLAV_1476* have predicted functional partners such as *SCLAV_3712* and *SCLAV_4952* that are GMP synthase, *SCLAV_5509*, and *SCLAV_1148* which are G3P dehydrogenase.

SCLAV_p0815 (uncharacterized protein) was detected as -4.8-fold downregulated in DEPA. According to the STRING data, *cas1*, *cas2*, and *cvm7* are represented as *SCLAV_p0815*'s predicted functional partners. Moreover, *SCLAV_p0812*, AfsA domain-containing protein, is also indicated as a predicted functional partner of *SCLAV_p0815*. As a result, *SCLAV_p0815* may have a direct or indirect regulatory role on the CA-CC supercluster.

SCLAV_p0816 (putative dehydrogenase) was detected as -4.0-fold downregulated in DEPA. In the STRING database, its predicted functional partners are PEP synthase, NADH:flavin oxidoreductase/NADH oxidase, and Oxidoreductases. *SCLAV_p0816* could have a role in carbohydrate metabolism for supply energy to the cell.

3.2 Gene Expression Analysis for Validation of RNA-seq Results

Data validation is known to be necessary for RNA-seq analysis that has been performed with no replication of the biological sample. Real-time quantitative polymerase chain reaction (RT-qPCR) experiments have been suggested to do for validation of the RNA-Sequencing results.

In this study, RT-qPCR experiments were performed with fifteen specifically chosen DEGs. Eight of these genes have been found as upregulated by RNA-sequencing. Also, gene expression levels of the 8 genes have been found as upregulated by RT-qPCR analysis. Upregulated genes are *SCLAV_2150*, *SCLAV_3003*, *cas2*, *SCLAV_5080*, *SCLAV_5662*, *SCLAV_0155*, *SCLAV_2475*, and *SCLAV_1592*. Detailed information about these genes, their transcription and gene expression results have been shown in Table 3.5. On the other hand, seven of these genes have been found as downregulated by RNA-sequencing. Also, gene expression levels of the 7 genes have been found as downregulated by RT-qPCR analysis. Downregulated genes are *SCLAV_p0742*, *SCLAV_p0747*, *SCLAV_p0757*, *SCLAV_p0718*, *SCLAV_p0750*, *SCLAV_p0717*, and *SCLAV_p0758* detailed information about these genes, their transcription, and gene expression results have been shown in Table 3.5.

In the result of the validation study, the transcriptional level that has been measured by RNA-sequencing of the chosen fifteen genes was verified via measuring consistent the gene expression level with them by RT-qPCR analysis (Figure 3.6 a-b).

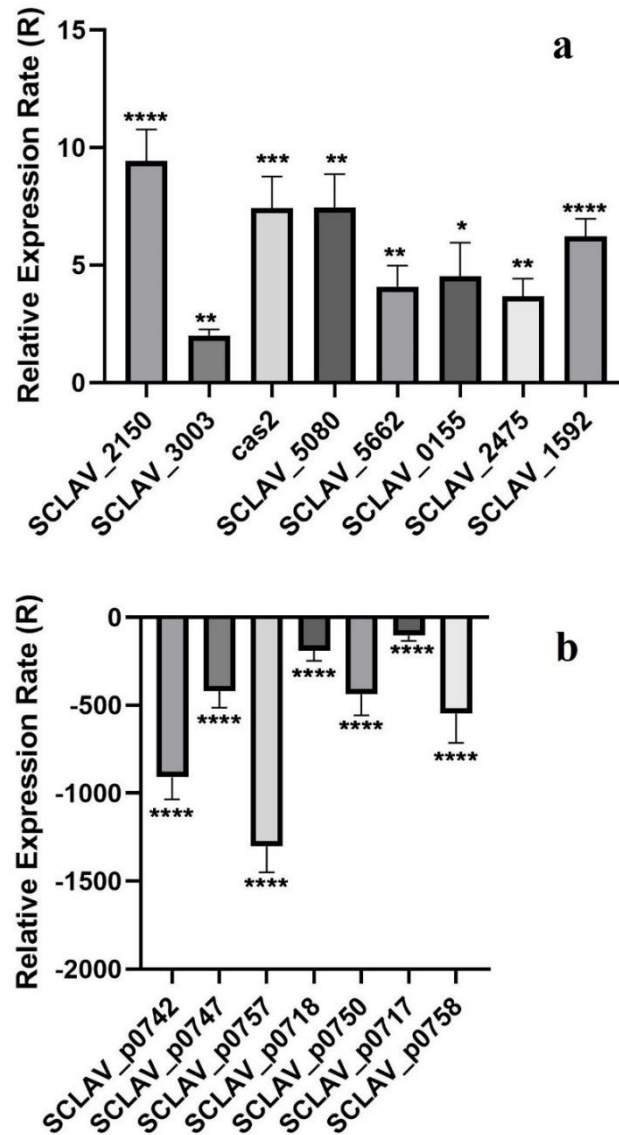


Figure 3.6 RT-qPCR results of selected a) upregulated genes b) downregulated genes. The significance levels are represented as ns (no significance, $p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.0001$).

Table 3.5 Detailed information about genes that are chosen for data validation.

Gene	Protein product	UNIPROT ACC. N.	RT-qPCR		RNA seq				
			Log2 relative fold change	Relative fold change	Log2 relative fold change	FPKM (DEPA)	FPKM (NRRL 3585)	FDR	P-VALUE
SCLAV_2150	Uncharacterized protein	E2Q699	3.22	9.32	6.66	3.04	0.03	6.56x10 ⁻⁰⁴	2.99x10 ⁻⁰⁴
SCLAV_3003	Hypothetical protein	B5GU72	1.00	2	7.76	6.52	0.03	1.82x10 ⁻⁰⁴	7.72x10 ⁻⁰⁵
cas2	CRISPR-associated endonuclease Cas2	B5GV89	2.89	7.43	7.66	6.07	0.03	9.53x10 ⁻⁰⁵	3.92x10 ⁻⁰⁵
SCLAV_5080	LapA_dom domain-containing protein	E2PX76	2.89	7.46	6.60	2.92	0.03	1.56x10 ⁻⁰²	8.85x10 ⁻⁰³
SCLAV_5662	Epimerase	B5GZD1	1.96	3.91	6.33	2.42	0.03	2.62x10 ⁻⁰⁵	1.01x10 ⁻⁰⁵
SCLAV_0155	Putative phosphotransferase	E2Q6Y4	2.17	4.51	5.82	1532.58	27.14	0	0
SCLAV_2475	Transcriptional regulator WhiB	B5GPH9	1.86	3.67	5.75	1.61	0.03	2.37x10 ⁻⁰³	1.16x10 ⁻⁰³
SCLAV_1592	Anti-sigma factor Ser/Thr protein kinase	B5GQQ0	2.64	6.23	6.09	2.05	139.45	9.82x10 ⁻¹²⁸	5.26x10 ⁻¹²⁹
SCLAV_p0742	Uncharacterized protein	B5GN86	-9.65	-908.8	-11.68	0.03	98.54	4.23x10 ⁻¹⁷²	1.76x10 ⁻¹⁷³

Table 3.5 Detailed information about genes that are chosen for data validation.(continued)

<i>SCLAV_p07</i> 47	Chitin-binding protein	B5GN81	-8.44	-419.6	-10.47	0.03	42.44	8.08x10 ⁻⁸²	5.97x10 ⁻⁸³
<i>SCLAV_p07</i> 57	Uncharacterized protein	B5GN71	-10.26	-1302	-7.85	0.03	6.92	1.19x10 ⁻¹³	2.78x10 ⁻¹⁴
<i>SCLAV_p07</i> 18	Putative FAD-dependent monooxygenase	D5SJW2	-8.70	-416.66	-13.29	0.03	302.16	0	0
<i>SCLAV_p07</i> 50	Subtilisin-like protease	D5SJJ4	-8.28	-1034.48	-12.70	0.04	267.39	0	0
<i>SCLAV_p07</i> 17	SnoaL-like domain-containing protein	B5GNB3	-6.20	-235.29	-12.17	0.03	138.03	1.82x10 ⁻¹⁸⁷	7.05x10 ⁻¹⁸⁹
<i>SCLAV_p07</i> 58	Putative DNA-binding protein	B5GN70	-8.29	-1176.47	-11.86	0.03	111.85	0	0

3.3 General Characterization of Nano-LC/MSMS Data

From nanoLC-MS/MS analysis, a total of 100341 spectra from NRRL3585 and 67220 spectra from DEPA samples were obtained. 77220 and 30332 spectra coming from our samples, NRRL3585 and DEPA, respectively, were involved in the identifications of peptides. The score that represented the significance of the results was decided as 1.3 for our study. After peptide identification, 1761 groups of proteins were found to belong to NRRL3585, and 878 groups of proteins were specific DEPA. After data cleaning, 746 unique proteins of NRRL3585, 23 unique proteins of DEPA, and 441 common proteins of both strains were detected (Figure 3.7). Our study focused on the common 441 differentially expressed proteins (DEPs) that had included 167 overrepresented proteins (larger than 1.3 score), and 134 underrepresented proteins (smaller than -1.3 score) in DEPA strain.

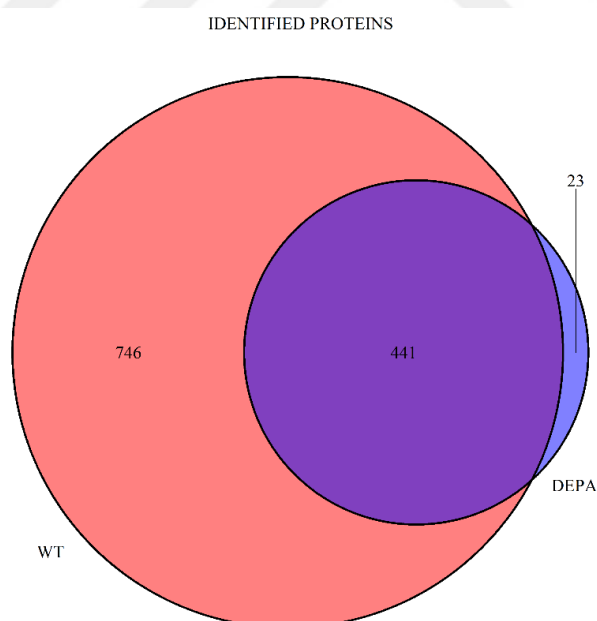


Figure 3.7 Venn Diagram representing the identified proteins via LC-MS/MS analysis of *S. clavuligerus* DEPA and NRRL3585 strains.

3.4 Integrative Analysis of Transcriptomic and Proteomic Data

A total of 311 data have been matched for integrative analysis of RNA-sequencing and nano-LCMS/MS data. 303 of these data have perfectly matched. According to nano-LCMS/MS data, in the DEPA strain of *S. clavuligerus*, 98 of the 303 proteins have been detected as no change in its production, 116 proteins have been shown overrepresentation in the production; lastly, 89 proteins have been shown underrepresentation in the production. On the other hand, according to RNA-seq data, in the DEPA strain of *S. clavuligerus*, 264 of the corresponding transcripts have been detected as no change in its expression, 29 have shown upregulation in the expression, and 10 have shown downregulation in the expression.

The distribution of the integrative data, concerning its functional categories, has shown as a Table 3.6. Comparison of the RNA-seq and nano-LCMS/MS data are discussed below.

Table 3.6 Integration of transcriptomic and proteomic data

Gene names	Protein Name	UNIPRO T ACC. N.	RNA-SEQUENCING			NANO-LC MS/MS					
			log ₂ FC Transcriptome	FPKM (DEPA)	FPKM (NRRL3 585)	FDR	P-VAL	log ₂ FC proteome	Cellular localization	Mass	pI
Clavam Gene Cluster											
SCLAV_2920 (cvm8)	Non-specific serine/threonine kinase	B5H356	0.042	260.62	253.12	8.58x10 ⁻⁰¹	8.20x10 ⁻⁰¹				
SCLAV_2921 (cvm9)	DNA-binding protein	A0PCK2	0.239	78.76	66.74	4.37x10 ⁻⁰²	2.70x10 ⁻⁰²				
SCLAV_2922 (cvm6)	Putative pyridoxal phosphate-dependent aminotransferase	E2PWJ4	-4.193	7.26	132.8	0	0				
SCLAV_2923 (cvm5)	Flavin-dependent oxidoreductase, F420-dependent tetrahydromethanopterin reductase	Q9X5G9	-3.668	4.49	57.07	2.16x10 ⁻¹⁵⁷	9.69x10 ⁻¹⁵⁹				
SCLAV_2924 (cvm4)	Alpha/beta hydrolase	E2PWJ6	-2.999	7.5	59.96	9.75x10 ⁻¹¹⁴	5.65x10 ⁻¹¹⁵				
SCLAV_2925 (cas1)	Clavaminate synthase 1	E2PWJ7	-4.812	2.19	61.53	1.01x10 ⁻¹⁶⁶	4.26x10 ⁻¹⁶⁸	-0.157	Cytoplasm	35,370	5.31
SCLAV_2926 (cvm1)	Aldo/keto reductase	E2PWJ8	-1.295	11.97	29.38	1.61x10 ⁻²⁰	2.93x10 ⁻²¹				
SCLAV_2927 (cvm2)	Putative ribulose-5-phosphate epimerase	Q9X5G6	-1.035	33.68	69.03	1.87x10 ⁻¹⁴	4.22x10 ⁻¹⁵				
SCLAV_2928 (cvm3)	Flavin reductase-like protein	E2PWK0	-1.199	37.41	85.86	1.15x10 ⁻²⁷	1.77x10 ⁻²⁸				
SCLAV_2929 (cvm7)	DmrI/RedD/AfsR-family transcriptional regulator	A0PCK1	-0.172	28.02	31.57	1.41x10 ⁻⁰²	7.93x10 ⁻⁰³				
SCLAV_2930 (cvm11)	Putative efflux protein	A0PCK0	0.387	17.94	13.72	8.72x10 ⁻⁰²	5.75x10 ⁻⁰²				

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_2931 (cvm12)	Putative transcriptional regulator	A0PCJ9	0.616	19.23	12.55	1.32x10 ⁻⁰⁵	4.95x10 ⁻⁰⁶
SCLAV_2932 (cvm13)	Beta-aspartyl-peptidase	A0PCJ8	-0.008	11.97	12.04	9.32x10 ⁻⁰¹	9.09x10 ⁻⁰¹
SCLAV_2933 (cvmH)	LanU-like protein	E2PWK5	-0.132	13.12	14.38	4.95x10 ⁻⁰¹	4.17x10 ⁻⁰¹
SCLAV_2934 (cvmP)	Putative peptidylarginine deiminase	A0PCJ6	0.657	618.25	391.98	3.71x10 ⁻²⁵⁴	1.04x10 ⁻²⁵⁵
SCLAV_2935 (cvmG)	Uncharacterized protein	E2PWK7	0.400	3.51	2.66	4.12x10 ⁻⁰¹	3.36x10 ⁻⁰¹
Alanylclavam cluster							
SCLAV_p1067	Membrane protein	D5SKW0	-3.373	4.48	5.66	3.34x10 ⁻⁰¹	2.64x10 ⁻⁰¹
SCLAV_p1069	Dehydratase	D5SKW2	-1.029	0.25	0.51	3.94x10 ⁻⁰¹	3.19x10 ⁻⁰¹
SCLAV_p1070	Aminotransferase	D5SKW3	-1.029	0.81	0.68	7.71x10 ⁻⁰¹	7.17x10 ⁻⁰¹
SCLAV_p1071	YjgF family regulator	B5GMQ 2	0.054	5.42	5.22	9.71x10 ⁻⁰¹	9.59x10 ⁻⁰¹
SCLAV_p1072	Serine hydroxymethyltransferase	D5SKW5	-0.571	7.44	11.05	3.56x10 ⁻⁰³	1.79x10 ⁻⁰³
SCLAV_p1074 (ceaSt)	Carboxyethyl arginine synthase isoenzyme 1	D5SKW7	-0.079	5.35	5.65	7.21x10 ⁻⁰¹	6.63x10 ⁻⁰¹
SCLAV_p1076 (pah1)	Proclavamate amidinohydrolase	D5SKW9	0.217	2.08	1.79	6.98x10 ⁻⁰¹	6.35x10 ⁻⁰¹
SCLAV_p1077 (oat1)	Probable glutamate N-acetyltransferase 1	D5SKX0	0.092	1.78	1.67	8.88x10 ⁻⁰¹	8.55x10 ⁻⁰¹
SCLAV_p1078 (cvm6P)	Putative pyridoxal phosphate-dependent aminotransferase	D5SKX1	-1.185	2.38	5.41	4.63x10 ⁻⁰⁵	1.83x10 ⁻⁰⁵
SCLAV_p1079 (cvm7P)	Putative transcriptional regulator	D5SKX2	0.397	3.91	2.97	7.32x10 ⁻⁰²	4.73x10 ⁻⁰²
Clavulanic Acid/Cepharmycin C Supercluster							
SCLAV_4181 (gccs)	N-glycyl-clavaminic acid synthetase	E2Q5P2	0.874	443.87	242.15	6.31x10 ⁻¹⁹²	2.41x10 ⁻¹⁹³

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_4182 (orf16)	DUF482 domain-containing protein	B5GLE0	0.985	204.02	103.1	4.80x10 ⁻⁹⁹	3.10x10 ⁻¹⁰⁰	0.198	Cytoplasm	43,518	5.63
SCLAV_4183 (oppA2)	ABC-type dipeptide transport system, solute-binding protein	Q8KRB4	1.146	228.81	103.37	1.80x10 ⁻²⁰²	6.00x10 ⁻²⁰⁴	0.073	Cell-Membrane	61,988	5.40
SCLAV_4184	Uncharacterized protein	E2Q5P5	-1.359	0.76	1.95	1.53x10 ⁻⁰²	8.635x10 ⁻⁰³				
SCLAV_4185 (orf14)	Acetyltransferase GNAT family protein	Q8KRB5	1.518	190.9	66.65	1.20x10 ⁻¹⁵³	5.30x10 ⁻¹⁵⁵	1.057	Cytoplasm	36,293	5.7
SCLAV_4186 (orf13)	Integral membrane protein DUF6	Q8KRB6	1.134	229.24	104.46	1.27x10 ⁻¹¹⁷	7.22x10 ⁻¹¹⁹				
SCLAV_4187 (orf12)	Beta-lactamase	B5GLD6	1.478	543.08	194.98	0	0				
SCLAV_4189 (cyp)	Cytochrome P450-SU2	B5GLD4	0.831	550.11	309.27	1.90x10 ⁻²⁰²	6.50x10 ⁻²⁰⁴	-0.388	Cytoplasm	45,038	5.41
SCLAV_4190 (car)	Clavaldehyde dehydrogenase	B5GLD3	1.077	592.63	280.95	1.50x10 ⁻¹⁹⁷	5.40x10 ⁻¹⁹⁹	1.186	Cytoplasm	26,456	6.17
SCLAV_4191 (claR)	Transcriptional activator	O66038	1.150	702.33	316.56	0	0				
SCLAV_4192 (oppA1)	ABC-type dipeptide transport system, periplasmic component	E2Q5Q3	1.522	687.85	239.53	0	0	1.434	Cell-Membrane	62,301	5.00
SCLAV_4193 (oat2)	Arginine biosynthesis bifunctional protein ArgJ 3	P0DJQ6	1.936	2277.56	595.02	0	0	1.990	Cytoplasm	42,198	5.48
SCLAV_4194 (cas/cas2)	Clavamate synthase	Q53939	1.283	4729.59	1943.04	0	0	1.015	Cytoplasm	35,796	5.18
SCLAV_4195 (pah)	Proclavamate amidohydrolase	B5GLC8	1.314	2189.8	881.03	0	0				
SCLAV_4196 (bis/bis2)	Carboxyethyl-arginine synthase	B5GLC7	1.356	2616.01	1021.91	0	0	1.324	Extracellular	54,530	5.92
SCLAV_4197 (ccaS2)	N(2)-(2-carboxyethyl)arginine synthase, CEA synthetase	Q9LCV9	1.354	6027.51	2358.42	0	0	0.860	Extracellular	60,907	5.01
SCLAV_4198 (pcbR)	PcbR (Secreted penicillin binding protein)	B5GLC4	0.279	1388.65	1144.79	1.67x10 ⁻⁹²	1.11x10 ⁻⁹³				
SCLAV_4200 (pcbAB)	N-5-amino-5-carboxypentanoyl-L-cysteiny-D-valine synthase	E2QSR1	1.400	646.07	244.84	0	0				

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_4201 (lat)	Lysine 6-aminotransferase	B5GLC1	1.399	1140.91	432.56	0	0	1.075	Cytoplasm	49,889	5.36
SCLAV_4202 (blp)	Beta-Lactamase Inhibitory Protein-Like Protein	B5GLC0	0.124	423.1	388.36	2.23x10 ⁻⁰²	1.30x10 ⁻⁰²				
SCLAV_4203	ORF12 protein (Secreted protein)	B5GLB9	-0.064	40.31	42.15	4.95x10 ⁻⁰¹	4.18x10 ⁻⁰¹				
SCLAV_4204 (ccaR)	Positive regulator	B5GLB7	0.242	1242.53	1050.68	5.52x10 ⁻²⁸	8.43x10 ⁻²⁹				
SCLAV_4205 (cmcH)	3'-hydroxymethylcephem-O-carbamoyltransferase	B5GLB6	0.890	1084.24	584.91	0	0				
SCLAV_4206 (cefF)	Deacetoxycephalosporin C hydroxylase	B5GLB5	0.908	757.86	403.77	1.10x10 ⁻²⁴⁸	3.00x10 ⁻²⁵⁰	1.943	Cytoplasm	34,599	4.79
SCLAV_4207 (cmcJ)	7-alpha-cephem-methoxylase P8 chain	B5GLB4	0.738	903.03	541.34	6.27x10 ⁻²⁰¹	2.22x10 ⁻²⁰²				
SCLAV_4208 (cmcI)	Rhamnosyl O-methyltransferase	B5GLB3	0.628	2143.94	1386.93	2.28x10 ⁻²⁶³	6.01x10 ⁻²⁶⁵				
SCLAV_4210 (cefD)	Isopenicillin N epimerase	B5GLB1	0.612	450.57	294.79	2.66x10 ⁻⁹⁴	1.76x10 ⁻⁹⁵				
SCLAV_4211 (cefE)	Deacetoxycephalosporin C synthetase	B5GLB0	0.822	1384.16	783.18	0	0	0.412	Cytoplasm	34,556	5.14
SCLAV_4212 (pcd)	Aldehyde dehydrogenase	B5GLA9	0.850	1424.06	789.85	0	0	0.317	Cytoplasm	54,001	5.08
SSCG_00147 (pcbC)	Isopenicillin N synthetase	B5GLC3	1.219	2620.51	1125.44	0	0	1.811	Cytoplasm	36,959	5.18
SCLAV_4213 (cmcT)	CmcT protein	E2Q5S4	1.391	43.34	113.68	2.25x10 ⁻¹¹⁸	1.27x10 ⁻¹¹⁹				
SCLAV_4214	Penicillin binding protein	E2Q5S5	0.684	118.94	74.03	5.06x10 ⁻⁵⁵	5.07x10 ⁻⁵⁶				
Arginine Biosynthesis											
SCLAV_0799 (argB)	Acetylglutamate kinase	E2PVC9	1.502	851.06	300.57	0	0				
SCLAV_0801 (argC)	N-acetyl-gamma-glutamylphosphate	E2PVD1	1.631	2929.14	945.47	0	0				
SCLAV_0798 (argD)	Acetylmethionine aminotransferase	B5H2S4	1.113	731.17	338.1	0	0	0.029	Cytoplasm	42,136	5.18

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_0800 (argJ)	Bifunctional ornithine	B5H252	1.734	1125,75	338,34	0	0	
SCLAV_0797 (argR)	Arginine repressor	B5H255	1.006	599,28	298,43	6.82x10 ⁻¹²⁴	3.72x10 ⁻¹²⁵	
SCLAV_0796 (argG)	Argininosuccinate synthase	E2PVC6	2.000	11646,79	2912,1	0	0	
SCLAV_0795 (argH)	Argininosuccinate lyase	B5H257	1.784	1920.19	557.74	0	0	-0.692
SCLAV_4815 (argF)	Ornithine carbamoyltransferase	E2PUZ1	1.492	853.4	303.35	0	0	
G3P Biosynthesis								
SCLAV_3958	Putative polyphosphate glucokinase	E2Q494	-0.421	76,68	102,67	3.07x10 ⁻¹⁰	8.39x10 ⁻¹¹	
SCLAV_1142 (pgi)	Glucose-6-phosphate isomerase	E2PYJ8	-0.200	82.48	94,72	7.40x10 ⁻⁰⁵	3.01x10 ⁻⁰⁵	
SCLAV_4332 (pfkA)	ATP-dependent 6-phosphofructokinase	B5GKZ5	2.998	6.39	0.8	6.87x10 ⁻⁰⁹	2.03x10 ⁻⁰⁹	-0.566
SCLAV_0508 (pfkA)	ATP-dependent 6-phosphofructokinase	B5H2K3	-0.241	57.75	68.26	1.81x10 ⁻⁰³	8.68x10 ⁻⁰⁴	-2.067
SCLAV_1059 (fucA)	Aldolase	E2PXL5	-0.283	67.84	82,53	1.39x10 ⁻³	6.60x10 ⁻⁴	
SCLAV_1148 (gap1)	Glyceraldehyde-3-phosphate dehydrogenase	B5GYJ0	-0.328	1803,8	2264,68	8.04x10 ⁻¹³⁹	3.95x10 ⁻¹⁴⁰	
SCLAV_5509 (gap2)	Glyceraldehyde-3-phosphate dehydrogenase	E2Q1F0	0.245	344,67	290,81	2.64x10 ⁻¹⁶	5.54x10 ⁻¹⁷	
SCLAV_1147 (pgk)	Phosphoglycerate kinase	B5GYJ1	-0.122	160.57	174.72	3.78x10 ⁻⁰³	1.92x10 ⁻⁰³	-0.550
SCLAV_2648	Fructose-bisphosphate aldolase	B5GTD3	-0.338	547.11	691.38	5.11x10 ⁻⁴⁶	5.78x10 ⁻⁴⁷	-0.792
Glycerol Metabolism								
SCLAV_0877 (glpF2)	Glycerol uptake facilitator protein	E2PWC1	0.581	261,4	174,71	4.14x10 ⁻³²	5.88x10 ⁻³³	

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_0878 (gIpK)	Glycerol kinase	B5H2G1	0.479	154.27	110.67	1.00x10 ⁻²⁷	1.55x10 ⁻²⁸
SCLAV_0879 (gIpD1)	Glycerol-3-phosphate dehydrogenase	B5U892	0.418	88.4	66.18	1.97x10 ⁻¹³	4.64x10 ⁻¹⁴
Holomycin Gene Cluster							
SCLAV_5267 (hImA)	Acetyltransferase	B5GVJ0	0.146	2.71	2.45	8.03x10 ⁻⁰¹	7.55x10 ⁻⁰¹
SCLAV_5268 (hImB)	Acyl-CoA dehydrogenase domain protein	E2PZ80	0.342	2.94	2.32	4.22x10 ⁻¹	3.45x10 ⁻⁰¹
SCLAV_5269 (hImC)	Gramicidin S biosynthesis protein grsT	B5GVJ3	0.643	1.78	1.14	3.55x10 ⁻⁰¹	2.83x10 ⁻⁰¹
SCLAV_5270 (hImD)	Glucose-methanol-choline oxidoreductase	E2PZ82	-0.112	1.85	2	7.68x10 ⁻⁰¹	7.13x10 ⁻⁰¹
SCLAV_5271 (hImE)	Non-ribosomal peptide synthetase	E2PZ83	-0.011	1.25	1.26	9.68x10 ⁻⁰¹	9.56x10 ⁻⁰¹
SCLAV_5272 (hImF)	Lantibiotic biosynthesis protein	B5GVJ6	0.332	8.95	7.11	3.42x10 ⁻⁰¹	2.71x10 ⁻⁰¹
SCLAV_5273 (hImG)	Globin	B5GVJ7	-0.408	5.32	7.06	3.22x10 ⁻⁰¹	2.51x10 ⁻⁰¹
SCLAV_5274 (hImH)	Putative transmembrane efflux protein	E2PZ86	-0.201	9.72	11.17	2.33x10 ⁻⁰¹	1.74x10 ⁻⁰¹
SCLAV_5275 (hImI)	Thioredoxin reductase	E2PZ87	-0.276	5.41	6.55	3.26x10 ⁻⁰¹	2.55x10 ⁻⁰¹
hImJ	Regulatory protein	B5GVK0	0.034	51.69	50.49	9.18x10 ⁻⁰¹	8.89x10 ⁻⁰¹
SCLAV_5276 (hImK)	Thioesterase	E2PZ88	1.188	0.82	0.36	3.18x10 ⁻⁰¹	2.48x10 ⁻⁰¹
SCLAV_5277 (hImL)	Condensation domain-containing protein	B5GVK2	0.508	3	2.11	1.63x10 ⁻⁰¹	1.16x10 ⁻⁰¹
SCLAV_5278 (hImM)	AmphRI-like transcriptional regulator	E2PZ90	1.198	104	45.33	1.41x10 ⁻¹⁶⁶	5.97x10 ⁻¹⁶⁸
Tunicamycin Gene cluster							
SCLAV_4276 (tunL)	Phosphatidylglycerophosphatase B	B5GL47	0.641	241.8	155.07	7.59x10 ⁻³⁰	1.12x10 ⁻³⁰

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_4277 (<i>tunK</i>)	Polyketide synthase	B5GL46	0.774	176.89	103.42	1.53x10 ⁻⁰⁹	4.33x10 ⁻¹⁰
SCLAV_4278 (<i>tunJ</i>)	Transport permease protein	E2Q6Q3	0.713	198.1	120.86	3.58x10 ⁻³⁵	4.85x10 ⁻³⁶
SCLAV_4279 (<i>tunI</i>)	ABC transporter related protein	E2Q6Q4	0.653	262.98	167.2	1.63x10 ⁻⁴⁶	1.83x10 ⁻⁴⁷
SCLAV_4280 (<i>tunH</i>)	Type I phosphodiesterase/nucleotide pyrophosphatase	E2Q6Q5	0.811	314.62	179.37	4.14x10 ⁻¹⁴³	2.01x10 ⁻¹⁴⁴
SCLAV_4281 (<i>tunG</i>)	Phosphoglycerate mutase	E2Q6Q6	0.897	238.81	128.24	7.85x10 ⁻⁴⁹	8.53x10 ⁻⁵⁰
SCLAV_4282 (<i>tunF</i>)	UDP-glucose 4-epimerase	B5GL42	0.951	545.14	281.99	1.52x10 ⁻¹⁹⁹	5.50x10 ⁻²⁰¹
SCLAV_4283 (<i>tunE</i>)	LmbE family protein	E2Q6Q8	0.904	859.87	459.62	8.62x10 ⁻²⁰²	3.02x10 ⁻²⁰³
SCLAV_4284 (<i>tunD</i>)	Glycosyl transferase group 1	E2Q6Q9	0.916	1239.6	657.13	0	0
SCLAV_4285 (<i>tunC</i>)	GCN5-related N-acetyltransferase	E2Q6R0	0.925	1143.08	601.85	0	0
SCLAV_4286 (<i>tunB</i>)	Radical SAM domain protein	E2Q6R1	0.881	1436.34	779.74	0	0
SCLAV_4287 (<i>tunA</i>)	NAD-dependent epimerase/dehydratase	E2Q6R2	0.916	633.64	335.8	1.55x10 ⁻²¹²	5.20x10 ⁻²¹⁴
Type I PKS							
SCLAV_0447	FkbH like protein	E2Q9A3	-0.600	2.58	3.91	2.10x10 ⁻⁰²	1.22x10 ⁻⁰²
SCLAV_0448	Uncharacterized protein	B5GU44	0.585	8.19	5.46	2.03x10 ⁻⁰¹	1.49x10 ⁻⁰¹
SCLAV_0449	AMP-binding domain-containing protein	E2Q9A5	0.415	1.2	0.9	4.32x10 ⁻⁰¹	3.55x10 ⁻⁰¹
SCLAV_0450	Acyl-CoA dehydrogenase	B5GU47	0.252	0.5	0.42	7.71x10 ⁻⁰¹	7.17x10 ⁻⁰¹
SCLAV_0451	Acyl-CoA dehydrogenase	E2Q9A7	-0.623	1.85	2.85	5.01x10 ⁻⁰²	3.13x10 ⁻⁰²
SCLAV_0452	Peptide carrier protein	B5GU49	-3.369	0.03	0.31	5.61x10 ⁻⁰¹	4.84x10 ⁻⁰¹
SCLAV_0453	Polyketide synthase	E2Q9A9	0.070	2.33	2.22	8.32x10 ⁻⁰¹	7.89x10 ⁻⁰¹

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_0454</i>	3-oxoacyl-acyl-carrier-protein synthase III	E2Q9B0	-1.931	0.59	2.25	1.25×10^{03}	5.91×10^{-04}
<i>SCLAV_0455</i>	Acyl carrier protein	E2Q9B1	0.417	11.75	8.8	4.95×10^{-01}	4.17×10^{-01}
<i>SCLAV_0456</i>	Hydrolase superfamily dihydrolipoamide acyltransferase-like protein	E2Q9B2	-0.778	4.13	7.08	1.02×10^{02}	5.55×10^{-03}
<i>SCLAV_0457</i>	4'-phosphopantetheinyl transferase	E2Q9B3	-0.014	6.07	6.13	9.33×10^{-01}	9.11×10^{-01}
<i>SCLAV_0459</i>	Alpha/beta hydrolase	E2Q9B5	0.459	1.87	1.36	4.04×10^{-01}	3.28×10^{-01}
<i>SCLAV_0460</i>	Putative hydrolase	E2Q9B6	0.209	0.52	0.45	8.55×10^{-01}	8.17×10^{-01}
<i>SCLAV_0461</i>	Uncharacterized protein	E2Q9B7	0.044	18.18	17.63	9.30×10^{-01}	9.05×10^{-01}
<i>SCLAV_0462</i>	Putative regulatory protein AfsR-like protein	E2Q9B8	0.803	0.82	0.47	1.21×10^{-01}	8.27×10^{-02}
<i>SCLAV_0463</i> (<i>avaA1</i>)	Putative gamma-butyrolactone biosynthesis protein	E2Q9B9	-0.662	0.79	1.25	2.98×10^{-01}	2.30×10^{-01}
<i>SCLAV_0464</i>	Ferredoxin	E2Q9C0	3.369	0.31	0.03	5.88×10^{-01}	5.16×10^{-01}
<i>SCLAV_0467</i>	SMP-30/gluconolactonase/LRE domain-containing protein	E2Q9C3	-0.163	0.92	1.03	8.24×10^{-01}	7.78×10^{-01}
<i>SCLAV_0468</i>	Putative membrane protein	E2Q9C4	0.007	2.14	2.13	9.96×10^{-01}	0.994×10^{-01}
<i>SCLAV_0469</i>	HAD-superfamily hydrolase, subfamily IA, variant 3	E2Q9C5	0.711	1.31	0.8	4.43×10^{-01}	3.66×10^{-01}
<i>SCLAV_0470</i>	Monooxygenase	E2Q9C6	0.911	1.41	0.75	8.43×10^{-02}	5.53×10^{-02}
<i>SCLAV_0471</i> (<i>avaA2</i>)	Gamma-butyrolactone biosynthesis protein	E2Q9C7	0.290	1.21	0.99	6.94×10^{-01}	6.30×10^{-01}
<i>SCLAV_0472</i>	LuxR response regulator receiver	E2Q9C8	0.885	1366.89	740.16	1.85×10^{259}	4.98×10^{-261}
<i>SCLAV_0473</i>	Putative two-component sensory kinase	E2Q9C9	0.255	48.91	40.99	4.35×10^{03}	2.22×10^{-03}
<i>SCLAV_0474</i>	Goadsporin biosynthetic protein	E2Q9D0	0.752	1.33	0.79	1.55×10^{-01}	1.09×10^{-01}
<i>SCLAV_0475</i>	Goadsporin biosynthetic protein	E2Q9D1	1.059	3.23	1.55	1.35×10^{-04}	5.65×10^{-05}
<i>SCLAV_0476</i>	Uncharacterized protein	E2Q9D2	4.891	0.89	0.03	3.33×10^{-01}	2.62×10^{-01}

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_0477	SARP family pathway specific regulatory protein	E2Q9D3	0.211	24.72	21.35	2.14x10 ⁻⁰¹	1.58x10 ⁻⁰¹
SCLAV_0478	Thioesterase domain protein	E2Q9D4	-0.259	4.27	5.11	4.89x10 ⁻⁰¹	4.11x10 ⁻⁰¹
SCLAV_0479	Integral membrane protein	E2Q9D5	-0.184	0.44	0.5	8.10x10 ⁻⁰¹	7.62x10 ⁻⁰¹
SCLAV_0480	Major facilitator superfamily permease	E2Q9D6	1.088	3.72	1.75	1.85x10 ⁻⁰³	8.93x10 ⁻⁰⁴
SCLAV_0481	Daurorubicin-doxorubicin polyketide synthase	B5H3Q8	0.194	2.31	2.02	7.23x10 ⁻⁰¹	6.65x10 ⁻⁰¹
SCLAV_0482	AMP-dependent synthetase and ligase	E2Q9D8	0.500	1.74	1.23	2.84x10 ⁻⁰¹	2.18x10 ⁻⁰¹
SCLAV_0483	Decarboxylase	E2Q9D9	-0.060	3.27	3.41	8.34x10 ⁻⁰¹	7.90x10 ⁻⁰¹
SCLAV_0484	3-oxoacyl-(Acyl carrier protein) synthase III	B5H3R1	-0.034	4.66	4.77	8.99x10 ⁻⁰¹	8.68x10 ⁻⁰¹
SCLAV_0485	Putative membrane protein	E2Q9E1	1.020	33.41	16.47	3.20x10 ⁻⁰⁶	1.13x10 ⁻⁰⁶
SCLAV_0486	Cytochrome P450-like enzyme	B5H3R3	0.047	6.26	6.06	8.96x10 ⁻⁰¹	8.64x10 ⁻⁰¹
SCLAV_0487	Monooxygenase	E2Q9E3	-0.323	4.11	5.14	2.52x10 ⁻⁰¹	1.89x10 ⁻⁰¹
SCLAV_0488	Cyclase/dehydrase	E2Q9E4	0.145	5.14	4.65	8.10x10 ⁻⁰¹	7.63x10 ⁻⁰¹
SCLAV_0489	MMPL domain protein	E2Q9E5	-0.115	2.78	3.01	6.69x10 ⁻⁰¹	6.04x10 ⁻⁰¹
SCLAV_0490	Glycosyl hydrolase family 109 protein	E2Q9E6	-0.466	15.6	21.55	9.74x10 ⁻⁰⁵	4.01x10 ⁻⁰⁵
SCLAV_0491	Putative ABC transporter ATPase and permease component	E2Q9E7	0.205	57.7	50.04	1.91x10 ⁻⁰³	9.24x10 ⁻⁰⁴
SCLAV_0492	ABC transporter	E2Q9E8	0.276	65.92	54.44	8.91x10 ⁻⁰⁶	3.27x10 ⁻⁰⁶
SCLAV_0493	Uncharacterized protein	E2Q9E9	0.303	16.53	13.4	5.84x10 ⁻⁰¹	5.10x10 ⁻⁰¹
SCLAV_0494	Integral membrane protein	B5H2L7	0.492	24.55	17.46	3.35x10 ⁻⁰³	1.68x10 ⁻⁰³
SCLAV_0495	ATP-dependent helicase	E2Q9F1	-0.315	110.56	137.56	1.49x10 ⁻²⁰	2.71x10 ⁻²¹
SCLAV_0496	Acyl-CoA thioesterase	B5H2L5	-0.349	114.35	145.61	7.66x10 ⁻¹⁰	2.14x10 ⁻¹⁰
SCLAV_0497	Glutathione peroxidase	B5H2L4	-0.321	264.63	330.65	2.35x10 ⁻¹⁰	6.32x10 ⁻¹¹
					0.082	Extracellular	17,242 4.73

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_p1225	Valine decarboxylase	D5SLB8	2.195	23.53	5.14	5.19x10 ⁻⁵⁵	5.22x10 ⁻⁵⁶	3.852	Cell-Wall	62,334	5.08
Type II PKS											
SCLAV_4911	Histidine kinase	E2PVZ4	0.410	53.44	40.22	1.33x10 ⁻¹³	3.11x10 ⁻¹⁴				
SCLAV_4912	Polyketide hydroxylase	E2PVZ5	-0.152	5.03	5.59	5.09x10 ⁻⁰¹	4.32x10 ⁻⁰¹				
SCLAV_4913	Putative WhiE I protein	E2PVZ6	-0.453	1.6	2.19	3.41x10 ⁻⁰¹	2.71x10 ⁻⁰¹				
SCLAV_4914	Putative WhiE II protein	E2PVZ7	5.719	1.58	0.03	8.37x10 ⁻⁰³	4.50x10 ⁻⁰³				
SCLAV_4915	Putative polyketide beta-ketoacyl synthase I	E2PVZ8	-0.527	0.34	0.49	6.12x10 ⁻⁰¹	5.42x10 ⁻⁰¹				
SCLAV_4916	Chain length factor	E2PVZ9	0.550	0.41	0.28	6.52x10 ⁻⁰¹	5.85x10 ⁻⁰¹				
SCLAV_4918	Polyketide cyclase	E2PW01	-0.178	1.75	1.98	8.15x10 ⁻⁰¹	7.69x10 ⁻⁰¹				
SCLAV_4919	Cyclase II	E2PW02	1.577	3.49	1.17	8.66x10 ⁻⁰²	5.70x10 ⁻⁰²				
SCLAV_4920	O-methyltransferase	E2PW03	-0.479	5.13	7.15	6.17x10 ⁻⁰²	3.92x10 ⁻⁰²				
SCLAV_4921	Secreted protein	E2PW04	0.173	1.77	1.57	7.72x10 ⁻⁰¹	7.19x10 ⁻⁰¹				
Type III PKS											
SCLAV_5463	PKS domain-like acyltransferase	B5GWR 0	-0.661	136.8	216.38	6.33x10 ⁻⁵⁰	6.74x10 ⁻⁵¹				
SCLAV_5464	Putative ABC transporter, ATP-binding protein	B5GWR 1	-0.049	9.6	9.93	8.25x10 ⁻⁰¹	7.80x10 ⁻⁰¹				
SCLAV_5465	Uncharacterized protein	B5GWR 2	-0.068	16.47	17.26	7.21x10 ⁻⁰¹	6.63x10 ⁻⁰¹				
SCLAV_5466	Uncharacterized protein	B5GWR 3	0.121	35.36	32.51	2.91x10 ⁻⁰¹	2.24x10 ⁻⁰¹				
SCLAV_5467	Uncharacterized protein	B5GWR 4	-0.230	162.29	190.37	1.05x10 ⁻⁰³	4.93x10 ⁻⁰⁴				
SCLAV_5468	Uncharacterized protein	E2Q0J2	0.427	13.19	9.81	5.39x10 ⁻⁰¹	4.61x10 ⁻⁰¹				
SCLAV_5469	Stress-inducible protein	E2Q0J3	-0.700	4.02	6.53	2.47x10 ⁻⁰²	1.45x10 ⁻⁰²				

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_5470	Uncharacterized protein	E2Q0I4	-1.118	1.23	2.67	2.25x10 ⁻⁰³	1.10x10 ⁻⁰³
SCLAV_5471	Uncharacterized protein	E2Q0I5	-0.585	0.86	1.29	7.18x10 ⁻⁰¹	6.58x10 ⁻⁰¹
SCLAV_5472	Glycosyl transferase	E2Q0I6	-1.170	1.16	2.61	1.00x10 ⁻⁰²	5.54x10 ⁻⁰³
SCLAV_5473	Uncharacterized protein	E2Q0I7	-0.363	6.32	8.13	8.50x10 ⁻⁰²	5.60x10 ⁻⁰²
SCLAV_5474	Uncharacterized protein	B5GWR	0.005	2.68	2.67	9.70x10 ⁻⁰¹	9.57x10 ⁻⁰¹
SCLAV_5476	Transcriptional regulator, SARP family protein	E2Q0K0	-0.564	8.71	12.88	1.34x10 ⁻⁰⁶	4.61x10 ⁻⁰⁷
SCLAV_5477	Uncharacterized protein	B5GWS1	0.226	18.27	15.62	1.41x10 ⁻⁰²	7.89x10 ⁻⁰³
SCLAV_5478	Uncharacterized protein	E2Q0K2	-0.016	8.27	8.36	8.72x10 ⁻⁰¹	8.37x10 ⁻⁰¹
SCLAV_5479	Transcriptional regulator, Fis family	B5GWS3	-0.381	6.32	8.23	9.21x10 ⁻⁰²	6.12x10 ⁻⁰²
SCLAV_5480	PDDEXK_1 domain-containing protein	B5GWS4	-0.338	7.65	9.67	3.95x10 ⁻⁰²	2.41x10 ⁻⁰²
SCLAV_5481	Protein kinase	B5GWS5	-0.311	29	35.98	4.54x10 ⁻⁰⁴	2.03x10 ⁻⁰⁴
SCLAV_5482	Site-specific DNA-methyltransferase (adenine-specific)	B5GWS6	-0.446	27.75	37.81	3.84x10 ⁻⁰⁹	1.11x10 ⁻⁰⁹
SCLAV_5483	2-hydroxyacid-family dehydrogenase	E2Q0K7	-0.322	40.98	51.23	6.02x10 ⁻⁰⁴	2.74x10 ⁻⁰⁴
SCLAV_5484	Non-ribosomal peptide synthetase	B5GWS8	0.398	3.36	2.55	1.84x10 ⁻⁰¹	1.33x10 ⁻⁰¹
SCLAV_5485	Non-ribosomal peptide synthetase	E2Q0K9	-0.329	6.12	7.69	1.15x10 ⁻⁰¹	7.81x10 ⁻⁰²
SCLAV_5486	Cupin family protein	B5GWT0	-2.693	1.28	8.28	7.43x10 ⁻⁰⁶	2.71x10 ⁻⁰⁶
SCLAV_5487	Thioesterase	E2Q0L1	-0.640	39.87	62.14	4.44x10 ⁻¹⁰	1.23x10 ⁻¹⁰
SCLAV_5488	3-hydroxyisobutyrate dehydrogenase	E2Q0L2	-0.572	2.18	3.24	1.96x10 ⁻⁰¹	1.42x10 ⁻⁰¹
SCLAV_5489	Uncharacterized protein	E2Q0L3	-4.824	0.03	0.85	3.07x10 ⁻⁰¹	2.38x10 ⁻⁰¹
SCLAV_5490	Putative transketolase	E2Q0L4	-1.469	20.3	56.19	5.58x10 ⁻⁸³	4.05x10 ⁻⁸⁴
SCLAV_5491	Cytochrome P450	E2Q0L5	-1.069	6.08	12.76	2.23x10 ⁻⁰⁸	6.87x10 ⁻⁰⁹

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_5492	Type III PKS, putative chalcone synthase	E2Q0L6	-0.660	9.64	15.23	1.88x10 ⁻⁰⁴	7.97x10 ⁻⁰⁵		
SCLAV_5493	Secreted protein	E2Q0L7	-0.134	2.56	2.81	8.30x10 ⁻⁰¹	7.85x10 ⁻⁰¹		
SCLAV_5494	Metallo cofactor biosynthesis protein	E2Q0L8	-0.413	4.67	6.22	7.36x10 ⁻⁰²	4.76x10 ⁻⁰²		
SCLAV_5495	Uncharacterized protein	B5GWT8	-1.889	0.88	3.26	4.86x10 ⁻⁰²	3.02x10 ⁻⁰²		
SCLAV_5496	Daunorubicin resistance ABC transporter ATPase subunit	B5GWT9	-3.110	0.55	4.75	5.46x10 ⁻¹⁰	1.51x10 ⁻¹⁰		
SCLAV_5497	Transport permease protein	B5GWU0	1.245	3.2	1.35	1.85x10 ⁻⁰²	1.06x10 ⁻⁰²		
PKS-NRPS									
SCLAV_p1007	Beta-lactamase domain protein	B5GMI9	2.082	35.13	8.3	5.53x10 ⁻⁸⁶	3.86x10 ⁻⁸⁷	Extracellular	70,620 4.9
SCLAV_4462 (<i>leuB</i>)	3-isopropylmalate dehydrogenase	B5H0M9	-0.585	153.65	230.52	5.40x10 ⁻⁴²	6.57x10 ⁻⁴³	Cytoplasm	36,772 5.01
SCLAV_4463	Branched-chain-amino-acid aminotransferase	B5H0M8	-0.393	110.44	145	2.66x10 ⁻¹⁴	6.04x10 ⁻¹⁵	Cell-Membrane	39,453 5.13
SCLAV_4468	Short-chain dehydrogenase/reductase SDR	E2Q807	1.862	191.07	52.58	2.40x10 ⁻¹⁸⁶	9.50x10 ⁻¹⁸⁸	Cell-Membrane	32,656 9.49
SCLAV_4472	Amidase	E2Q811	1.538	201.51	69.37	6.20x10 ⁻²³⁷	1.90x10 ⁻²³⁸	Extracellular	50,210 5.28
NRPS									
SCLAV_4757	DUF2236 domain-containing protein	B5GZG2	1.254	29.95	12.56	1.47x10 ⁻²²	2.54x10 ⁻²³	Extracellular	44,959 4.97
Secondary Metabolites Biosynthesis, Transport and Catabolism									
SCLAV_0832	Short chain dehydrogenase	E2PW76	-0.148	43.63	48.33	1.61x10 ⁻⁰¹	1.14x10 ⁻⁰¹	Cytoplasm	27,109 4.84
SCLAV_1673	Putative ferredoxin reductase	E2Q2Z5	-0.363	220.9	284.04	3.11x10 ⁻²⁷	4.85x10 ⁻²⁸	Cytoplasm	44,690 5.17
SCLAV_1760	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	B5H007	0.074	236.66	224.83	6.83x10 ⁻⁰²	4.38x10 ⁻⁰²	Cytoplasm	53,701 5.24
SCLAV_1944	Acetyl/propionyl CoA carboxylase alpha subunit	B5H1P3	0.964	28.8	14.76	3.15x10 ⁻²³	5.35x10 ⁻²⁴	Cell-Membrane, Extracellular	67,249 5.44
SCLAV_1946	Acyl-CoA dehydrogenase	E2Q4I1	0.416	38.73	29.03	7.31x10 ⁻⁰⁵	2.97x10 ⁻⁰⁵	Cytoplasm	41,851 5.35

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_1948</i> (<i>sidD</i>)	Pyridoxal-dependent decarboxylase	B5HIN9	-1.488	30.04	84.26	2.71x10 ⁻⁹⁸	1.80x10 ⁻⁹⁹	0.829	Cell-Wall	52,172	5.12
<i>SCLAV_1949</i>	L-lysine N6-monooxygenase	B5HIN8	-1.036	32.5	66.64	1.75x10 ⁻³⁹	2.22x10 ⁻⁴⁰	0.056	Cytoplasm	47,833	5.08
<i>SCLAV_0152</i>	Lysine N-acyltransferase MbtK	E2Q668	1.461	15.66	5.69	1.48x10 ⁻⁰⁷	4.78x10 ⁻⁰⁸	0.415	Extracellular	21,434	5.37
<i>SCLAV_0166</i>	Alpha/beta hydrolase	E2Q6Z5	0.031	71.62	70.09	7.83x10 ⁻⁰¹	7.33x10 ⁻⁰¹	1.374	Extracellular	46,813	5.36
Energy Production and Conversion											
<i>SCLAV_0466</i>	Oxidoreductase	E2Q9C2	0.015	288.06	285.17	8.20x10 ⁻⁰¹	7.75x10 ⁻⁰¹	-2.407	Cytoplasm	81,784	8.41
<i>SCLAV_0905</i>	Oxidoreductase	B5H000	-0.100	97.78	104.77	8.55x10 ⁻⁰²	5.62x10 ⁻⁰²	-1.030	Cell-Membrane, Cytoplasm	37,801	4.95
<i>SCLAV_1230</i> (<i>gltD</i>)	Glutamate synthase subunit beta	E2PZJ9	0.401	409.84	310.49	1.03x10 ⁻⁴⁸	1.12x10 ⁻⁴⁹	-0.976	Cytoplasm	52,473	5.40
<i>SCLAV_1399</i>	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex	E2Q0T5	-0.494	317.33	447.06	5.4x10 ⁻¹⁰²	3.4x10 ⁻¹⁰³	0.682	Cytoplasm	58,965	4.64
<i>SCLAV_1401</i>	Pyruvate dehydrogenase E1 component	E2Q0T7	-0.247	256.1	303.82	3.26x10 ⁻³⁰	4.77x10 ⁻³¹	-2.867	Cytoplasm	103,394	5.77
<i>SCLAV_1807</i> (<i>sodF</i>)	Superoxide dismutase	B5H2Y5	-0.313	1108.23	1376.93	1.86x10 ⁻⁴⁹	2.01x10 ⁻⁵⁰	0.514	Cytoplasm	23,505	5.15
<i>SCLAV_1907</i> (<i>gltA</i>)	Citrate synthase	E2Q4E2	-0.459	799.99	1099.53	1.20x10 ⁻¹⁵⁸	5.60x10 ⁻¹⁶⁰	-0.284	Cell-Membrane	50,012	6.03
<i>SCLAV_2067</i>	Malate oxidoreductase	E2Q599	-0.276	147.88	179.09	2.17x10 ⁻¹²	5.39x10 ⁻¹³	-1.952	Cytoplasm	48,951	5.03
<i>SCLAV_2248</i> (<i>ppc</i>)	Phosphoenolpyruvate carboxylase	B5GPS1	-0.200	135.25	155.39	2.47x10 ⁻¹¹	6.32x10 ⁻¹²	-1.656	Cell-Membrane, Cell-Wall, Extracellular	100,623	5.88
<i>SCLAV_2425</i>	Aldehyde dehydrogenase	B5GPD0	-0.077	433.75	457.53	6.28x10 ⁻⁰⁴	2.86x10 ⁻⁰⁴	-1.531	Cytoplasm	52,052	5.07
<i>SCLAV_2973</i> (<i>padZ</i>)	Dehydrogenase	B5GZV5	-0.243	566.13	669.85	4.87x10 ⁻⁴⁰	6.09x10 ⁻⁴¹	2.380	Cytoplasm	59,495	5.11
<i>SCLAV_2898</i> (<i>trxB</i>)	Thioredoxin reductase	B5GX94	-0.249	1071.77	1273.97	3.18x10 ⁻⁴⁶	3.59x10 ⁻⁴⁷	-0.234	Cytoplasm	34,146	4.98

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_2979</i> (<i>bkdC</i>)	Dihydroliipoamide component of complex	acetyltransferase pyruvate dehydrogenase	E2PWQ1	0.310	167.07	134.75	1.68x10 ⁻¹³	3.94x10 ⁻¹⁴	1.997	Cytoplasm	51,335	5.65
<i>SCLAV_2998</i> (<i>bkdB2</i>)	Branched-chain dehydrogenase E1 beta subunit	alpha keto acid	B5GU66	-0.845	191.71	344.45	2.10x10 ⁻¹⁰⁹	1.20x10 ⁻¹¹⁰	-0.356	Cytoplasm	35,599	5.20
<i>SCLAV_2999</i>	Dihydroliipoamide component of complex	acetyltransferase pyruvate dehydrogenase	E2PWS1	-0.599	157.57	238.64	9.97x10 ⁻⁶⁶	8.60x10 ⁻⁶⁷	-0.067	Cytoplasm	51,496	5.85
<i>SCLAV_3274</i>	Aldehyde dehydrogenase		E2PZ14	-0.335	167.58	211.36	2.66x10 ⁻²⁰	4.91x10 ⁻²¹	-0.599	Cytoplasm	50,853	4.97
<i>SCLAV_3535</i>	Monooxygenase FAD-binding protein		E2Q1A2	-1.437	43.83	118.68	8.00x10 ⁻¹³³	4.20x10 ⁻¹³⁴	3.143	Cytoplasm	52,028	8.07
<i>SCLAV_3570</i> (<i>nuoG1</i>)	NADH-quinone oxidoreductase		B5GWF3	-0.893	215.47	400.22	0	0	-0.162	Cytoplasm	87,947	5.78
<i>SCLAV_3696</i>	MOSC domain-containing protein		B5GYU8	0.330	130.33	103.69	2.31x1 ⁻⁰⁵	8.87x10 ⁻⁰⁶	-0.011	Extracellular	24,162	6.09
<i>SCLAV_3730</i>	Succinate--CoA subunit beta	ligase [ADP-forming]	E2Q387	-0.555	417.98	614.09	1.50x10 ⁻¹¹³	8.50x10 ⁻¹¹⁵	0.739	-	40,845	4.74
<i>SCLAV_3731</i> (<i>sucD</i>)	Succinate--CoA subunit alpha	ligase [ADP-forming]	E2Q388	-0.572	480.67	714.61	4.00x10 ⁻¹⁰⁴	2.50x10 ⁻¹⁰⁵	0.291	Cell- Membrane, E xtracellular	29,860	5.30
<i>SCLAV_3742</i> (<i>mdh</i>)	Malate dehydrogenase		B5H2X3	-0.375	383.09	496.94	6.01x10 ⁻³⁹	7.66x10 ⁻⁴⁰	0.461	Cytoplasm	34,586	5.10
<i>SCLAV_3770</i>	Succinate dehydrogenase subunit	flavoprotein	B5H3R8	-1.031	183.29	374.57	1.20x10 ⁻²⁹⁹	2.80x10 ⁻³⁰¹	0.058	Cell- Membrane	64,752	5.33
<i>SCLAV_3929</i>	Oxidoreductase		E2Q465	-0.073	66.13	69.54	3.18x10 ⁻⁰¹	2.48x10 ⁻⁰¹	-1.234	Cytoplasm	35,967	5.36
<i>SCLAV_3994</i>	Ferredoxin		B5GQ20	-0.402	2173.65	2872.09	8.21x10 ⁻⁷⁸	6.26x10 ⁻⁷⁹	0.891	Cell- Membrane	11,736	4.20
<i>SCLAV_4071</i>	Catalase		E2Q4Y6	-0.302	1421.87	1752.92	2.90x10 ⁻¹³⁴	1.50x10 ⁻¹³⁵	0.529	Cytoplasm	54,223	5.53
<i>SCLAV_4123</i>	Dehydrogenase		E2Q5I4	-0.486	110.56	154.81	2.63x10 ⁻¹⁹	4.98x10 ⁻²⁰	-1.765	Cell- Membrane, C ytoplasm	33,830	6.32
<i>SCLAV_4122</i>	Putative malate dehydrogenase		E2Q5I3	-0.032	110.21	112.67	4.94x10 ⁻⁰¹	4.17x10 ⁻⁰¹	0.656	Cytoplasm	42,394	4.97
<i>SCLAV_4267</i> (<i>atpF</i>)	ATP synthase subunit b		B5GL56	-0.681	1114.06	1785.72	6.40x10 ⁻²¹¹	2.10x10 ⁻²¹²	0.041	Cell- Membrane	19,960	5.02

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_4268 (<i>atpH</i>)	ATP synthase subunit delta	B5GL55	-0.621	469.72	722.21	1.80×10^{-111}	1.10×10^{-112}	-0.273	Cell- Membrane	28,602	5.52
SCLAV_4270 (<i>atpG</i>)	ATP synthase gamma chain	B5GL53	-0.642	1120.2	1747.8	0	0	0.328	Cell- Membrane	32,810	8.60
SCLAV_4272	ATP synthase epsilon chain	E2Q6P7	-0.566	352.5	521.98	8.81×10^{-32}	1.26×10^{-32}	0.417	Cell- Membrane	13,303	5.02
SCLAV_4445 (<i>rocA</i>)	L-glutamate gamma-semialdehyde dehydrogenase	B5H0P5	-0.085	82.48	87.49	1.01×10^{-01}	6.75×10^{-02}	0.604	Cytoplasm	58,696	5.16
SCLAV_4899	Assimilatory sulfite reductase	B5H1L3	-0.142	1278.1	1410.75	4.69×10^{-32}	6.68×10^{-33}	-1.117	Cytoplasm	62,969	5.77
SCLAV_5208	Fatty acid-CoA racemase	E2PYA4	1.245	373.36	157.56	3.80×10^{-259}	1.00×10^{-260}	-0.382	Extracellular	40,563	5.66
SCLAV_5569	Electron transfer flavoprotein	B5H3W2	-0.657	58.45	92.14	1.33×10^{-15}	2.86×10^{-16}	-0.058	Cell- Membrane	27,036	4.52
Amino Acid Transport and Metabolism											
SCLAV_0727 (<i>aroC</i>)	Chorismate synthase	E2PV56	-0.574	81.15	120.84	8.45×10^{-25}	1.38×10^{-25}	-1.459	Cytoplasm	41,660	5.98
SCLAV_0902	Putative M20/M25/M40-family peptidase	E2PWE6	-0.332	78.38	98.65	3.66×10^{-09}	1.06×10^{-09}	-0.639	Cell- Membrane	47,812	4.92
SCLAV_1115 (<i>dapD</i>)	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase (Tetrahydropicolinate succinylase)	B5GYL9	-0.297	126.66	155.62	7.63×10^{-09}	2.26×10^{-09}	-0.690	Cytoplasm	33,876	5.18
SCLAV_1118	Cysteine desulfurase	B5GYL6	-0.387	412.07	538.96	1.96×10^{-56}	1.92×10^{-57}	-0.989	Cytoplasm	45,601	5.29
SCLAV_1197	Branched chain amino acid binding protein	B5GXJ7	-0.241	696.5	823.14	5.51×10^{-36}	7.36×10^{-37}	-0.438	Cytoplasm	43,189	7.80
SCLAV_1207	Alanine aminopeptidase	E2PZH6	-0.126	74.38	81.17	3.75×10^{-03}	1.90×10^{-03}	-1.290	Cytoplasm	92,965	4.87
SCLAV_1243	Tryptophan synthase alpha chain	E2PZL3	-0.639	139.27	216.87	9.22×10^{-37}	1.21×10^{-37}	0.289	Cytoplasm	28,064	5.08
SCLAV_1257 (<i>hisA</i>)	Phosphoribosyl isomerase A	B5GXT2	-0.479	71.99	100.31	1.10×10^{-09}	3.09×10^{-10}	0.536	Cytoplasm	25,221	4.67
SCLAV_1331 (<i>aroF</i>)	Phospho-2-dehydro-3-deoxyheptonate aldolase	B5HIQ6	-0.520	308.81	442.84	1.85×10^{-84}	1.31×10^{-85}	-0.228	Cytoplasm	49,593	5.17
SCLAV_1397	Probable cytosol aminopeptidase	E2Q0T3	-0.173	115.02	129.65	9.77×10^{-05}	4.03×10^{-05}	0.211	Cytoplasm	51,763	5.48
SCLAV_1434	Integral membrane protein	E2Q0X0	-0.822	146.44	258.92	7.50×10^{-119}	4.20×10^{-120}	-0.703	Extracellular	53,605	7.92

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_1707</i> (<i>leuA</i>)	2-isopropylmalate synthase	E2Q329	-0.444	446.18	607.16	1.20x10 ⁻¹¹⁶	7.00x10 ⁻¹¹⁸	0.329	Cell- Membrane, Cell-Wall, Extracellular	66,309	4.98
<i>SCLAV_2046</i> (<i>hpd</i>)	4-hydroxyphenylpyruvate dioxygenase	B5GSL5	0.355	531.96	416.02	1.07x10 ⁻³⁸	1.37x10 ⁻³⁹	1.374	Cytoplasm	41,873	5.11
<i>SCLAV_2088</i>	Glutamate dehydrogenase	E2Q5C0	-0.867	94.38	172.14	3.50x10 ⁻²⁹⁶	8.50x10 ⁻²⁹⁸	-0.512	Cytoplasm	183,296	5.41
<i>SCLAV_2199</i>	Beta-thionase	B5GZQ3	0.353	66.58	52.14	8.74x10 ⁻⁴⁷	2.97x10 ⁻⁴⁷	-0.714	Cell- Membrane	48,918	4.91
<i>SCLAV_2609</i>	Putative peptide hydrolase	E2Q9V4	-0.243	50.43	59.67	6.80x10 ⁻⁴⁵	2.75x10 ⁻⁴⁵	-1.303	Cytoplasm	65,285	4.80
<i>SCLAV_2614</i>	Aspartokinase	B5H3B4	-0.539	528.98	768.76	4.40x10 ⁻¹⁴⁶	2.10x10 ⁻¹⁴⁷	-0.130	Cell- Membrane	44,706	4.95
<i>SCLAV_2697</i> (<i>arcB</i>)	Ornithine cyclodeaminase	B5GTG5	-0.354	9.39	12	6.97x10 ⁻⁴²	4.48x10 ⁻⁴²	0.744	Cytoplasm	37,070	5.36
<i>SCLAV_3250</i>	Threonine synthase	E2PYZ0	-1.437	168.11	455.19	0	0	-0.757	Cytoplasm	47,294	4.98
<i>SCLAV_3352</i> (<i>serC</i>)	Phosphoserine aminotransferase	E2Q004	-0.352	265.11	338.43	4.26x10 ⁻²⁷	6.65x10 ⁻²⁸	-0.596	Cytoplasm	39,457	5.09
<i>SCLAV_3851</i> (<i>metB</i>)	Cystathionine gamma-synthase	E2Q3Y5	-0.012	235.14	237.08	6.14x10 ⁻⁴¹	5.44x10 ⁻⁴¹	0.171	Cytoplasm	43,356	6.28
<i>SCLAV_3976</i> (<i>bldKB</i>)	ABC transport system lipoprotein	B5GTX3	-0.033	656.25	671.4	3.25x10 ⁻⁴²	1.96x10 ⁻⁴²	-0.120	Extracellular	65,261	6.76
<i>SCLAV_4077</i> (<i>araA</i>)	3-phosphoshikimate carboxyvinyltransferase	1- B5GQA8	0.279	68.54	56.5	1.29x10 ⁻⁴⁴	5.39x10 ⁻⁴⁵	-0.834	Cytoplasm	46,003	5.25
<i>SCLAV_4401</i> (<i>gcvT</i>)	Aminomethyltransferase	B5GVF5	-0.063	50.87	53.15	4.29x10 ⁻⁴¹	3.52x10 ⁻⁴¹	-0.579	Cytoplasm	39,287	5.00
<i>SCLAV_4406</i>	Oligopeptide-binding lipoprotein	B5GVG2	-0.070	401.14	421.02	1.05x10 ⁻⁴³	4.90x10 ⁻⁴⁴	-0.993	Cell- Membrane	63,840	8.50
<i>SCLAV_4438</i> (<i>ilvB1</i>)	Acetolactate synthase	E2Q7X7	-0.780	32.9	56.48	4.64x10 ⁻³¹	6.71x10 ⁻³²	-0.200	Cell- Membrane, C ell-Wall	65,779	5.65
<i>SCLAV_4439</i> (<i>ilvH</i>)	Acetohydroxy-acid synthase small subunit	B5H0Q1	-0.448	61.77	84.29	1.04x10 ⁻⁴⁵	3.88x10 ⁻⁴⁶	-0.012	Cytoplasm	19,080	8.05
<i>SCLAV_4662</i> (<i>gluB</i>)	Glutamate binding periplasmic protein	B5GYA8	-0.225	614.78	718.78	2.53x10 ⁻¹⁹	4.80x10 ⁻²⁰	-0.434	Cell- Membrane	30,016	5.88

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_5076</i>	Acyl-peptide hydrolase	E2PX72	0.037	78.83	76.86	6.10x10 ⁻⁰¹	5.39x10 ⁻⁰¹	0.156	Cell-Membrane	73,147	5.25
<i>SCLAV_4585 (gabT)</i>	4-aminobutyrate aminotransferase	E2Q943	-0.956	12.66	24.56	3.97x10 ⁻¹⁴	9.12x10 ⁻¹⁵	0.551	Cytoplasm	46,313	5.46
<i>SCLAV_5559</i>	O-acetylhomoserine aminocarboxypropyltransferase	E2Q1K0	-0.441	871.68	1183.14	2.50x10 ⁻¹⁶⁷	1.10x10 ⁻¹⁶⁸	-0.205	Cytoplasm	53,426	5.81
<i>proA</i>	Gamma-glutamyl phosphate reductase	B5H776	-0.401	69.27	91.45	2.27x10 ⁻¹¹	5.81x10 ⁻¹²	-0.616	Cytoplasm	45,269	5.05
Carbohydrate Transport and Metabolism											
<i>SCLAV_0620</i>	Mannose-1-phosphate guanyltansferase	B5GYY7	-0.610	456	696.07	0	0	-2.326	Cell-Membrane	89,672	5.02
<i>SCLAV_0890</i>	Phosphatase	E2PWD4	-0.342	67.52	85.59	5.90x10 ⁻⁰⁵	2.37x10 ⁻⁰⁵	-0.083	Cell-Membrane, Extracellular	24,440	5.96
<i>SCLAV_1133</i>	Transketolase	B5GYK3	-1.362	120.91	310.82	0	0	-2.590	Cytoplasm	75,570	5.48
<i>SCLAV_1134</i>	Transaldolase	E2PYJ0	-0.930	135.6	258.37	2.40x10 ⁻¹¹⁰	1.40x10 ⁻¹¹¹	-1.488	Cytoplasm	40,664	4.85
<i>SCLAV_1136 (opcA)</i>	Oxppcycle protein	B5GYK0	-0.915	58.92	111.11	4.81x10 ⁻⁴⁵	5.55x10 ⁻⁴⁶	-1.347	Extracellular	37,739	5.39
<i>SCLAV_1161</i>	Zn-dependent hydrolase	B5GXN1	-0.210	117.91	136.43	1.70x10 ⁻⁰³	8.14x10 ⁻⁰⁴	0.171	Extracellular	23,256	5.41
<i>SCLAV_1444</i>	Alpha-amylase	B5GXE1	-0.803	322.57	562.79	0	0	0.413	Extracellular	192,665	6.34
<i>SCLAV_1447</i>	Carbohydrate ABC transporter substrate-binding protein, CUT1 family	B5GXE3	-0.682	970.63	1557.48	0	0	-0.947	Extracellular	45,619	5.68
<i>SCLAV_1830</i>	Putative sugar hydrolase	B5H3Q2	0.068	26.9	25.67	6.52x10 ⁻⁰¹	5.85x10 ⁻⁰¹	-1.065	Extracellular	44,795	5.21
<i>SCLAV_3252 (otsA)</i>	Putative trehalose-6-phosphate synthase	B5GNY9	-0.542	606.35	882.56	5.40x10 ⁻¹⁸⁷	2.10x10 ⁻¹⁸⁸	-1.016	Cytoplasm	51,384	5.56
<i>SCLAV_3671 (glmM)</i>	Phosphoglucosamine mutase	B5GX14	-0.396	81.42	107.13	1.72x10 ⁻¹³	4.05x10 ⁻¹⁴	-0.320	Cell-Membrane, Cell-Wall, Extracellular	46,621	5.03
<i>SCLAV_4838</i>	Solute-binding protein	E2PVS1	-0.051	151.98	157.45	2.43x10 ⁻⁰¹	1.81x10 ⁻⁰¹	0.440	Extracellular	38,846	5.39
Lipid Transport and Metabolism											

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_0815</i>	3-hydroxybutyryl-CoA dehydrogenase	E2PW59	-0.487	206.1	288.88	1.48×10^{-31}	2.12×10^{-32}	-0.153	Cell-Membrane	31,151	4.9
<i>SCLAV_1027 (fabI)</i>	Enoyl-[acyl-carrier-protein] reductase [NADH]	B5H0X6	-0.209	137.77	159.26	2.30×10^{-04}	9.87×10^{-05}	-1.641	Cytoplasm	27,606	5.47
<i>SCLAV_1733</i>	Acyl-CoA synthetase	B5H010	-0.645	222.65	348.11	6.10×10^{-136}	3.10×10^{-137}	-2.279	Cell-Membrane, Extracellular	67,652	5.58
<i>SCLAV_2155</i>	Acyl-CoA dehydrogenase	E2Q6A4	-0.127	299.21	326.81	3.69×10^{-05}	1.45×10^{-05}	0.649	Cytoplasm	42,077	5.58
<i>SCLAV_2890</i>	1L-myo-inositol-1-phosphate synthase	B5GXA2	0.356	436.35	340.84	2.42×10^{-30}	3.53×10^{-31}	0.071	Cell-Wall	39,508	5.05
<i>SCLAV_3008</i>	Acyl-CoA dehydrogenase	B5GU77	0.010	2969.11	2947.83	7.89×10^{-01}	7.39×10^{-01}	-1.592	Cytoplasm	66,495	4.9
<i>SCLAV_3830</i>	JadJ-like biotin carboxylase	B5GSX0	-0.216	102.7	119.29	6.16×10^{-07}	2.07×10^{-07}	-1.292	Cell-Membrane, Extracellular	61,671	5.15
<i>SCLAV_4309</i>	Acetyl-CoA acetyltransferase	B5GL15	-0.227	191.81	224.55	1.97×10^{-09}	5.60×10^{-10}	-0.952	Cytoplasm	41,208	5.14
<i>SCLAV_4367</i>	Putative enoyl-CoA hydratase	E2Q7Q8	0.389	72.48	55.34	4.98×10^{-05}	1.98×10^{-05}	-0.404	Cytoplasm	27,326	5.38
<i>SCLAV_5596</i>	DUF385 domain-containing protein	E2Q1N7	-0.624	151.96	234.23	3.07×10^{-20}	5.68×10^{-21}	0.731	Cell-Membrane, Cell-Wall	16,318	6.09
Coenzyme Transport and Metabolism											
<i>SCLAV_0705 (metK)</i>	S-adenosylmethionine synthase	B5GX10	-0.304	361.28	445.92	1.49×10^{-29}	2.21×10^{-30}	0.004	Cytoplasm	43,523	4.92
<i>SCLAV_1064</i>	Mg-protoporphyrin IX chelatase	E2PXM0	-0.415	27.96	37.28	8.71×10^{-09}	2.60×10^{-09}	1.698	Cell-Membrane, Cell-Wall	76,916	5.84
<i>SCLAV_2107</i>	Adenosylhomocysteinase	E2Q5D9	-0.061	349.52	364.52	1.53×10^{-02}	8.60×10^{-03}	0.211	Cytoplasm	55,742	5.07
<i>SCLAV_2410 (folK)</i>	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokinase	B5GPB5	-0.472	179.13	248.39	9.26×10^{-18}	1.85×10^{-18}	-0.342	Cell-Membrane	21,265	4.58
<i>SCLAV_2771</i>	Putative methyltransferase	E2PUR4	-0.561	1067.23	1574.67	3.30×10^{-245}	9.40×10^{-247}	0.017	Cytoplasm	34,385	4.55
<i>SCLAV_2863</i>	Phosphomethylpyrimidine synthase	B5GXD1	0.207	2386.34	2066.94	1.70×10^{-95}	1.12×10^{-96}	1.862	Cell-Membrane, Cell-Wall	65,821	5.31

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_3512 (hemBI)</i>	Delta-aminolevulinic acid dehydratase	B5GNL9	-0.408	362	480.26	1.37×10^{-43}	1.61×10^{-44}	-1.632	Cytoplasm	35,281	4.83
<i>SCLAV_4041</i>	UBA/THIF-type protein	B5GQ72	-0.913	15.6	29.37	4.00×10^{-35}	5.42×10^{-36}	0.283	Cell-Membrane, Cell-Wall	42,611	4.72
<i>SCLAV_4635 (rni)</i>	Ribonuclease J	E2QA08	-0.288	157.95	192.86	1.44×10^{-16}	2.98×10^{-17}	-1.008	Cytoplasm	63,024	6.2
Inorganic Ion Transport and Metabolism											
<i>SCLAV_0132</i>	Putative iron-siderophore ABC transporter substrate-binding protein	E2Q648	-0.245	173.3	205.38	9.55×10^{-09}	2.85×10^{-09}	-0.758	Cell-Membrane	37,660	5.53
<i>SCLAV_0390</i>	Extracellular binding protein	B5GTZ2	-0.703	61.97	100.91	1.97×10^{-24}	3.24×10^{-25}	0.694	Extracellular	34,724	4.95
<i>SCLAV_0843</i>	Siderophore-interacting protein	B5H160	-0.524	134.03	192.75	4.50×10^{-24}	7.48×10^{-25}	-0.110	Cell-Membrane	31,519	5.7
<i>SCLAV_1010</i>	ABC transporter ATP-binding protein	B5H0W0	-0.175	132	149.01	2.41×10^{-03}	1.18×10^{-03}	-0.364	Extracellular	28,983	5.37
<i>SCLAV_1495</i>	Heme oxygenase	E2Q1U4	-0.030	362.62	370.2	3.11×10^{-01}	2.42×10^{-01}	-0.468	Cytoplasm	25,305	5.46
<i>SCLAV_3193</i>	Sulfurtransferase	B5GP52	-0.353	4911.68	6274.74	0	0	0.993	Cytoplasm	31,672	4.68
<i>SCLAV_3978 (BldKD)</i>	ABC transporter intracellular subunit	B5GTX5	-0.089	115.32	122.67	9.57×10^{-02}	6.39×10^{-02}	-0.344	Cell-Membrane	37,838	5.56
<i>SCLAV_4408</i>	Oligopeptide ABC transporter binding protein	B5GVG4	-0.305	80.29	99.2	5.16×10^{-07}	1.73×10^{-07}	0.573	Cell-Membrane	41,031	5.87
Nucleotide Transport and Metabolism											
<i>SCLAV_0131</i>	DUF574 domain-containing protein	B5H0U3	-0.068	59.92	62.83	4.21×10^{-01}	3.45×10^{-01}	0.814	Cell-Membrane	30,332	4.82
<i>SCLAV_0712 (carB)</i>	Carbamoyl-phosphate synthase large chain	B5GX03	-0.354	69.63	89.02	9.68×10^{-22}	1.70×10^{-22}	-1.452	Cell-Membrane, Cell-Wall, Extracellular	118,416	4.90
<i>SCLAV_0713 (carA)</i>	Carbamoyl-phosphate chain	B5GX02	-0.324	60.88	76.23	2.93×10^{-06}	1.03×10^{-06}	0.047	Cell-Membrane, Cell-Wall, Extracellular	41,162	6.01
<i>SCLAV_1876</i>	Phosphoribosylaminoimidazolesuccinocarboxamide synthase	B5GV74	0.902	28.86	15.44	7.54×10^{-08}	2.39×10^{-08}	2.603	Cell-Membrane	26,666	4.80

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_2244</i> (<i>kprS</i>)	Ribose-phosphate pyrophosphokinase	B5GPR7	-0.707	317.46	518.19	1.60x10 ⁻¹²⁰	9.10x10 ⁻¹²²	-0.645	Cytoplasm	35,292	5.62
<i>SCLAV_3080</i>	Nucleoside diphosphate kinase	B5GT76	-0.312	249.22	309.48	9.32x10 ⁻¹⁰	2.61x10 ⁻¹⁰	-0.546	Cytoplasm	18,716	5.24
<i>SCLAV_3089</i> (<i>purD</i>)	Phosphoribosylamine--glycine ligase	B5GT67	-0.297	58.86	72.29	1.27x10 ⁻⁰⁵	4.77x10 ⁻⁰⁶	-1.906	Cell-Membrane, Cell-Wall, Extracellular	43,096	5.01
<i>SCLAV_3092</i> (<i>purC</i>)	Phosphoribosylaminoimidazole-succinocarboxamide synthase	E2PXS3	-0.418	125.8	168.08	3.02x10 ⁻¹⁵	6.61x10 ⁻¹⁶	-0.428	Cell-Membrane, Cell-Wall, Extracellular	30,674	4.70
<i>SCLAV_3099</i> (<i>PurQ</i>)	Phosphoribosylformylglycinamide synthase subunit	B5GT55	-0.906	177.21	332.04	2.09x10 ⁻⁸⁵	1.47x10 ⁻⁸⁶	0.373	Cytoplasm	25,815	5.52
<i>SCLAV_3100</i> (<i>purL</i>)	Phosphoribosylformylglycinamide synthase subunit	E2PXT1	-0.784	130.32	224.34	1.20x10 ⁻¹⁴⁶	5.40x10 ⁻¹⁴⁸	-0.297	Cytoplasm	79,579	4.60
<i>SCLAV_3106</i> (<i>purM</i>)	Phosphoribosylformylglycinamide cyclo-ligase	B5GT48	-0.974	81.42	159.98	4.08x10 ⁻⁷³	3.24x10 ⁻⁷⁴	-0.034	Cytoplasm	38,183	4.75
<i>SCLAV_3446</i>	D-alanine--D-alanine ligase	E2Q112	0.406	46.28	34.93	1.90x10 ⁻⁰⁴	8.07x10 ⁻⁰⁵	0.997	Cytoplasm	33,372	4.76
<i>SCLAV_3658</i>	Adenylate kinase	B5GX28	-0.334	684.46	862.51	1.70x10 ⁻³⁵	2.29x10 ⁻³⁶	0.698	Cytoplasm	23,270	4.87
<i>SCLAV_3701</i> (<i>guaB</i>)	Inosine-5'-monophosphate dehydrogenase	B5GYV3	-0.589	333.91	502.13	2.90x10 ⁻¹³²	1.60x10 ⁻¹³³	-0.242	Cytoplasm	52,779	5.99
<i>SCLAV_3712</i> (<i>guaA</i>)	GMP synthase [glutamine-hydrolyzing]	E2Q369	-0.422	207.75	278.38	1.91x10 ⁻⁴³	2.27x10 ⁻⁴⁴	0.911	Cell-Membrane, Cell-Wall, Extracellular	59,213	5.00
<i>SCLAV_3736</i> (<i>purH</i>)	Bifunctional purine biosynthesis protein	E2Q393	-0.548	53.77	78.6	1.98x10 ⁻²⁰	3.61x10 ⁻²¹	-0.212	Cytoplasm	55,796	5.34
<i>SCLAV_3739</i> (<i>folD</i>)	Bifunctional protein	E2Q396	-0.351	362.57	462.52	2.64x10 ⁻²⁸	4.00x10 ⁻²⁹	0.070	Cytoplasm	30,143	6.26
Translation, Ribosomal Structure and Biogenesis											
<i>SCLAV_0701</i>	Methionyl-tRNA formyltransferase	E2PV30	-0.355	42.26	54.04	1.09x10 ⁻⁰⁴	4.50x10 ⁻⁰⁵	-0.930	Cytoplasm	33,258	5.84

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_0709	Guanylate kinase	B5GX06	0.181	1097.05	967.44	7.85×10^{-05}	3.20×10^{-05}	0.697	Cytoplasm	11,505	10.4
SCLAV_0731 (<i>alsS</i>)	Alanine--tRNA ligase	E2PV60	0.097	193.95	181.37	1.46×10^{-03}	6.91×10^{-04}	-2.362	Cytoplasm	95,307	5.20
SCLAV_0734 (<i>rpsD</i>)	30S ribosomal protein S4	B5GWY 5	-0.092	582.54	621.04	1.37×10^{-03}	6.49×10^{-04}	1.251	Cytoplasm	23,533	10.2
SCLAV_0819 (<i>syfB</i>)	Phenylalanine--tRNA ligase beta subunit	E2PW63	0.078	101.55	96.18	8.67×10^{-02}	5.71×10^{-02}	-0.112	Cytoplasm	89,571	5.16
SCLAV_0820 (<i>syfA</i>)	Phenylalanine--tRNA ligase alpha subunit	E2PW64	-0.027	166.55	169.66	4.75×10^{-01}	3.98×10^{-01}	-0.478	Cytoplasm	40,806	4.91
SCLAV_0823 (<i>rplT</i>)	50S ribosomal protein L20	E2PW67	-0.368	530.11	684.23	2.26×10^{-20}	4.13×10^{-21}	2.394	Cytoplasm	14,442	10.8
SCLAV_0825 (<i>infC</i>)	Translation initiation factor IF-3	E2PW69	-0.326	805.85	1009.95	1.36×10^{-42}	1.64×10^{-43}	0.483	Cytoplasm	25,617	9.41
SCLAV_1188 (<i>rpsA</i>)	30S ribosomal protein S1	B5G XK8	-0.104	4586.71	4931.02	2.92×10^{-55}	2.92×10^{-56}	1.366	Cell-Wall, Cytoplasm	54,731	4.53
SCLAV_1764 (<i>rpmA</i>)	50S ribosomal protein L27	B5H010	-0.271	529.07	638.31	3.52×10^{-08}	1.10×10^{-08}	1.647	Cytoplasm	9,003	11.6
SCLAV_1765 (<i>rplU</i>)	50S ribosomal protein L21	B5H011	-0.163	825.78	924.24	1.44×10^{-06}	4.97×10^{-07}	2.953	Cytoplasm	11,522	9.64
SCLAV_1769	Ribonuclease, Rne/Rng family	E2Q3M3	-0.089	86.71	92.24	4.66×10^{-03}	2.40×10^{-03}	1.065	Cytoplasm	154,870	6.15
SCLAV_2103	Methylthioribose-1-phosphate isomerase	E2Q5D5	-0.228	41.84	49	8.42×10^{-03}	4.53×10^{-03}	-0.236	Extracellular	39,637	5.21
SCLAV_2879 (<i>rplI</i>)	50S ribosomal protein L9	B5GXB4	-0.234	1179.55	1387.32	1.21×10^{-21}	2.13×10^{-22}	0.496	Cytoplasm	15,747	9.79
SCLAV_2880 (<i>rpsR</i>)	30S ribosomal protein S18	B5GXB3	-0.407	953.56	1264.07	3.19×10^{-26}	5.09×10^{-27}	0.873	Cytoplasm	8,990	10.3
SCLAV_3013 (<i>aspS1</i>)	Aspartate--tRNA ligase	B5GU84	-0.520	166.24	238.36	1.92×10^{-60}	1.79×10^{-61}	-1.524	Cytoplasm	66,046	5.27
SCLAV_4604	Ribosome-binding factor A	E2Q962	0.390	439.83	335.65	2.01×10^{-13}	4.75×10^{-14}	1.448	Cytoplasm	15,680	4.88
SCLAV_3626 (<i>rplA</i>)	50S ribosomal protein L1	B5GXB4	-0.202	848.91	976.58	7.45×10^{-19}	1.43×10^{-19}	0.331	Cytoplasm	25,779	9.63
SCLAV_3627 (<i>rplJ</i>)	50S ribosomal protein L10	B5GXB3	-0.459	1484.74	2041.33	1.10×10^{-18}	6.00×10^{-20}	0.947	Cytoplasm	18,622	9.12

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_3632</i> (<i>rpsG</i>)	30S ribosomal protein S7	B5GX57	-0.099	781.96	837.67	3.68x10 ⁻⁰⁴	1.63x10 ⁻⁰⁴	-0.460	Cytoplasm	17,408	10.4
<i>SCLAV_3633</i> (<i>fusA2</i>)	Elongation factor G	B5GX56	0.068	1267.46	1209.09	5.19x10 ⁻⁰⁷	1.74x10 ⁻⁰⁷	0.433	Cytoplasm	77,548	4.99
<i>SCLAV_3637</i> (<i>rplC</i>)	50S ribosomal protein L3	B5GX49	-0.111	1637.6	1768.92	2.84x10 ⁻¹¹	7.28x10 ⁻¹²	0.537	Cytoplasm	22,701	10.1 7
<i>SCLAV_3638</i> (<i>rplD</i>)	50S ribosomal protein L4	E2Q2C2	0.092	909.69	853.26	6.50x10 ⁻⁰³	3.43x10 ⁻⁰³	2.412	Cytoplasm	23,141	10.9
<i>SCLAV_3640</i> (<i>rplB</i>)	50S ribosomal protein L2	B5GX46	0.059	992.34	952.84	5.65x10 ⁻⁰²	3.56x10 ⁻⁰²	0.402	Cytoplasm	30,412	11.3 3
<i>SCLAV_3641</i> (<i>rpsS</i>)	30S ribosomal protein S19	B5GX45	0.107	632.68	587.28	2.29x10 ⁻⁰¹	1.70x10 ⁻⁰¹	1.440	Cytoplasm	10,557	10.8 1
<i>SCLAV_3643</i> (<i>rpsC</i>)	30S ribosomal protein S3	B5GX43	0.044	620.9	602.19	3.32x10 ⁻⁰¹	2.61x10 ⁻⁰¹	0.370	Cytoplasm	30,077	10.4 5
<i>SCLAV_3646</i> (<i>rpsQ</i>)	30S ribosomal protein S17	B5GX40	-0.149	678.57	752.49	1.23x10 ⁻⁰⁴	5.12x10 ⁻⁰⁵	0.666	Cytoplasm	10,833	9.84
<i>SCLAV_3648</i> (<i>rplX</i>)	50S ribosomal protein L24	B5GX38	-0.057	588.81	612.6	8.21x10 ⁻⁰²	5.37x10 ⁻⁰²	1.927	Cytoplasm	11,634	10.0 5
<i>SCLAV_3649</i> (<i>rplE</i>)	50S ribosomal protein L5	B5GX37	-0.207	571.62	659.69	7.97x10 ⁻¹¹	2.09x10 ⁻¹¹	1.113	Cytoplasm	20,923	9.73
<i>SCLAV_3651</i> (<i>rpsH</i>)	30S ribosomal protein S8	B5GX35	0.086	1029.09	969.34	9.40x10 ⁻⁰²	6.26x10 ⁻⁰²	-0.666	Cytoplasm	14,322	9.74
<i>SCLAV_3652</i> (<i>rplF</i>)	50S ribosomal protein L6	B5GX34	0.066	978.91	935.34	1.38x10 ⁻⁰¹	9.58x10 ⁻⁰²	0.747	Cytoplasm	19,119	9.85
<i>SCLAV_3653</i> (<i>rplR</i>)	50S ribosomal protein L18	B5GX33	-0.017	426.89	431.92	4.48x10 ⁻⁰¹	3.72x10 ⁻⁰¹	0.639	Cytoplasm	13,486	10.7 5
<i>SCLAV_3654</i> (<i>rpsE</i>)	30S ribosomal protein S5	B5GX32	-0.110	842.15	908.7	6.32x10 ⁻⁰⁶	2.30x10 ⁻⁰⁶	0.275	Cytoplasm	20,485	10.2
<i>SCLAV_3656</i> (<i>rplO</i>)	50S ribosomal protein L15	B5GX30	-0.022	908.43	922.49	1.95x10 ⁻⁰¹	1.42x10 ⁻⁰¹	3.307	Cytoplasm	15,939	10.1 4
<i>SCLAV_3662</i> (<i>rpsM</i>)	30S ribosomal protein S13	B5GX24	-0.116	1349.89	1463.31	5.77x10 ⁻⁰⁷	1.94x10 ⁻⁰⁷	2.054	Cytoplasm	14,228	10.7 4
<i>SCLAV_3663</i> (<i>rpsK</i>)	30S ribosomal protein S11	B5GX23	-0.480	1192.71	1663.93	4.50x10 ⁻⁷⁹	3.38x10 ⁻⁸⁰	0.810	Cytoplasm	14,384	11.5 4

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_3665 (rplQ)	50S ribosomal protein L17	B5GX21	-0.132	948.37	1039.01	1.50x10 ⁻⁰⁷	4.86x10 ⁻⁰⁸	-0.087	Cytoplasm	18,094	9.58
SCLAV_3669 (rplM)	50S ribosomal protein L13	B5GX16	-0.479	853.55	1189.56	3.34x10 ⁻⁶²	3.02x10 ⁻⁶³	2.796	Cytoplasm	16,364	9.86
SCLAV_3670 (rpsI)	30S ribosomal protein S9	B5GX15	-0.426	712.61	957.27	3.15x10 ⁻⁴⁸	3.45x10 ⁻⁴⁹	1.246	Cytoplasm	18,856	9.30
SCLAV_3960 (ychF)	Ribosome-binding ATPase YchF	B5GTV7	-0.438	12.1	16.39	3.39x10 ⁻⁰²	2.05x10 ⁻⁰²	0.121	CellMembrane, CellWall, Extracellular	39,286	4.73
SCLAV_4424 (gatA)	Glutamyl-tRNA(Gln) amidotransferase subunit A	B5GV10	-0.046	87.25	90.09	3.67x10 ⁻⁰¹	2.94x10 ⁻⁰¹	0.144	CellMembrane, CellWall, Extracellular	52,147	4.66
SCLAV_4498 (gltX2)	Glutamate--tRNA ligase	E2Q8V5	-0.487	104.78	146.86	5.85x10 ⁻²⁸	8.94x10 ⁻²⁹	-1.583	Cytoplasm	52,408	4.91
SCLAV_4541 (rpsP)	30S ribosomal protein S16	B5H3T5	-0.103	1791.39	1924.21	3.24x10 ⁻⁰⁸	1.01x10 ⁻⁰⁸	0.804	Cytoplasm	15,886	9.27
SCLAV_4545 (rplS)	50S ribosomal protein L19	B5H3T1	0.840	994.48	555.53	1.11x10 ⁻⁸⁹	7.54x10 ⁻⁹¹	0.235	Cytoplasm	13,147	10.9
SCLAV_4556 (rpsB)	30S ribosomal protein S2	B5GZ26	0.209	918.05	794.28	1.79x10 ⁻¹⁸	3.49x10 ⁻¹⁹	0.753	Cytoplasm	33,138	5.22
SCLAV_4557	Elongation factor Ts	B5GZ27	0.062	336.75	322.69	2.52x10 ⁻⁰¹	1.89x10 ⁻⁰¹	0.638	Cytoplasm	30,002	5.38
SCLAV_4559	Ribosome-recycling factor	E2Q917	-0.118	271.43	294.53	1.06x10 ⁻⁰²	5.81x10 ⁻⁰³	1.635	Cytoplasm	19,352	5.85
SCLAV_4626 (pnp)	Polyribonucleotide nucleotidyltransferase	B5H1T0	-0.074	305.58	321.75	7.57x10 ⁻⁰⁴	3.47x10 ⁻⁰⁴	0.382	Cytoplasm	79,508	5.00
SCLAV_5070	Polyketide cyclase / dehydrase and lipid transport	E2PX66	-0.757	115.25	194.78	1.17x10 ⁻²³	1.97x10 ⁻²⁴	-0.490	Cytoplasm	16,608	4.81
Transcription, Replication, Recombination and Repair											
SCLAV_0254	Putative acetyltransferase	E2Q783	2.001	12.57	3.14	1.65x10 ⁻¹³	3.87x10 ⁻¹⁴	1.517	Extracellular	30,907	5.01
SCLAV_0719	DNA-binding protein	B5GWZ7	-0.389	321.36	420.96	3.59x10 ⁻¹⁸	7.07x10 ⁻¹⁹	1.112	Cell-Membrane, Cell-Wall, Extracellular	18,131	6.31
SCLAV_0752	Probable transcriptional regulatory protein	B5GWW	-0.387	463.81	606.55	6.55x10 ⁻³⁸	8.43x10 ⁻³⁹	0.792	Cytoplasm	26,641	4.68

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_1957</i> (<i>adpA</i>)	AraC-family transcriptional regulator	B5HIM7	0.330	619.33	492.85	1.38×10^{-40}	1.71×10^{-41}	-2.002	Cytoplasm	42,601	9.46
<i>SCLAV_3146</i>	TetR-family transcriptional regulator	B5GPA7	0.996	839.4	420.85	1.50×10^{-271}	3.70×10^{-273}	1.240	Cytoplasm	28,388	5.39
<i>SCLAV_3224</i>	Transcriptional factor regulator	B5GP19	-0.344	10605.49	13459.58	0	0	1.073	Extracellular	17,829	5.54
<i>SCLAV_3415</i>	DtxR family transcriptional regulator, putative Mn-dependent transcriptional regulator (Iron repressor)	B5GNF7	-0.520	718.2	1029.98	1.50×10^{-98}	9.70×10^{-100}	0.765	Cytoplasm	25,244	4.86
<i>SCLAV_3500</i>	DNA-binding protein	E2Q167	-0.024	393.38	399.97	3.32×10^{-61}	2.62×10^{-61}	0.434	Extracellular	29,540	5.42
<i>SCLAV_3630</i> (<i>rpoC</i>)	DNA-directed RNA polymerase subunit beta'	B5GX60	-0.207	863.36	996.65	7.60×10^{-101}	4.80×10^{-102}	-0.643	Cytoplasm	144,888	6.77
<i>SCLAV_3664</i> (<i>rpoA</i>)	DNA-directed RNA polymerase subunit alpha	B5GX22	-0.180	522.53	592.11	2.32×10^{-13}	5.50×10^{-14}	0.707	Cytoplasm	36,712	4.66
<i>SCLAV_4254</i> (<i>rho</i>)	Transcription termination factor	B5GL69	-0.278	309.25	374.85	1.01×10^{-34}	1.38×10^{-35}	-0.867	Cell-Membrane	72,534	9.07
<i>SCLAV_4470</i>	Putative secreted lipase	E2Q809	1.745	378.07	112.83	0	0	0.604	Extracellular	33,675	5.36
<i>SCLAV_4500</i>	AreB protein	A7DWG0	-0.435	39.14	52.91	7.24×10^{-05}	2.94×10^{-05}	0.328	Cytoplasm	25,051	11.24
<i>SCLAV_4535</i> (<i>glnB</i>)	Nitrogen regulatory protein P-II	E2Q8Z2	0.265	21.96	18.28	4.29×10^{-01}	3.52×10^{-01}	-1.757	Cytoplasm	12,285	5.69
<i>SCLAV_0583</i>	Uracil-DNA glycosylase	B5GZ23	-0.696	226.81	367.37	5.09×10^{-59}	4.84×10^{-60}	0.105	Cytoplasm	25,528	5.91
<i>SCLAV_1848</i>	Energy-dependent translational throttle protein	E2Q3V3	-0.277	477.16	578.29	8.19×10^{-44}	9.63×10^{-45}	-0.557	Cell-Membrane	61,180	5.26
<i>SCLAV_2065</i>	DNA-binding protein	B5GS15	-0.564	183.59	271.37	2.20×10^{-12}	5.45×10^{-13}	-0.067	Cell-Wall, Cytoplasm	9,803	9.45
<i>SCLAV_2881</i>	Single-stranded DNA-binding protein	B5GXB2	-0.248	772.99	917.7	6.96×10^{-21}	1.25×10^{-21}	1.436	Cell-Wall, Cytoplasm	20,039	5.29
<i>SCLAV_2912</i>	Beta sliding clamp	B5H364	-0.762	581.61	986.31	8.60×10^{-303}	2.00×10^{-304}	0.096	Cytoplasm	39,823	4.66
<i>SCLAV_4026</i>	ATP-dependent RNA helicase	E2Q4U1	0.724	617.36	373.7	0	0	0.284	Cell-Membrane	87,198	9.91
<i>SCLAV_4708</i>	Zinc protease	E2QA81	0.083	177.5	167.55	8.34×10^{-02}	5.47×10^{-02}	-0.689	Cell-Membrane	48,953	4.88

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_4709	Protease	E2QA82	-0.092	216.31	230.57	6.38x10 ⁻⁰³	3.36x10 ⁻⁰³	0.185	Cytoplasm	48,291	4.97
Posttranslational Modification, Protein Turnover, Chaperones											
SCLAV_0857 (<i>paA</i>)	Pup--protein ligase	B5H174	-0.008	1116.47	1122.81	3.30x10 ⁻⁰¹	2.59x10 ⁻⁰¹	-2.504	Cell-Membrane, Cell-Wall, Extracellular	52,121	8.74
SCLAV_0860 (<i>prcA</i>)	Proteasome subunit alpha (20S proteasome alpha subunit)	E2PWA4	-0.049	762.97	789.24	1.80x10 ⁻⁰²	1.03x10 ⁻⁰²	0.129	Cytoplasm	27,591	4.88
SCLAV_0861 (<i>prcB</i>)	Proteasome subunit beta	E2PWA5	0.055	715.21	688.56	1.43x10 ⁻⁰¹	9.94x10 ⁻⁰²	0.058	Cytoplasm	27,496	4.91
SCLAV_1119	ABC transporter ATP-binding subunit	E2PYH6	-0.268	372.19	448.18	2.07x10 ⁻¹⁵	4.50x10 ⁻¹⁶	1.630	Cell-Membrane	27,623	5.38
SCLAV_1752	Putative Zn-dependent protease	B5H380	0.858	223.56	123.34	1.58x10 ⁻⁷⁶	1.22x10 ⁻⁷⁷	-0.571	Cell-Membrane	39,351	6.38
SCLAV_1785	ATP-dependent Clp protease proteolytic subunit	B5H031	-0.284	354.44	431.48	2.83x10 ⁻¹⁴	6.42x10 ⁻¹⁵	-0.663	Cytoplasm	23,981	5.02
SCLAV_2010	Bacterioferritin comigratory protein	B5GSQ0	-0.154	138.86	154.48	3.42x10 ⁻⁰²	2.07x10 ⁻⁰²	0.134	Cell-Membrane	16,716	5.74
SCLAV_2464	L-isoaspartyl protein carboxyl methyltransferase	B5GPG8	0.773	59.81	34.99	2.79x10 ⁻²⁰	5.14x10 ⁻²¹	-0.377	Cytoplasm	43,342	5.02
SCLAV_2665 (<i>grpE</i>)	Protein GrpE (HSP-70 cofactor)	E2PUF8	1.584	4819.87	1607.16	0	0	0.674	Cytoplasm	22,968	4.54
SCLAV_2666 (<i>dnaK</i>)	Chaperone protein DnaK (HSP70)	B5GTB1	1.643	12335.7	3949.82	0	0	1.045	Cell-Membrane, Cell-Wall, Extracellular	66,533	4.79
SCLAV_3676	Uncharacterized protein	E2Q2G0	-0.640	26.8	41.77	1.01x10 ⁻⁰⁵	3.72x10 ⁻⁰⁶	0.793	Cytoplasm	19,153	7.02
SCLAV_3692 (<i>groES</i>)	10 kDa chaperonin (GroES protein)	B5GYU4	1.001	2689.08	1343.3	5.00x10 ⁻²⁷⁸	1.20x10 ⁻²⁷⁹	1.647	Cytoplasm	10,989	4.67
SCLAV_3693 (<i>groEL</i>)	60 kDa chaperonin (GroEL protein)	B5GYU5	0.852	2795.16	1548.06	0	0	0.830	Cytoplasm	56,807	4.83
SCLAV_4323	Thioredoxin	E2Q6U8	0.541	409.28	281.32	4.50x10 ⁻⁵⁵	4.50x10 ⁻⁵⁶	-0.016	Cell-Membrane	34,128	4.43
SCLAV_4864	ATP/GTP-binding protein	B5H202	-0.191	177.2	202.24	4.52x10 ⁻⁰³	2.32x10 ⁻⁰³	-1.039	Cytoplasm	14,509	5.10

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_p0820	Thiol peroxidase	B5GN05	-0.360	269.48	345.93	2.95x10 ⁻¹³	7.03x10 ⁻¹⁴	0.193	Cytoplasm	17,084	4.47
Cell wall/Membrane/Envelope Biogenesis											
SCLAV_1724	Tricom protease homolog	E2Q346	0.799	72.51	41.68	8.36x10 ⁻⁶⁹	6.96x10 ⁻⁷⁰	0.012	Cytoplasm	114,839	5.64
SCLAV_2243	Bifunctional protein GlnU (glnU)	B5GPR6	-0.554	267.35	392.47	1.57x10 ⁻⁸⁹	1.07x10 ⁻⁹⁰	-1.071	Cytoplasm	49,548	5.94
SCLAV_2836	Secreted protein	E2PVJ5	-2.321	20.39	101.87	3.60x10 ⁻¹⁰³	2.20x10 ⁻¹⁰⁴	-0.461	Cytoplasm	25,314	5.00
SCLAV_3256	Putave phosphosugar isomerase	E2PYZ6	-0.268	139.27	167.75	2.31x10 ⁻⁰⁷	7.54x10 ⁻⁰⁸	-0.605	Cytoplasm	31,474	4.75
SCLAV_3582	Putative membrane protein	B5GWG6	0.439	3664.1	2703.07	0	0	0.336	Cytoplasm	48,021	4.82
SCLAV_4762	Methyltransferase	B5GZG7	-0.089	23.52	25.01	5.26x10 ⁻⁰¹	4.49x10 ⁻⁰¹	-0.396	Cell-Membrane	30,471	4.85
SCLAV_4982	DUF1906 domain-containing protein	B5GRL8	-0.801	851.37	1483.41	0	0	-0.803	Extracellular	81,717	6.92
SCLAV_p0841	DUF1906 domain-containing protein	B5GMV4	-0.714	1117.74	1833.25	0	0	-1.690	Extracellular	80,708	7.25
Cell Cycle Control, Cell Division, Chromosome Partitioning											
SCLAV_0742	DUF349 domain-containing protein	B5GWY0	-0.153	379.22	421.79	7.11x10 ⁻⁰⁹	2.11x10 ⁻⁰⁹	0.341	Extracellular	44,615	6
SCLAV_1287	Antigen 84 (Cell wall synthesis protein Wag31)	B5GXQ5	-0.002	1098.69	1100.33	5.35x10 ⁻⁰¹	4.57x10 ⁻⁰¹	2.113	Cytoplasm	38,601	5.32
SCLAV_3043	Putative M28-family peptidase	E2PWW5	-0.902	501.3	937.02	0	0	-2.322	Extracellular	52,975	6.17
SCLAV_3831	Nucleoside triphosphate pyrophosphatase	B5GSW9	-0.321	130.42	162.94	7.14x10 ⁻⁰⁷	2.41x10 ⁻⁰⁷	-0.244	Cytoplasm	21,108	5.29
Signal transduction mechanisms											
SCLAV_0615	FHA domain protein	E2PU72	0.512	672.46	471.62	9.57x10 ⁻⁸⁰	7.16x10 ⁻⁸¹	0.992	Extracellular	34,128	6.97
SCLAV_1094	Putative transport associated protein	E2PYF1	1.057	357.93	172.09	1.60x10 ⁻²⁰⁰	5.70x10 ⁻²⁰²	0.558	Cytoplasm	42,506	5.33
SCLAV_2541	Anti-sigma factor	B5GPP5	-0.241	247.16	292.1	1.73x10 ⁻⁰⁵	6.58x10 ⁻⁰⁶	0.944	Cell-Membrane, Cell-Wall, Extracellular	14,964	4.55

Table 3.6 Integration of transcriptomic and proteomic data (continued)

SCLAV_2542	Anti-sigma factor antagonist (bldG)	B5GPP6	-0.061	793.41	827.53	2.89×10^{-02}	1.72×10^{-02}	1.295	Cytoplasm, Extracellular	12,646	5.07
SCLAV_3974	GTP-binding elongation factor	B5GTX1	-0.135	238.31	261.69	6.82×10^{-07}	2.30×10^{-07}	-0.836	Cell-Membrane	69,342	5.17
SCLAV_4121	Secreted protein	E2Q5I2	-0.266	189.59	227.97	2.28×10^{-10}	6.13×10^{-11}	-1.964	Extracellular	34,464	5.01
SCLAV_4134	Two-component system response regulator	B5H4B5	-0.105	146.07	157.05	6.31×10^{-02}	4.01×10^{-02}	0.332	Cytoplasm	26,802	5.67
SCLAV_4305	Cellulose-binding protein	B5GLI9	-0.380	267.25	347.74	1.20×10^{-26}	1.89×10^{-27}	1.692	Extracellular	34,642	5.29
Intracellular Trafficking, Secretion, and Vesicular Transport											
SCLAV_2094 (secA)	Protein translocase subunit SecA	E2Q5C6	0.046	534.41	517.59	1.72×10^{-02}	9.82×10^{-03}	0.233	Cell-Membrane	104,817	5.1
SCLAV_3020	Toxic cation resistance protein	B5GU90	-0.379	100.22	130.33	1.47×10^{-25}	2.37×10^{-26}	0.941	Extracellular	79,775	4.44
SCLAV_4537	Signal recognition particle protein	E2Q8Z4	-0.152	136.3	151.45	1.63×10^{-04}	6.90×10^{-05}	-1.237	Cell-Membrane	55,264	9.31
Defense Mechanism											
SCLAV_2769	Argininosuccinate containing protein	B5GY86	-0.565	221.38	327.42	3.01×10^{-67}	2.55×10^{-68}	-3.267	Cell-Membrane, Cell-Wall, Extracellular	44,428	5.77
SCLAV_4822	Alkaline d-peptidase	B5GUT9	1.045	986.34	478.11	0	0	-1.331	Extracellular	42,034	8.91
SCLAV_5557	Phage protein	E2QIJ8	-0.418	450.66	602.22	2.22×10^{-52}	2.3×10^{-53}	0.207	Cytoplasm	32,710	9.77
Function Unknown											
SCLAV_0126	Secreted protein	B5H0T8	0.889	395.16	213.45	1.50×10^{-243}	4.40×10^{-245}	-0.498	Extracellular	63,250	9.04
SCLAV_0317	Uncharacterized protein	E2Q861	1.528	239.16	82.93	0	0	1.240	Cell-Membrane	68,068	6.95
SCLAV_0518	Secreted tripeptidylaminopeptidase	E2Q9H4	-0.148	176.06	195.07	2.35×10^{-05}	9.00×10^{-07}	-1.195	Extracellular	56,903	8.49
SCLAV_0604	Phenazine biosynthesis protein	B5GZ01	-0.187	65.8	74.91	2.20×10^{-02}	1.28×10^{-02}	-0.049	Extracellular	28,815	5.12
SCLAV_1030	Predicted Zn-dependent protease-like protein	E2PXI6	-0.136	73.56	80.85	2.15×10^{-02}	1.25×10^{-02}	-0.074	Cell-Membrane	49,319	5.34

Table 3.6 Integration of transcriptomic and proteomic data (continued)

<i>SCLAV_1055</i>	Transcriptional regulator	E2PXL1	0.169	1605.76	1428.44	3.37×10^{-04}	1.48×10^{-04}	0.333	Cytoplasm	11,396	10.75
<i>SCLAV_1152</i>	Nucleotide-binding protein	E2PYK8	-0.127	143.81	157.09	1.19×10^{-02}	6.55×10^{-03}	-0.157	Cell-Membrane, Cell-Wall, Extracellular	34,157	5.09
<i>SCLAV_1320</i>	Transcriptional regulatory protein	E2PZU0	-0.436	1458.97	1974.08	1.81×10^{-71}	1.47×10^{-72}	-0.300	Cytoplasm	13,377	5.01
<i>SCLAV_1535</i>	Possibly nucleic acid-binding protein	E2Q1Y4	-0.302	85.4	105.3	1.44×10^{-05}	5.41×10^{-06}	0.732	Cell-Membrane, Cell-Wall	27,572	5.18
<i>SCLAV_1797</i>	Uncharacterized N-acetyltransferase	E2Q3Q1	0.040	774.61	753.68	1.85×10^{-01}	1.33×10^{-01}	-0.983	Cytoplasm	44,545	5.44
<i>SCLAV_2090</i>	Protease	B5HI89	-0.424	240.16	322.14	4.85×10^{-18}	9.61×10^{-19}	-0.323	Cytoplasm	20,453	5.11
<i>SCLAV_2658</i>	Uncharacterized protein	B5GTC1	-0.195	508.13	581.68	1.00×10^{-08}	3.00×10^{-09}	-0.095	Cell-Membrane	20,312	4.2
<i>SCLAV_2829</i>	SEC-C motif domain protein	B5GWJ0	-0.021	79.75	80.91	6.97×10^{-01}	6.34×10^{-01}	-0.386	Cell-Membrane	34,776	5.46
<i>SCLAV_2835</i>	DUF1775 domain-containing protein	E2PVJ4	-1.814	38.37	134.93	3.00×10^{-122}	1.60×10^{-123}	0.628	Cell-Membrane	29,511	8
<i>SCLAV_2904</i>	R3H domain-containing protein	B5GX88	-0.370	264.94	342.42	4.17×10^{-14}	9.59×10^{-15}	1.464	Cytoplasm	18,354	4.52
<i>SCLAV_3194</i>	DUF1416 domain-containing protein	B5GP51	-0.362	2254.05	2897.61	1.42×10^{-59}	1.34×10^{-60}	0.946	Cell-Wall	9,676	4.69
<i>SCLAV_3223</i>	Lipoprotein (Fragment)	E2PYW3	-0.538	185.58	269.45	1.05×10^{-25}	1.70×10^{-26}	0.148	Cell-Membrane	20,365	4.59
<i>SCLAV_3612</i>	UPF0234 protein	B5GX76	-0.143	163.34	180.38	2.77×10^{-02}	1.64×10^{-02}	1.145	Extracellular	17,984	6.37
<i>SCLAV_4298</i>	Uncharacterized protein	B5GL26	0.567	78.35	52.9	5.77×10^{-05}	2.31×10^{-05}	2.010	Cell-Membrane	14,465	4.83
<i>SCLAV_4781</i>	Uncharacterized protein	B5GUP6	0.104	561.14	522.28	4.95×10^{-03}	2.56×10^{-03}	0.981	Extracellular	27,741	4.38
<i>SCLAV_4853</i>	Chlorite dismutase	E2PV76	-0.399	482.28	635.94	7.42×10^{-40}	9.34×10^{-41}	-0.434	Extracellular	27,557	5.75
<i>SCLAV_5138</i>	Uncharacterized protein	E2PY35	0.804	22.17	12.7	2.08×10^{-09}	5.93×10^{-10}	2.941	Cytoplasm	47,919	4.66
<i>SCLAV_5522</i>	Uncharacterized protein	E2Q1G3	-1.152	1153.41	2563.87	0	0	1.525	Cytoplasm	26,147	5.28
<i>SSCG_04568</i> <i>sufD</i>	Fe-S cluster assembly protein SufD	B5GYL4	0.089	398.96	375.05	9.80×10^{-03}	5.33×10^{-03}	1.452	Cytoplasm, Extracellular	41,969	4.83

3.4.1 Comparison of the RNA-seq and nano-LCMS/MS data

3.4.1.1 Secondary Metabolism

In the secondary metabolism, a total of 42 differentially expressed genes (DEGs) and proteins (DEPs) were detected in DEPA. 1 of them is in the clavam gene cluster, 17 of them are in clavulanic acid-cephamycin C supercluster, 19 of them are related to other secondary metabolism products of *S. clavuligerus*. Moreover, 2 were detected in the arginine biosynthetic pathway, and 4 were detected in the G3P biosynthetic pathway. These are known as C₅ (Arginine) and C₃ (G3P) precursors of clavulanic acid biosynthesis.

3.4.1.1.1 Clavam Gene Cluster

In the clavam gene cluster, clavimate synthase 1 (*cas1*) has been detected downregulated (-4.2-fold) at the transcriptomic level by RNA-seq; on the other hand, there has been no significant change (-0.2-fold) detected at the proteomic level by nano-LC-MS/MS. In addition, *cvm6*, *cvm5*, *cvm4*, *cvm1*, *cvm2* and *cvm3* genes have been detected as downregulated (-4.2, -3.7, -3.0, -1.3, -1.0, -1.2, respectively) by RNA-seq analysis. Other genes in the clavam gene cluster did not show any significant change. Any other proteins, except Cas1, produced in the clavam gene cluster have not been detected by nano-LCMS/MS.

Clavam gene cluster has been discussed in section 3.1.3.3. Downregulation of all these compulsory genes for 5S clavam metabolites production in our overproducer DEPA could lead to increasing CA production by lowering the other clavam products.

3.4.1.1.2 CA-CC Supercluster

From the integrative analysis, a total of 17 differentially expressed genes (DEGs) and proteins (DEPs) were detected. *oppA2*, *orf14*, *orf12*, *car*, *oppA1*, *oat2*, *cas2*, *bls2*, *ceaS2*, *lat*, and *pcbC* showed upregulation in gene expression in a range of 1.2 to 1.9-fold; remaining 6 genes (*orf16*, *cyp*, *cefF*, *cmcI*, *cefE*, *pcd*) showed a non-significant change at the expression level, but their expression level was detected in a range of 0.99 to 0.63-fold which looks very high.

In the protein level, acetyltransferase GNAT family protein (Orf14), clavaldehyde dehydrogenase (Car), ABC-type dipeptide transport system periplasmic component (OppA1), arginine biosynthesis bifunctional protein (Oat2), clavamate synthase (Cas), carboxyethyl-arginine beta-lactam synthase (Bls), N(2)-(2-carboxyethyl)arginine synthase (CeaS2), lysine 6-aminotransferase (Lat), deacetoxycephalosporin C hydroxylase (CefF), rhamnosyl O-methyltransferase (CmcI), deacetoxycephalosporin C synthetase (CefE) and isopenicillin N synthetase (PcbC) were detected 1.1, 1.2, 1.4, 2.0, 1.1, 1.3, 1.1, 1.9, 0.9, 0.4, 1.8-fold overrepresented, respectively. There were no significant changes detected in the production of the remaining 5 proteins, which are DUF482 domain-containing protein (Orf16), ABC-type dipeptide transport system, solute binding protein (OppA2), beta-lactamase (Orf12), cytochrome P450-SU2 (Cyp), and aldehyde dehydrogenase (Pcd). RNA-sequencing analysis, *orf13*, *claR*, *pah2*, *pcbAB*, and *cmcT* genes have been shown to increase in expression level in a range of 1.1 to 1.40-fold. Previous studies related to the enhancement of the clavulanic acid production in *S. clavuligerus* showed that increased expression of the genes *ceaS2*, *bls2*, *cas2*, and *pah2* directly affect the yield of CA about 3.8 to 8.7-fold, positively (Jnawali *et al.*, 2011a; Kurt *et al.*, 2013).

OppA1, which was detected increasing level in both transcriptomic and proteomic analysis, has a role in the transportation of oligopeptide signaling molecules being essential for CA production (Lorenzana *et al.*, 2004).

As mentioned before, arginine and G3P are the precursors for clavulanic acid. In the gene cluster, *argB*, *argC*, *argD*, *argJ*, *argR*, *argG*, *argH*, and *argF* genes were detected as significantly increased by transcriptomic analysis. However, only ArgD and ArgH proteins were detected by proteomic analysis, and the production levels were 0.02-fold and -0.7-fold, which were not consistent with the RNA-seq results. In the G3P biosynthetic pathway, the first step of glycolysis is catalyzed by ATP-dependent 6-phosphofructokinase, encoded by *pfkA* gene. *pfkA* was detected as increasing 3.0-fold at the transcriptional level, but it was detected as decreasing -0.6-fold at the protein level. Phosphoglycerate kinase, encoded by *pgk* gene, and fructose-biphosphate aldose, encoded by *SCLAV_2648* gene, proteins were identified as respectively -0.6-fold and -0.8-fold decreased in DEPA by the proteomic analysis. Any significant changes were not detected by the transcriptomic analysis in the expression level of *pgk* and *SCLAV_2648* genes.

3.4.1.1.3 Others in Secondary Metabolism

In this chapter, 18 differentially expressed genes (DEGs) and proteins (DEPs) related to other secondary metabolism products have been detected by transcriptomic and proteomic analysis are discussed.

In the tunicamycin biosynthetic gene cluster, glycosyltransferase group 1 protein encoded by *tunI* gene was detected. At the transcriptional level, a non-significant 0.9-fold increase was detected, and significant 0.8-fold increase was detected at the proteomic level.

In polyketide synthase (PKS) I biosynthetic gene cluster, valine decarboxylase, encoded by *SCLAV_p1225* gene, was detected as increasing at the level of transcriptional expression (2.2-fold) and protein expression production (3.9-fold).

Two members of the PKS/NRPS hybrid gene cluster, beta-lactamase domain protein, encoded by *SCLAV_p1007* gene, and short-chain dehydrogenase/reductase, encoded by *SCLAV_4468* gene, were detected 2-fold and 1-fold increase at the level of transcriptional expression, respectively. They were detected 3-fold and 7-fold overproduced at the protein level, respectively. In this gene cluster, branched-chain-amino-acid aminotransferase protein, encoded by *SCLAV_4463* gene, was detected -1.0-fold underrepresentation at the protein level. In contrast, there was no significant change detected at the transcriptional level. On the other hand, *SCLAV_4472* gene, which produces amidase, was detected as 1.5-fold upregulated by RNA-seq analysis. However, there was no significant change detected at the protein level by nano-LCMS/MS analysis.

Putative ferredoxin reductase was 0.8-fold, acetyl/propionyl CoA carboxylase alpha subunit was 1.0-fold, acetyl-CoA dehydrogenase was 0.7-fold and alpha/beta hydrolyze was 1.4-fold overrepresented at the proteomic level, but the expression level of the genes that express these proteins have not shown any significant change in the transcriptomic analysis.

Pyridoxal-dependent decarboxylase, encoded by *SCLAV_1948* (*sidD*) gene, was detected 0.8-fold overrepresented at the proteomic level and -1.5-fold downregulated at the transcriptomic level. *SCLAV_1949* gene that produces L-lysine N6-monooxygenase was detected -1.0-fold downregulated at the transcriptomic level, but no significant change was detected in their corresponding protein level. *sidD* and *SCLAV_1949* locate in the siderophore desferrioxamine gene cluster known as secondary metabolite gene cluster 6 (SMC6) (Medema *et al.*, 2010).

Lysine N-acyltransferase, encoded by *SCLAV_0152* gene, was detected 0.4-fold overrepresented in proteome level and 1.5-fold upregulated in transcriptomic level. *SCLAV_0152* locates in the siderophore gene cluster known as secondary metabolite gene cluster 3 (SMC3) (Medema *et al.*, 2010).

3.4.1.2 Energy Production and Conversion

In this category, a total of 36 DEGs and DEPs were detected. 3 of these 36 DEGs have shown significant changes, and 23 of these DEPs have shown significant changes in DEPA.

SCLAV_3535, *SCLAV_3770* and *SCLAV_5208* were only significant DEGs (-1.4-fold, -1.03-fold, 1.3-fold) in this category. Their corresponding protein production level was detected as 3.1-fold for only monooxygenase FAD-binding protein (*SCLAV_3535*). Others did not show any significant changes at the proteomic level.

Across the remaining 22 DEPs, 11 were reported as underrepresented, and 11 were reported as overrepresented.

Underrepresented proteins are 3 of oxidoreductase (*SCLAV_0466*; -2.4-fold, *SCLAV_0905*; -1.0-fold and *SCLAV_3929*; -1.2-fold), glutamate synthase subunit beta (*SCLAV_1230* known as GltD; -1.0-fold), pyruvate dehydrogenase E1 component (*SCLAV_1401*; -2.9-fold), malate oxidoreductase (*SCLAV_2067*; -2.0-fold), PEP carboxylase (*SCLAV_2248* known as Ppc; -1.7-fold), 2 of aldehyde dehydrogenase (*SCLAV_2425*; -1.5-fold and *SCLAV_3274*; -0.6-fold), dehydrogenase (*SCLAV_4123*; -1.8-fold) and assimilatory sulfite reductase (*SCLAV_4899*; -1.1-fold) in DEPA.

Overrepresented proteins are 2 of dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex (*SCLAV_1399*; 0.7-fold and *SCLAV_2979*

known as BkdC; 2.0-fold), superoxide dismutase (SCLAV_1807 known as SodF; 0.5-fold), dehydrogenase (SCLAV_2973 known as PaaZ; 2.4-fold), succinate--CoA ligase [ADP-forming] subunit beta (SCLAV_3730 known as SucC; 0.7-fold), malate dehydrogenase (SCLAV_3742; 0.5-fold), ferredoxin (SCLAV_3994; 0.9-fold), catalase (SCLAV_4071; 0.5-fold), putative malate dehydrogenase (SCLAV_4122; 0.7-fold), ATP synthase epsilon chain (SCLAV_4272; 0.4-fold) and L-glutamate gamma-semialdehyde dehydrogenase (SCLAV_4445; 0.6-fold) in DEPA.

For the biosynthesis of the primary amino acid anabolic precursors to produce CA and CC, such as aspartate and lysine, oxaloacetate and acetyl-CoA participation are required. Overrepresented SCLAV_3742 and SCLAV_4122 might have participated in the oxaloacetate formation.

3.4.1.3 Amino Acid Transport and Metabolism

In this category, a total of 32 DEPs and DEGs were detected. Only one of these DEGs was significant in DEPA. *SCLAV_3250* was detected -1.4-fold downregulated in DEPA. Threonine synthase protein, encoded by *SCLAV_3250* gene, was also detected as -0.8-fold underrepresented in protein level. The remaining 31 genes detected in this category were not showed any significant changes.

By the proteomic analysis, a total of 21 proteins were detected as significantly changed in DEPA. Across the 21 DEPs, 17 were reported as downrepresented, and 4 were reported as overrepresented in DEPA.

Chorismate synthase, encoded by *aroC* (*SCLAV_0727*) gene, was detected as -1.5-fold underrepresented. *aroC* catalyzes the last step of the shikimate pathway, and chorismate production has occurred. 3-phosphoshikimate 1-carboxyvinyltransferase, encoded by *aroA* gene, is also involved in the shikimate pathway was detected as -

0.8-fold underrepresented. Using the shikimate pathway, *Streptomyces* produce precursors needed for the biosynthesis of some secondary metabolites such as aromatic polyketide antibiotics (Wilson *et al.*, 1998).

Tetrahydropicolinate succinylase, encoded by *dapD* (SCLAV_1115) gene, was detected as -0.6-fold underrepresented in DEPA. *dapD* catalyzes the reaction of (S)-tetrahydrodipicolinate to LL-2,6-diaminopimelate formation in the succinylase route. Later, L-Lysine formation occurs from DAP. L-Lysine is one of the precursors of the CC.

Cysteine desulfurase, encoded by *SCLAV_1118* gene, was detected as -1.0-fold underrepresented. The result of the transfer of the sulfur residue in the L-cysteine by cysteine desulfurase, L-alanine forms.

Ornithine cyclodeaminase is encoded by *arcB* gene was detected as 0.8-fold overrepresented in DEPA. Ornithine cyclodeaminase has roles in breaking L-arginine into L-proline and L-ornithine. Moreover, 4-hydroxyphenylpyruvate dioxygenase, encoded by *hdp* gene, catalyzes the tyrosine catabolism (Moran, 2005) was detected as 1.4-fold overrepresented in DEPA. Saudagar and Singhal (2007) stated that for getting a higher yield of clavulanic acid, L-arginine, L-proline, and L-threonine are a better choice as C₅ precursor rather than L-ornithine.

Glutamate dehydrogenase (SCLAV_2088; -0.5-fold), gamma-glutamyl phosphate reductase (proA; -0.6-fold), glutamate binding periplasmic protein (SCLAV_4662; -4.4-fold), branched chain amino acid binding protein (SCLAV_1197; -0.4-fold), alanine aminopeptidase (SCLAV_1207; -1.3-fold), integral membrane protein (SCLAV_1434; -0.7-fold), beta-thionase (SCLAV_2199; -0.7-fold), putative peptide hydrolase (SCLAV_2609; -1.3-fold), phosphoserine aminotransferase (SCLAV_3352; -0.6-fold), aminomethyltransferase (SCLAV_4401; -0.6-fold) and pligopeptide-binding lipoprotein (SCLAV_4406; -1.0-fold) were other detected underrepresented proteins in DEPA. Phosphoribosyl isomerase A (SCLAV_1257;

0.5-fold) and 4-aminobutyrate aminotransferase (SCLAV_4585; 0.6-fold) were the remaining overrepresented proteins in DEPA.

3.4.1.4 Translation, Ribosomal Structure, and Biogenesis

In this category, a total of 49 DEPs and DEGs were detected. None of these DEGs showed significant expressions at the transcriptional level, and 38 of the DEPs were significantly differentially expressed at the proteomic level. 8 of the DEPs have shown overrepresentation, and 30 of these DEPs have shown underrepresentation in DEPA.

This category was dominated by 30S and 50S ribosomal proteins S or L subunits. Between SCLAV_3626 and SCLAV_3670, there have been 21 proteins that were mainly overrepresented in a range of 0.4 to 2.4-fold. Only SCLAV_3632 (-0.5-fold) and SCLAV_3651 (-0.7-fold) have been detected as underrepresented.

Methionyl-tRNA formyltransferase (SCLAV_0701), phenylalanine—tRNA ligase alpha subunit (SCLAV_0820), aspartate tRNA—ligase (SCLAV_3013), glutamate—tRNA liagse (SCLAV_4498) were identified as underrepresented -0.9, -2.4, -0.5, -1.5 and -1.6-fold, respectively. In addition, polyketide cyclase was detected as -0.5-fold underrepresented.

Other 9 overrepresented proteins described as 30S and 50S ribosomal proteins S or L subunits were SCLAV_0734 (1.3-fold), SCLAV_0823 (2.4-fold), SCLAV_1188 (1.4-fold), SCLAV_1764 (1.6-fold), SCLAV_1765 (3.0-fold), SCLAV_2879 (0.5-fold), SCLAV_2880 (0.9-fold), SCLAV_4541 (0.8-fold) and SCLAV_4556 (0.8-fold).

Lastly, guanylate kinase (SCLAV_0709), translation initiation factor IF-3 (SCLAV_0825), and elongation factor Ts (SCLAV_4557) proteins were detected as

overrepresented between 0.5-0.7-fold. Ribonuclease, Rne/Rng family (SCLAV_1769) and ribosome-recycling factor (SCLAV_4559) were detected as overrepresented 1.1- and 1.6-fold, respectively.

3.4.1.5 Carbohydrate Transport and Metabolism

In the carbohydrate metabolism category, 12 DEGs and DEPs were detected in DEPA. Among them, only *SCLAV_1133* gene, which encodes transketolase protein, was detected -1.4-fold downregulated at the transcriptional level. Other DEGs have not shown any statistically significant fold-changes in DEPA. The corresponding protein of *SCLAV_1133* was found -2.5-fold underrepresented by nano-LCMS/MS. In addition to that, transaldose (*SCLAV_1134*) catalyzes the reaction of conversion of G3P into D-fructose 6-phosphate was found -1.5-fold underrepresented; and oxppcycle protein (*SCLAV_1136*), has a relation with glucose-6-phosphate dehydrogenase subunit, was found -1.4-fold underrepresented at the proteomic level. Mannose-1-phosphate guanylyltransferase (*SCLAV_0620*) was detected -2.3-fold underrepresented. *SCLAV_0620* catalyzes a reaction; the end-products are GDP-mannose and phosphate. Howlett and his co-workers (2018) reported that in *S.coelicolor*, phenotypic changes were observed when they disrupt the GDP-mannose synthesis pathway.

Carbohydrate ABC transporter substrate-binding protein (*SCLAV_1447*), putative sugar hydrolase (*SCLAV_1830*), and putative trehalose-6-phosphate synthase (*SCLAV_3252*) were detected -1.0-fold underrepresented. Lastly, alpha-amylase (*SCLAV_1444*) and solute-binding protein (*SCLAV_4838*) were detected 0.4-fold overrepresented by nano-LCMS/MS.

3.4.1.6 Lipid Transport and Metabolism

In the lipid metabolism category, we found 10 DEGs and DEPs in DEPA. All DEGs were shown to display non-significant changes at a transcriptional level. A total of 7 DEPs were found to be significantly altered in their expression in DEPA.

Enoyl-[acyl-carrier-protein] reductase [NADH] (FabI) was detected -1.6-fold underrepresented in DEPA. FabI is essential for type II fatty acid biosynthesis system (FASII) and crucial for the survival of bacteria because it has a role in cell envelope synthesis, phospholipids synthesis, lipoproteins synthesis, lipopolysaccharide synthesis, etc. Because the FASII system is a much-conserved system among pathogens, antimicrobial studies have been focused on the disruption of the FASII system (Rana *et al.*, 2020).

Acyl-CoA synthetase (SCLAV_1733), acyl-CoA dehydrogenase (SCLAV_3008), acetyl-CoA acetyltransferase (SCLAV_4309), and JadJ-like biotin carboxylase (SCLAV_3830), which is the part of an acetyl-CoA carboxylase complex were other identified proteins that are involved in the fatty acid biosynthesis have been detected -2.3, -1.6, -1.0 and -1.3-fold underrepresented in DEPA. However, another acyl-CoA dehydrogenase (SCLAV_2155) has been detected 0.7-fold overrepresented. Finally, DUF385 domain-containing protein (SCLAV_5596), catalyzes the reaction of a quinol to quinone by reducing oxidized coenzyme F₄₂₀-(γ -Glu), has been found 0.7-fold overrepresented in DEPA.

3.4.1.7 Posttranslational Modification, Protein Turnover, and Chaperones

In this category, a total of 16 DEGs and DEPs were detected in DEPA. Among DEGs, SCLAV_2665, SCLAV_2666, and SCLAV_3692 have shown significant changes, with 1.6, 1.6, and 1.0-fold upregulation. Proteins that are encoded by these

genes are protein GrpE (HSP-70 cofactor), chaperone protein DnaK (HSP70), and 10kDa chaperonin (GroES), have also been detected to be significantly overrepresented by 0.7, 1.0, and 1.7-fold increments at a proteomic level in DEPA. In addition to GroES, 60 kDa chaperonin GroEL was detected as 0.8-fold overrepresented, as found by nanoLC-MS/MS, and contrarily, by RNA-seq, no significant change was detected for encoding genes of GroEL/GroES chaperonins. The latter are involved in protein folding under stress conditions to prevent the aggregation of misfolded/unfolded proteins in the cell/cell envelope (Goloubinoff *et al.*, 1997). Heat shock proteins (HSP), such as GrpE and DnaK are also known to be produced by the cell when exposed to stressful conditions, especially high temperatures to reduce the protein damage in the cell (Schröder *et al.*, 1993). Secondary metabolite production in *S. clavuligerus* is known to be induced when environmental/physiological stress factors increased. Other overrepresented proteins detected by nano-LCMS/MS are ABC transporter ATP-binding subunit (SCLAV_1119) (1.7-fold) and an uncharacterized protein which is encoded by SCLAV_3676 (0.8-fold).

Pup-protein ligase (PafA), encoded by SCLAV_0857, Putative Zn-dependent protease, encoded by SCLAV_1752, ATP-dependent Clp protease proteolytic subunit, encoded by SCLAV_1785, and ATP/GTP binding protein, encoded by SCLAV_4864 were underrepresented (-2.5, -0.6, -0.7 and -1.0-fold, respectively) proteins detected by nanoLC-MS/MS in DEPA strain.

3.4.1.8 Cell Wall/ Membrane/ Envelope Biogenesis

In this category, there were 8 DEGs and DEPs detected in DEPA. Secreted protein product of SCLAV_2836 was found to be significantly underrepresented at both proteomic (-0.5-fold) and transcriptomic (-2.3-fold) levels.

There were more significant results obtained from nano-LCMS/MS analysis: Bifunctional protein GlmU was found to be -1.1-fold decreased at the proteomic level. According to the UniprotKB database, GlmU has roles in two different biosynthetic pathways: LPS lipid A biosynthesis and UDP-N-acetyl-alpha-D-glucosamine (UDP-GlcNAc) biosynthesis. UDP-GlcNAc is one of the essential components of the peptidoglycan layer formation in *S. clavuligerus*. Two DUF1906 domain-containing proteins, encoded by *SCLAV_4982* and *SCLAV_p0841* genes, respectively, were found to be decreased by -0.8 and -1.7-fold. According to information in the STRING and EggNog databases about DUF1906 domain-containing proteins, these genes might associate with clavam metabolites synthesis or peptidoglycan binding proteins. Lastly, putative phosphosugar isomerase, encoded by *SCLAV_3256*, has been shown to be -0.6-fold decreased in DEPA.

3.4.1.9 Nucleotide Transport and Metabolism

In the nucleotide metabolism category, there were 17 DEGs and proteins (DEPs) detected in DEPA. All the detected DEGs have been shown to reflect non-significant changes at the transcriptional level in DEPA. Nonetheless, 10 of the detected DEPs were found as significantly changed in DEPA; 5 of them are found to be overrepresented, and 5 of them are found to be underrepresented.

Carbamoyl-phosphate synthase large chain (CarB), encoded by *SCLAV_0712*, was -1.5-fold, ribose-phosphate pyrophosphokinase (KprS), encoded by *SCLAV_2244*, was -0.7-fold, nucleoside diphosphate kinase, encoded by *SCLAV_3080*, was -0.6-fold, phosphoribosylamine-glycine kinase (PurD), encoded by *SCLAV_3089*, was -2.0-fold and phosphoribosylaminoimidazole- succinocarboxamide synthase (PurC), encoded by *SCLAV_3092*, was -0.4-fold underrepresented proteins detected by nanoLC-MS/MS in DEPA. PurC and PurD are involved in the inosine

monophosphate biosynthesis pathway (IMP) which is part of purine metabolism. PurD catalyzes the second step of IMP, which is the formation of glycineamide ribonucleotide (GAR) from glycine and phosphoribosylamine (5PRA); PurC catalyzes the seventh step of the IMP, the formation of phosphoribosylaminoimidazolesuccinocarboxamide (SAICAR) from 5'-phosphoribosyl-4-carboxy-5-aminoimidazole (CAIR), and L-Aspartate which is the precursor of CC biosynthesis. In addition to this, SAICAR synthase, encoded by *SCLAV_1876*, was found to be 2.6-fold increased at the proteomic level in DEPA. SAICAR synthase is known to be essential for pentostatin-related nucleoside antibiotics (Wu *et al.*, 2017). CarB is involved in the reaction of conversion of L-glutamine into carbomoyl phosphate, which is involved in arginine biosynthetic pathway, and L-glutamate, which is involved in ornithine biosynthetic pathway known as one of the arginine precursors.

D-alanine-D-alanine ligase, encoded by *SCLAV_3446*, has a role in cross-linkage of peptidoglycan, adenylate kinase, encoded by *SCLAV_3658*, is responsible for the formation of cAMP from ATP, and GMP synthase [glutamine-hydrolyzin] (GuaA), encoded by *SCLAV_3712*, has a role in purine metabolism within the GMP biosynthesis were overrepresented proteins in a range 0.7 to 1.0-fold in DEPA. Nitta and co-workers (2020) have been reported that the increasing level of cAMP in the *S.coelicolor* leads to an increasing level of actinorhodin that is one of the secondary metabolites produced in *S. coelicolor*.

3.4.1.10 Transcription, DNA Replication, Recombination and Repair

In this category, a total of 22 DEGs and DEPs were detected in DEPA. 2 of the genes were reported as significantly increased, as well as 16 of the proteins were reported

as changed significantly; 6 of the 16 proteins showed underrepresentation and 10 of the 16 proteins showed overrepresentation in production.

SCLAV_0254 and *SCLAV_4470* genes, and their protein products putative acetyltransferase and putative secreted lipase were found to be increased in both transcriptomic (2.0 and 1.8-fold, respectively) and proteomic (1.5 and 0.6-fold, respectively) analysis. TetR-family transcriptional regulator, encoded by *SCLAV_3146*, has been detected 1.2-fold overrepresented at the proteomic level, and 0.996-fold upregulated at the transcriptomic level.

DtxR family transcriptional regulator (iron repressor), encoded by *SCLAV_3415*, was found to be 0.8-fold overrepresented at the proteomic level in DEPA. In *S. avermitilis*, IdeR, which is one of the DxtR family transcriptional regulators, has been role as a global regulator that regulates iron homeostasis, metabolism of secondary metabolites, morphologic differentiation, and oxidative stress response (Cheng *et al.*, 2018).

DNA-directed RNA polymerase subunit alpha (RpoA) and subunit beta (RpoC) were detected respectively 0.7-fold increased and -0.6-fold decreased at the proteomic level in DEPA.

DNA-binding protein (1.1-fold) encoded by *SCLAV_0719*, probable transcriptional regulatory protein (0.8-fold) encoded by *SCLAV_0752*, transcriptional factor regulator (1.1-fold) encoded by *SCLAV_3224*, DNA-binding protein (0.4-fold) encoded by *SCLAV_3500*, and single-stranded DNA-binding protein (1.4-fold) encoded by *SCLAV_2881* have been recorded as overrepresented at the proteomic level in DEPA.

AraC-family transcriptional regulator (AdpA), encoded by *SCLAV_1957*, was detected -2.0-fold underrepresented at the proteomic level in DEPA. AdpA is responsible for regulating morphologic differentiation and antibiotic production in *Streptomyces* spp. López-García *et al.* (2010) reported that the deletion of *adpA* gene

resulted that an extreme decreasing in the CA production of *S. clavuligerus*. On the contrary, the same research showed that an increase in *adpA* gene expression also leads to an increase the production of CA in *S. clavuligerus*.

Transcription termination factor (Rho), encoded by *SCLAV_4254*, -0.9-fold, nitrogen regulatory protein P-II (GlnB), encoded by *SCLAV_4535*, -1.8-fold, energy-dependent translational throttle protein, encoded by *SCLAV_1848*, -0.6-fold and zinc protease, encoded by *SCLAV_4708*, -0.7-fold have recorded as underrepresented at the proteomic level in DEPA.

3.4.1.11 Coenzyme Transport and Metabolism

In this category, there are 9 DEGs and DEPs detected in DEPA.

Mg-protoporphyrin IX chelatase, encoded by *SCLAV_1064* gene, and phosphomethylpyrimidine synthase (ThiA), encoded by *SCLAV_2863* gene, were detected to be increased at protein levels (1.7-fold and 1.9-fold, respectively). ThiA is involved in thiamine biosynthesis by catalyzing the synthesis reaction of (HMP-P) moiety of thiamine.

Delta-aminolaevulinic acid dehydratase, encoded by *SCLAV_3512* gene, and ribonuclease J, encoded by *SCLAV_4635* gene, were detected to be decreased at the protein levels (-1.6-fold and -1.0-fold, respectively).

3.4.1.12 Cell Cycle Control, Dell Division, Chromosome Partitioning

In this category, a total of 4 DEGs and DEPs detected in DEPA. However, only cell wall synthesis protein antigen 84, encoded by *SCLAV_1287*, and putative M28-

family peptidase, encoded by *SCLAV_3043*, were reported as changed in their expression by 2.1 and -2.3-fold, respectively as determined by our nanoLC-MS/MS analysis.

3.4.1.13 Signal Transduction Mechanism

A total of 8 differentially expressed genes (DEGs) and proteins (DEPs) were detected in DEPA, regarding signal transduction mechanisms. Among the relevant DEGs, only *SCLAV_1094* has shown a significant change (1.1-fold) in its expression. Its corresponding protein, putative transport associated protein, has also shown a significant overrepresentation (0.6-fold) in the proteome.

Sigma factors have key regulatory roles by switching on/off at a transcriptional level for the secondary metabolite synthesis in *Streptomyces* spp. (Sun *et al.*, 2017). Anti-sigma factor, encoded by *SCLAV_2541*, and anti-sigma factor antagonist, encoded by *SCLAV_2542*, were detected 0.9 and 1.3-fold overrepresented by nanoLC-MS/MS. Other significantly upregulated proteins in this category were FHA domain protein (*SCLAV_0615*) and cellulose-binding protein (*SCLAV_4305*), which were detected respectively 1.0, and 1.7-fold.

GTP-binding elongation factor, encoded by *SCLAV_3974*, and secreted protein, encoded by *SCLAV_4121*, were underrepresented proteins, -0.8 and -2.0-fold, respectively.

3.4.1.14 Intracellular Trafficking, Secretion, and Vesicular Transport

In this category, there are 3 differentially expressed genes (DEGs) and proteins (DEPs) detected in DEPA. Toxic cation resistance protein, encoded by *SCLAV_3020*, was found 0.9-fold overrepresented, and signal recognition particle protein (SRP), encoded by *SCLAV_4537*, was found -1.2-fold underrepresented by nanoLC-MS/MS. SRP is needed for transferring newly synthesized membrane proteins to the correct location on the membrane. Hydrophobic signal sequence on the new protein leads to a translocation event, SRP is being able to recognize and bind to this sequence. The Srp-protein complex then moves toward from ribosome to its actual location on the membrane (Akopian *et al.*, 2013).

3.4.1.15 Inorganic/Organic Molecule Transport

In this category, a total of 8 differentially expressed genes (DEGs) and proteins (DEPs) were detected in DEPA. Among DEPs, 3 significantly overrepresented and 2 significantly underrepresented proteins were detected.

Putative iron-siderophore ABC transporter substrate-binding protein, encoded by *SCLAV_0132*, and heme oxygenase, encoded by *SCLAV_1495* gene, were found to be underrepresented -0.8 and -0.5-fold at proteomic level. In a comparative transcriptomic study of CA production in a favorable medium condition versus an unfavorable medium condition, *SCLAV_0132* gene was reported that downregulated -3.5-fold (Pinilla *et al.*, 2019).

Extracellular binding protein, encoded by *SCLAV_0390*, Sulfurtransferase (RhIA), encoded by *SCLAV_3193*, and Oligopeptide ABC transporter ATP-binding protein, encoded by *SCLAV_4408*, were found to be overrepresented, respectively, 0.7, 1.0, and 0.6-fold in DEPA. In 2011, to understand the function of RhIA, *rhIA* gene is

deleted from *S. clavuligerus* ATCC 27064. Deletion of *rhlA* has been found to be led to a decrease in the production of CC, CA, and Holomycin (Nárdiz *et al.*, 2011).

3.4.1.16 Defense Mechanism

In this category, 3 DEGs and DEPs were detected in DEPA. *SCLAV_4822*, the gene responsible for encoding alkaline d-peptidase, was detected 1.1-fold upregulated at the transcriptional level. On the contrary, at the proteomic level, the gene encoding an alkaline d-peptidase was found to be -1.3-fold underrepresented. Argininosuccinate lyase domain-containing protein, encoded by *SCLAV_2769* gene, is the other protein that was reported as significantly changed (-3.3-fold underrepresented) at the proteomic level.

3.4.1.17 Functions Unknown

In this category, a total of 24 differentially expressed genes (DEGs) and proteins (DEPs) were detected in DEPA. 1 of the detected genes was reported as significantly increased, and 2 of them were reported as significantly decreased, as well as 15 of the detected proteins were reported as changed significantly; 4 showed underrepresentation, and 11 of them showed overrepresentation in production.

SCLAV_0317 was found to be 1.5-fold upregulated at the transcriptional level, its corresponding protein (uncharacterized protein) was found to be 1.2-fold overrepresented at the protein level. *SCLAV_2835* and *SCLAV_5522* were found to be respectively -1.8 and -1.2-fold downregulated in the transcriptional expression; however, at the proteomic level, their corresponding proteins DUF1755 domain-

containing protein and uncharacterized protein were found to be overrepresented respectively 0.6 and 1.5-fold.

Secreted protein, encoded by *SCLAV_0126*, was found -0.5-fold underrepresented. This protein has been annotated as lysyl oxidase in the EggNOG database. Lysyl oxidase (LOX) enzyme function in the animal cell involves the formation of an extracellular matrix by crosslinking collagen and elastin (Grau-Bové *et al.*, 2015). However, in bacteria, it is not possible to crosslink collagenous materials with LOX enzymes, therefore a more comprehensive understanding the LOX enzyme's function is needed for further investigations.

Secreted tripeptidylaminopeptidase, encoded by *SCLAV_0518*, was found to be -1.2-fold downrepresented in DEPA. According to the EggNOG database, secreted tripeptidylaminopeptidase has a hydrolyzing activity on ester bonds. According to the STRING database, this enzyme might relate to the superfamily of hydrolase or acetyltransferases alpha/beta hydrolase.

Other proteins that were significantly overrepresented in DEPA included possible nucleic acid-binding ZN-ribbon protein (0.7-fold), encoded by *SCLAV_1535*, R3H domain-containing protein (1.5-fold), encoded by *SCLAV_2904*, DUF1416 domain-containing protein (1.0-fold), encoded by *SCLAV_3194*, UPF0234 protein (1.2-fold), encoded by *SCLAV_3612*, and Fe-S cluster assembly protein (SufD) (1.5-fold), encoded by *sufD*.

The remaining 3 overrepresented proteins we yet uncharacterized proteins, encoded by *SCLAV_4298*, *SCLAV_4781*, and *SCLAV_5138* respectively, that are believed to provide the increments in CA yields from 2.0 to 3.0-fold

3.4.2 Correlation Analysis

Correlation analysis is needed for an integrative analysis of proteomic and transcriptomic data to understand the expression dynamics of mRNA and protein molecules in an organism. As mentioned in the Material and Method section, correlation analysis was done using non-parametric Spearman's rank-order method (with minimum %95 confidence level) via R Studio. Between 311 distinct mRNA and their corresponding proteins, the correlation coefficient (R) value was found as 0.266 with a p-value of 1.99×10^{-6} . It is known that the acceptable range of correlation coefficient change between different groups of organisms; for bacteria, this range has been reported as 0.2 to 0.47, for yeast 0.34 to 0.87, and for multi-cellular organisms 0.09 to 0.47 (de Sousa Abreu *et al.*, 2009). Thus $R=0.266$ that we found in the present study falls within an acceptable range.

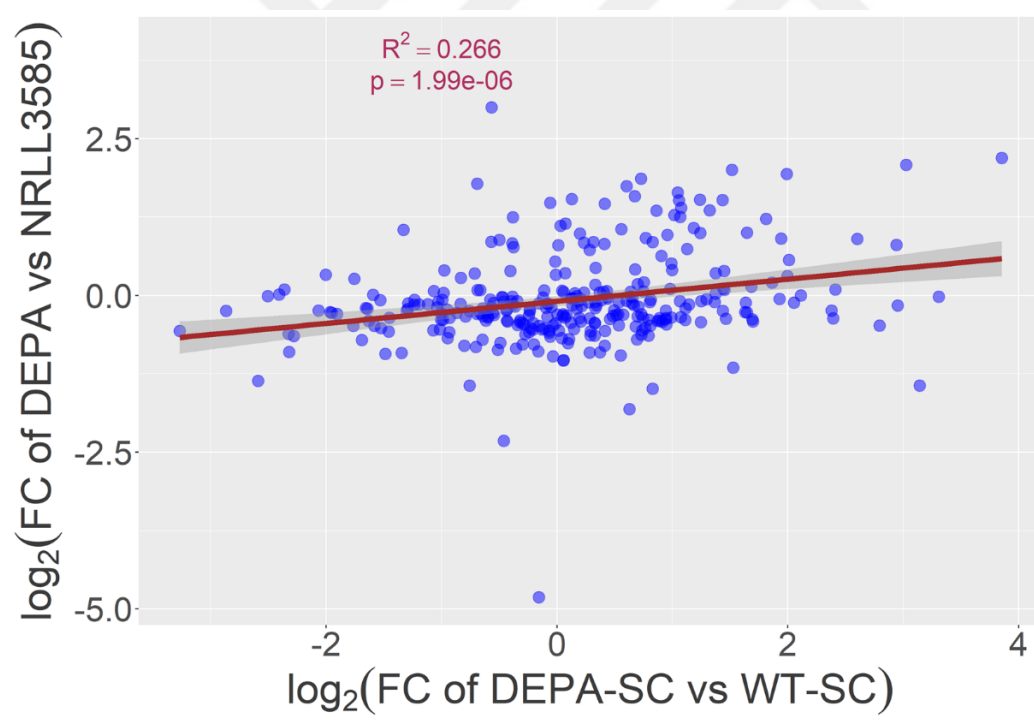


Figure 3.8 Correlation analysis of the transcriptomic and proteomic data

There are some biological reasons for a low correlation between proteomic and transcriptomic results; some of these reasons including the half-life difference between protein and mRNA molecules in the cell, codon usage-bias, weak ribosome binding site, and regulatory proteins. In addition to the biological reasons, technical reasons like experimental noises and insufficient statistical tools could reduce the correlation coefficient (Kumar *et al.*, 2016).



CHAPTER 4

CONCLUSION

- Comparative RNA sequencing analysis of *Streptomyces clavuligerus* standard strain NRRL3585 and industrial CA over-producer strain DEPA showed that the strains share 6115 common genes. On the other hand, DEPA has 60 unique genes and NRRL3585 has 188 unique genes that are not expressed in the strain used for comparison. Total 6363 genes were detected through the whole analysis. According to differential expression analysis of the identified genes, DEPA has 372 significantly upregulated, 552 significantly downregulated, and 5439 non-regulated genes, with respect to reference strain NRRL3585.
- KEGG pathway analysis revealed that the category “Metabolism” dominated the other categories, by having a total of 340 DEGs. “Environmental Information Processing”, “Cellular Processing”, “Genetic Information Processing”, and “Organismal Systems” followed the “Metabolism” category, by having 47, 14, 12, and 1 DEG(s), respectively.
- In the “Metabolism” pathways, the genes that are related to “Global and overview maps” have the highest number of DEGs (124) across the others. “Amino acid metabolism” with 52 DEGs in “Metabolism” category and “Membrane transport” with 41 DEGs in “Environmental Information Processing” category were the most prominent subcategories.

- According to the most enriched pathway analysis, “Biosynthesis of antibiotics” belonging to “Global and overview maps” was found as the most enriched pathway in DEPA, followed by "ABC transporters" belonging to “Membrane transport”, “Biosynthesis of amino acids” and “Carbon metabolism” belonging to “Global and overview maps”.
- Functional classification of DEGs was done by using Gene Ontology (GO) knowledgebase. The category “Molecular function” has the highest number of DEGs (622), followed by “Cellular component” (490) and “Biological process” (489) categories. “Catalytic activity” and “Binding” subcategories of “Molecular function” have the highest number of DEGs within all subcategories. “Metabolic process” and “Cellular process” subcategories have the highest number of DEGs within the “Biological process” class. Lastly, “Membrane” and “Membrane part” subcategories have the highest number of DEGs within “Cellular component” category.
- RNA-seq results were validated via RT-qPCR analysis. RT-qPCR experiments were performed for 15 distinct genes. By RNA-seq analysis, 8 of these genes have been detected as upregulated, and 7 have been detected as downregulated in DEPA. The transcript levels of these selected genes were consistent with their expression levels obtained by RNA-seq.
- For the differentially expressed genes data analysis, the genes with $\log_2FC$ values higher than 1.5 and lower than -1.5 were chosen. Of a total of 372 DEGs were focused after all, 158 were found as upregulated (in a range of 1.5 to 7.8-fold), and 214 were found as downregulated (in a range of -1.5 to -13.3-fold).

DEGs were also grouped according to their functional categories determined by COG database. Accordingly, “Function Unknown” category had the highest numbers of DEGs (126), followed by “Secondary metabolite biosynthesis, transport, and catabolism” (87).

- *amfT*, *amfS* and *amfB* genes of *amfTSBA* operon, which is responsible for aerial mycelium formation, and *whiB* gene, which is responsible for septation of aerial mycelium in DEPA strain showed an increase in transcriptional level more than 2-fold. *amfTSBA* operon is known to be regulated by *claR*, the transcriptional level of which was also significantly increased in DEPA. Secondary metabolites production and the effectiveness of morphological differentiation of *Streptomyces* spp are known to be linked to each other, as further proved by the results of our current study.
- Pathway-specific regulator *claR*, which is TetR-family transcriptional regulator needed for clavulanic acid production, was increased by only 1.15-fold in DEPA. γ -butyrolactones receptor *scaR* (also known as *brp*), which has an inhibitory effect on both CA and CC production, was increased 1.43-fold in DEPA. According to the results of the former studies on the regulation of CA-CC production, *scaR* affects the expression of very important regulatory genes, namely *adpA*, *ccaR*, and *claR* negatively; thus, CA and CC production levels were decreased. In our study, *claR* and *scaR* regulators were found to be significantly upregulated in DEPA, but the other important regulatory genes like *adpA*, *ccaR*, *areB*, *afsA* were not found to display very remarkable changes.

- When the transcriptomic data obtained in this study via RNA seq was integrated into the proteomic data of our group's previous research via nano-LCMS/MS analysis, we could obtain a more comprehensive understanding of the gene regulations that resulted in a very enhanced CA production in an industrial strain of *S. clavuligerus*.
- Of a total of 311 entries matched for integrative analysis. 303 perfectly matched. 116 proteins were overrepresented and 89 were underrepresented in DEPA at varying levels, while 98 proteins did not show much profound differences in DEPA strain.
- Many different transcriptional regulators belonged to “Transcription, Replication, Recombination and Repair” , “Translation, Ribosomal Structure and Biogenesis” and “Function Unknown” categories were detected as significantly up- or downregulated genes in DEPA strain.
- As shown by our former studies, the industrial strain DEPA produces at least 100 folds more clavulanic acid and 1.3-2.5 folds more cephamycin C than the standard strain. The most important genes to produce clavulanic acid in CA-CC supercluster such as *oat2*, *oppA1*, *cas2*, *bls2*, *claR* were found to be significantly increased in DEPA. Yet, the present -omics study, well-demonstrated the cumulative positive impacts of several mutations in previously unknown targets that we could otherwise never know their impacts.

- Iterative random mutagenesis as well as genetic engineering as a targeted approach can be used for eliminating undesirable pathways or overexpressing the genes or pathways known to increase the desired metabolites in industrial strains. Yet, the present study demonstrated the power of combining both approaches, In DEPA strain, we found that the genes that are located in the secondary metabolite biosynthetic gene clusters such as, clavam gene cluster, terpene gene cluster, siderophore gene cluster, polyketide synthase-non-ribosomal peptide synthase gene cluster downregulated at the transcriptional level. Moreover, at the proteomic level, there was no proteins detected from the holomycin gene cluster, alanylclavam gene cluster, type II PKS and type III PKS. Only one protein could be detected from tunicamycin and clavam gene clusters and lastly, only 2 proteins were detected from type I PKS gene cluster. It follows that the iterative classical mutagenesis/screening program utilized by bioindustry can eliminate many of the cellular elements acting as a burden against CA overproduction. The up- and-downregulated genes are to be the targets for rational approaches; via overexpression or silencing with respect to their function, in order to obtain very well-defined and more stable industrial producers.
- Correlation analysis between proteomic and transcriptomic data is needed for the understand the expression dynamics of mRNA and proteins in an organism and data validation The correlation coefficient between our data obtained via RNA-sequencing and nano-LCMS/MS analyses was found as 0.23, which is an acceptable value for bacteria.



REFERENCES

- Akopian, D., Shen, K., Zhang, X., and Shan, S. O. (2013). Signal recognition particle: an essential protein-targeting machine. *Annual review of biochemistry*, 82, 693-721.
- Álvarez-Álvarez, R., Martínez-Burgo, Y., Rodríguez-García, A., and Liras, P. (2017). Discovering the potential of *S. clavuligerus* for bioactive compound production: cross-talk between the chromosome and the pSCL4 megaplasmid. *BMC genomics*, 18(1), 1-13
- Álvarez- Álvarez, R., Rodríguez- García, A., Santamarta, I., Pérez- Redondo, R., Prieto- Domínguez, A., Martínez- Burgo, Y., and Liras, P. (2014). Transcriptomic analysis of *Streptomyces clavuligerus* $\Delta ccaR::tsr$: effects of the cephamycin C- clavulanic acid cluster regulator CcaR on global regulation. *Microbial biotechnology*, 7(3), 221-231.
- Arulanantham, H., Kershaw, N. J., Hewitson, K. S., Hughes, C. E., Thirkettle, J. E. and Schofield, C. J. (2006). ORF17 from the clavulanic acid biosynthesis gene cluster catalyzes the ATP-dependent formation of N-Glycyl-clavaminic Acid. *The Journal of Biological Chemistry*, 281:279-287.7
- Atsushi, M., Soon-Kwang, H., Hiroshi, I., Sueharu, H., and Teruhiko, B. (1994). Phosphorylation of the AfsR protein involved in secondary metabolism in *Streptomyces* species by a eukaryotic-type protein kinase. *Gene*, 146(1), 47-56.
- Bachmann, B. O., Li, R., and Townsend, C. A. (1998). β -Lactam synthetase: a new biosynthetic enzyme. *Proceedings of the National Academy of Sciences of the United States of America*, 95(16), 9082-6.

- Baggaley, K. H., Brown, A. G., and Schofield, C. J. (1997). Chemistry and biosynthesis of clavulanic acid and other clavams. *Natural product reports*, 14(4), 309-333.
- Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H. P., Clement, C., Ouhdouch, Y., and van Wezel, G. P. (2016). Taxonomy, physiology, and natural products of *Actinobacteria*. *Microbiology and Molecular Biology Reviews*, 80(1), 1-43.
- Benjdia, A., Balty, C., and Berteau, O. (2017). Radical SAM enzymes in the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs). *Frontiers in Chemistry*, 5, 87.
- Bignell, D. R., Tahlan, K., Colvin, K. R., Jensen, S. E., and Leskiw, B. K. (2005). Expression of *ccaR*, encoding the positive activator of cephamycin C and clavulanic acid production in *Streptomyces clavuligerus*, is dependent on *bldG*. *Antimicrobial agents and chemotherapy*, 49(4), 1529-1541.
- Brown, A. G., Butterworth, D., Cole, M., Hanscomb, G., Hood, J. D., Reading, C., and Rolinson, G. N. (1976). Naturally occurring β -lactamase inhibitors with antibacterial activity. *The Journal of antibiotics*, 29(6), 668-669.
- Bush, M. J., Chandra, G., Bibb, M. J., Findlay, K. C., and Buttner, M. J. (2016). Genome-wide chromatin immunoprecipitation sequencing analysis shows that WhiB is a transcription factor that cocontrols its regulon with WhiA to initiate developmental cell division in *Streptomyces*. *MBio*, 7(2), e00523-16.
- Campbell, R. E., Mosimann, S. C., Tanner, M. E., and Strynadka, N. C. (2000). The structure of UDP-N-acetylglucosamine 2-epimerase reveals homology to phosphoglycosyl transferases. *Biochemistry*, 39(49), 14993-15001.

- Capstick, D. S., Willey, J. M., Buttner, M. J., and Elliot, M. A. (2007). SapB and the chaplins: connections between morphogenetic proteins in *Streptomyces coelicolor*. *Molecular microbiology*, 64(3), 602-613.
- Cashel, M., and Kalbacher, B. (1970). The control of ribonucleic acid synthesis in *Escherichia coli*: V. characterization of a nucleotide associated with the stringent response. *Journal of Biological Chemistry*, 245(9), 2309-2318.
- Challis, G. L. (2005). A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. *Chembiochem*, 6(4), 601-611.
- Chater, K. F. (2001). Regulation of sporulation in *Streptomyces coelicolor* A3 (2): a checkpoint multiplex?. *Current opinion in microbiology*, 4(6), 667-673.
- Cheng, Y., Yang, R., Lyu, M., Wang, S., Liu, X., Wen, Y., Song, Y., Li, J., and Chen, Z. (2018). IdeR, a DtxR family iron response regulator, controls iron homeostasis, morphological differentiation, secondary metabolism, and the oxidative stress response in *Streptomyces avermitilis*. *Applied and environmental microbiology*, 84(22), e01503-18
- Cho, M. A., Han, S., Lim, Y. R., Kim, V., Kim, H., and Kim, D. (2019). *Streptomyces* cytochrome P450 enzymes and their roles in the biosynthesis of macrolide therapeutic agents. *Biomolecules and therapeutics*, 27(2), 127.
- Claessen, D., De Jong, W., Dijkhuizen, L., and Wösten, H. A. (2006). Regulation of *Streptomyces* development: reach for the sky!. *Trends in microbiology*, 14(7), 313-319.

- Coats, J. H., Li, G. P., Ming-St, K., and Yurek, D. A. (1989). Discovery, production, and biological assay of an unusual flavenoid cofactor involved in lincomycin biosynthesis. *The Journal of antibiotics*, 42(3), 472-474.
- Cuthbertson, L., and Nodwell, J. R. (2013). The TetR family of regulators. *Microbiology and Molecular Biology Reviews*, 77(3), 440-475.
- Davis, N. K., and Chater, K. F. (1992). The *Streptomyces coelicolor whiB* gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. *Molecular and General Genetics MGG*, 232(3), 351-358.
- De la Fuente, A., Martin, J. F., Rodriguez-Garcia, A., and Liras, P. (2004). Two proteins with ornithine acetyltransferase activity show different functions in *Streptomyces clavuligerus*: Oat2 modulates clavulanic acid biosynthesis in response to arginine. *Journal of bacteriology*, 186(19), 6501-6507.
- De Sousa Abreu, R., Penalva, L. O., Marcotte, E. M., and Vogel, C. (2009). Global signatures of protein and mRNA expression levels. *Molecular BioSystems*, 5(12), 1512-1526.
- Demain, A. L. (2002). Prescription for an ailing pharmaceutical industry. *Nature biotechnology*, 20(4), 331-331.
- Deng, W., Li, C., and Xie, J. (2013). The underling mechanism of bacterial TetR/AcrR family transcriptional repressors. *Cellular signalling*, 25(7), 1608-1613.
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1), 15-21.

- Drawz, S. M., and Bonomo, R. A. (2010). Three decades of β -lactamase inhibitors. *Clinical microbiology reviews*, 23(1), 160-201.
- Egan, L. A., Busby, R. W., Iwata-Reuyl, D., and Townsend, C. A. (1997). Probable role of clavaminic acid as the terminal intermediate in the common pathway to clavulanic acid and the antipodal clavam metabolites. *Journal of the American Chemical Society*, 119(10), 2348-2355.
- Elliot, M. A., Buttner, M. J., and Nodwell, J. R. (2007). Multicellular development in *Streptomyces*. *Myxobacteria: multicellularity and differentiation*, 419-438.
- Elliot, M. A., Karoonuthaisiri, N., Huang, J., Bibb, M. J., Cohen, S. N., Kao, C. M., and Buttner, M. J. (2003). The chaplins: a family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. *Genes and development*, 17(14), 1727-1740.
- Elson, S. W., Baggaley, K. H., Gillett, J., Holland, S., Nicholson, N. H., Sime, J. T., and Woroniecki, S. R. (1987). Isolation of two novel intracellular β -lactams and a novel dioxygenase cyclising enzyme from *Streptomyces clavuligerus*. *Journal of the Chemical Society. Chemical Communications*, 1736-1738.
- Farrugia, M. A., Macomber, L., and Hausinger, R. P. (2013). Biosynthesis of the urease metallocenter. *Journal of biological chemistry*, 288(19), 13178-13185.
- Flärdh, K. (2003). Growth polarity and cell division in *Streptomyces*. *Current opinion in microbiology*, 6:564-571.
- Fu, J., Qin, R., Zong, G., Liu, C., Kang, N., Zhong, C., and Cao, G. (2019a). The CagRS two-component system regulates clavulanic acid metabolism via

- multiple pathways in *Streptomyces clavuligerus* F613-1. *Frontiers in microbiology*, 10, 244.
- Fu, J., Qin, R., Zong, G., Zhong, C., Zhang, P., Kang, N., Oi, X., and Cao, G. (2019b). The two-component system CepRS regulates the cephamycin C biosynthesis in *Streptomyces clavuligerus* F613-1. *Amb express*, 9(1), 1-9.
- Georgopoulos, C., Liberek, K., Zylicz, M., and Ang, D. (1994) Properties of the heat-shock proteins of *Escherichia coli* and the autoregulation of the heat-shock response. In *The Biology of Heat Shock Proteins and Molecular Chaperones*. Morimoto, R.I., Tissières, A., and Georgopoulos, C. (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp. 209–249.
- Gill, D. R., and Salmond, G. P. (1987). The *Escherichia coli* cell division proteins FtsY, FtsE and FtsX are inner membrane-associated. *Molecular and general genetics MGG*, 210(3), 504-508.
- Ginther, C. L. (1979). Sporulation and the production of serine protease and cephamycin C by *Streptomyces lactamdurans*. *Antimicrobial agents and chemotherapy*, 15(4), 522-526.
- Glatz, E., Nilsson, R. P., Rutberg, L., and Rutberg, B. (1996). A dual role for the *Bacillus subtilis glpD* leader and the GlpP protein in the regulated expression of *glpD*: antitermination and control of mRNA stability. *Molecular microbiology*, 19(2), 319-328.
- Goloubinoff, P., Diamant, S., Weiss, C., and Azem, A. (1997). GroES binding regulates GroEL chaperonin activity under heat shock. *FEBS letters*, 407(2), 215-219.

- Gomez-Escribano, J. P., Martin, J. F., Hesketh, A., Bibb, M. J., and Liras, P. (2008). *Streptomyces clavuligerus* *relA*-null mutants overproduce clavulanic acid and cephamycin C: negative regulation of secondary metabolism by (p)ppGpp. *Microbiology*, 154(3), 744-755.
- Grau-Bové, X., Ruiz-Trillo, I., and Rodriguez-Pascual, F. (2015). Origin and evolution of lysyl oxidases. *Scientific reports*, 5(1), 1-11.
- Gross, C. (1996) Function and regulation of the heat shock proteins. In *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. Neidhardt, F.C., Curtiss R., III, Ingraham, J.L., Lin, E.C.C, Low, K.B., Magasanik, B., et al. (eds). Washington, DC: *American Society for Microbiology Press*, pp. 1382–1399.
- Hablesreiter, R. (2017). *Establishment of an Analysis Pipeline for RNA-seq Datasets* (Master's Thesis, Graz University of Technology, Graz, Austria). Retrieved from <https://diglib.tugraz.at/download.php?id=5a1deffd77db4&location=search>
- Haseltine, W. A., Block, R., Gilbert, W. and Weber, K. (1972). MSI and MSII are made on the ribosome in an idling reaction of protein synthesis. *Nature*. 238, 381–385.
- Hayashi, T., Tanaka, Y., Sakai, N., Watanabe, N., Tamura, T., Tanaka, I., and Yao, M. (2013). Structural and genomic DNA analysis of a putative transcription factor SCO5550 from *Streptomyces coelicolor* A3 (2): regulating the expression of gene *sco5551* as a transcriptional activator with a novel dimer shape. *Biochemical and biophysical research communications*, 435(1), 28-33.

- Heath, R. J., White, S. W., and Rock, C. O. (2001). Lipid biosynthesis as a target for antibacterial agents. *Progress in lipid research*, 40(6), 467-4
- Higgins, C. E., and Kastner, R. E. (1971). *Streptomyces clavuligerus* sp. nov., a β -lactam antibiotic producer. *International journal of systematic and evolutionary microbiology*, 21(4), 326-331.
- Hindle, Z., and Smith, C. P. (1994). Substrate induction and catabolite repression of the *Streptomyces coelicolor* glycerol operon are mediated through the GyIR protein. *Molecular microbiology*, 12(5), 737-745.
- Hodgson, J. E., Fosberry, A. P., Rawlinson, N. S., Ross, H. N., Neal, R. J., Arnell, J. C., Earl A.J., and Lawlor, E. J. (1995). Clavulanic acid biosynthesis in *Streptomyces clavuligerus*: gene cloning and characterization. *Gene*, 166(1), 49-55.
- Hofmann, K., and Bucher, P. (1995). The FHA domain: a putative nuclear signalling domain found in protein kinases and transcription factors. *Trends in biochemical sciences*, 20(9), 347-349.
- Hölzer, M. (2017). *The Dark Art of Next-Generation Sequencing Fundamental: approaches for genomics, transcriptomics, and differential gene expression* (Doctoral dissertation, Friedrich-Schiller University of Jena, Jena, Germany). Retrieved from <https://hoelzer-lab.github.io/assets/hoelzer-thesis-2017.pdf>
- Horinouchi, S., and Beppu, T. (1994). A- factor as a microbial hormone that controls cellular differentiation and secondary metabolism in *Streptomyces griseus*. *Molecular microbiology*, 12(6), 859-864.

- Howlett, R., Anttonen, K., Read, N., and Smith, M. C. (2018). Disruption of the GDP-mannose synthesis pathway in *Streptomyces coelicolor* results in antibiotic hyper-susceptible phenotypes. *Microbiology*, 164(4), 614.
- Hwang, S., Lee, N., Jeong, Y., Lee, Y., Kim, W., Cho, S., Palsson, O. B., and Cho, B. K. (2019). Primary transcriptome and translome analysis determines transcriptional and translational regulatory elements encoded in the *Streptomyces clavuligerus* genome. *Nucleic acids research*, 47(12), 6114-6129.
- Ives, P. R., and Bushell, M. E. (1997). Manipulation of the physiology of clavulanic acid production in *Streptomyces clavuligerus*. *Microbiology*, 143(11), 3573-3579.
- Janošík, M., Kery, V., Gaustadnes, M., Maclean, K. N., and Kraus, J. P. (2001). Regulation of human cystathionine β -synthase by S-adenosyl-L-methionine: evidence for two catalytically active conformations involving an autoinhibitory domain in the C-terminal region. *Biochemistry*, 40(35), 10625-10633.
- Jensen, S. E., Elder, K. J., Aidoo, K. A., and Paradkar, A. S. (2000). Enzymes catalyzing the early steps of clavulanic acid biosynthesis are encoded by two sets of paralogous genes in *Streptomyces clavuligerus*. *Antimicrobial Agents and Chemotherapy*, 44(3), 720-6.
- Jensen, S., Paradkar, A., and Mosher, R. (2004). Five additional genes are involved in clavulanic acid biosynthesis in *Streptomyces clavuligerus*. *Antimicrobial agents and chemotherapy*, 48(1), 192-202.
- Jin, W., Ryu, Y. G., Kang, S. G., Kim, S. K., Saito, N., Ochi, K., Lee, S. H., and Lee, K. J. (2004). Two *relA/spoT* homologous genes are involved in the

morphological and physiological differentiation of *Streptomyces clavuligerus*. *Microbiology*, 150(5), 1485-1493.

Jnawali, H. N., Liou, K., and Sohng, J. K. (2011a). Role of σ -factor (*orf21*) in clavulanic acid production in *Streptomyces clavuligerus* NRRL3585. *Microbiological research*, 166(5), 369-379.

Jnawali, H. N., Oh, T. J., Liou, K., Park, B. C. and Sohng, J. K. (2008). A two-component regulatory system involved in clavulanic acid production. *The journal of antibiotics*, 61(11):651-659.

Jnawali, H. N., Yoo, J. C. and Sohng, J. K. (2011b). Improvement of clavulanic acid production in *Streptomyces clavuligerus* by genetic manipulation of structural biosynthesis genes. *Biotechnology letters*, 33(6):1221-1226.

Jones, S. E., and Elliot, M. A. (2018). 'Exploring' the regulation of *Streptomyces* growth and development. *Current opinion in microbiology*, 42, 25-30.

Kakeshita, H., Takamatsu, H., Amikura, R., Nakamura, K., Watabe, K., and Yamane, K. (2001). Effect of depletion of FtsY on spore morphology and the protein composition of the spore coat layer in *Bacillus subtilis*. *FEMS microbiology letters*, 195(1), 41-46.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayana, T., Kawashima, S., Okuda, S., Tokimatsu, T., and Yamanishi, Y. (2007). KEGG for linking genomes to life and the environment. *Nucleic acids research*, 36, D480-D484.

Kannenber, E. L., and Poralla, K. (1999). Hopanoid biosynthesis and function in bacteria. *Naturwissenschaften*, 86(4), 168-176.

- Kato, F., Hino, T., Nakaji, A., Tanaka, M., and Koyama, Y. (1995). Carotenoid synthesis in *Streptomyces setonii* ISP5395 is induced by the gene *crtS*, whose product is similar to a sigma factor. *Molecular and general genetics MGG*, 247(3), 387-390.
- Kavanagha, K. L., Jçrnvallb, H., Perssonc, B., and Oppermanna, U. (2008). The SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. *Cellular and molecular life sciences*, 65, 3895-3906.
- Kenig, M., and Reading, C. (1979). Holomycin and an antibiotic (MM 19290) related to tunicamycin, metabolites of *Streptomyces clavuligerus*. *The journal of antibiotics*, 32:549-554.
- Kershaw, N. J., McNaughton, H. J., Hewitson, K. S., Hernandez, H., Griffin, J., Hughes, C., Greaves, P., Barton, B., Robinson, C. V., and Schofield, C. J. (2002). ORF6 from the clavulanic acid gene cluster of *Streptomyces clavuligerus* has ornithine acetyltransferase activity. *European journal of biochemistry*, 269(8), 2052-2059.
- Khaleeli, N. (1999). Origin of the β -lactam carbons in clavulanic acid from an unusual thiamine pyrophosphate-mediated reaction. *Journal of the american chemical society*, 121:9223-9224.
- Kieser, T., Bibb M. J., Buttner, M. J., Chater K. F., and Hopwood, D. A. (2000). Practical *Streptomyces* genetics. The John Innes Foundation, Norwich, United Kingdom
- Kim, D., Langmead, B., and Salzberg, S. L. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nature methods*, 12(4), 357-360.

- King, D. T., Sobhanifar, S., and Strynadka, N. C. (2017). The mechanisms of resistance to β lactam antibiotics. *Handbook of antimicrobial resistance*. New York: Springer.
- Kong, K. F., Schneper, L., and Mathee, K. (2010). Beta- lactam antibiotics: from antibiosis to resistance and bacteriology. *Apmis*, 118(1), 1-36.
- Kukurba, K. R., and Montgomery, S. B. (2015). RNA sequencing and analysis. *Cold spring harbor protocols*, 2015(11), pdb-top084970.
- Kumar, D., Bansal, G., Narang, A., Basak, T., Abbas, T., and Dash, D. (2016). Integrating transcriptome and proteome profiling: Strategies and applications. *Proteomics*, 16(19), 2533-2544.
- Kurt, A., Álvarez-Álvarez, R., Liras, P., and Özcengiz, G. (2013). Role of the *cmcH-ccaR* intergenic region and *ccaR* overexpression in cephamycin C biosynthesis in *Streptomyces clavuligerus*. *Applied microbiology and biotechnology*, 97(13), 5869-5880.
- Lamb, A. L. (2015) Breaking a Pathogen's Iron Will: Inhibiting Siderophore Production as an Antimicrobial Strategy, *Biochim Biophys Acta* 1854, 1054-1070.
- Lambalot, R. H., Gehring, A. M., Flugel, R. S., Zuber, P., LaCelle, M., Marahiel, M. A., Marahiel, M. A., Reid, R., Khosla, C., and Walsh, C. T. (1996). A new enzyme superfamily—the phosphopantetheinyl transferases. *Chemistry & biology*, 3(11), 923-936.
- Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods*, 9(4), 357-359.

- Le, H. S., Schulz, M. H., McCauley, B. M., Hinman, V. F., and Bar-Joseph, Z. (2013). Probabilistic error correction for RNA sequencing. *Nucleic acids research*, 41(10), e109-e109.
- Li, B., and Walsh, C. T. (2010). Identification of the gene cluster for the dithiolopyrrolone antibiotic holomycin in *Streptomyces clavuligerus*. *Proceedings of the national academy of sciences*, 107(46), 19731-19735.
- Li, R., Khaleeli, N., and Townsend, C. A. (2000). Expansion of the clavulanic acid gene cluster: identification and in vivo functional analysis of three new genes required for biosynthesis of clavulanic acid by *Streptomyces clavuligerus*. *Journal of bacteriology*, 182(14), 4087-95.
- Liang, K.H. (2013). Transcriptomics. *Bioinformatics for biomedical science and clinical applications*, 49–82.
- Lima, L. M., da Silva, B. N. M., Barbosa, G., and Barreiro, E. J. (2020). β -lactam antibiotics: An overview from a medicinal chemistry perspective. *European journal of medicinal chemistry*, 112829.
- Liras, P., and Martín, J. F. (2006). Gene clusters for beta-lactam antibiotics and control of their expression: why have clusters evolved, and from where did they originate?. *International microbiology*, 9(1), 9.
- Liras, P., Gomez-Escribano, J. P., and Santamarta, I. (2008). Regulatory mechanisms controlling antibiotic production in *Streptomyces clavuligerus*. *Journal of industrial microbiology and biotechnology*, 35(7), 667.

- López-Agudelo, V. A., Gómez-Ríos, D., and Ramirez-Malule, H. (2021). Clavulanic acid production by *Streptomyces clavuligerus*: insights from systems biology, strain engineering, and downstream processing. *Antibiotics*, 10(1), 84.
- López-García, M. T., Santamarta, I., and Liras, P. (2010). Morphological differentiation and clavulanic acid formation are affected in a *Streptomyces clavuligerus* *adpA*-deleted mutant. *Microbiology*, 156(8), 2354-2365.
- Lorenzana, L. M., Pérez-Redondo, R., Santamarta, I., Martín, J. F., and Liras, P. (2004). Two oligopeptide-permease-encoding genes in the clavulanic acid cluster of *Streptomyces clavuligerus* are essential for production of the β -lactamase inhibitor. *Journal of bacteriology*, 186(11), 3431-3438.
- Lowe, R., Shirley, N., Bleackley, M., Dolan, S., and Shafee, T. (2017). Transcriptomics technologies. *PLoS computational biology*, 13(5), e1005457.
- Lu, F., Hou, Y., Zhang, H., Chu, Y., Xia, H., and Tian, Y. (2017). Regulatory genes and their roles for improvement of antibiotic biosynthesis in *Streptomyces*. *3 Biotech*, 7(4), 1-15.
- MacKenzie. (2007). Studies on the biosynthetic pathways of clavulanic acid and cephamycin C in *Streptomyces clavuligerus*. (PHD), Acta Universitatis, Uppsala, Sweden.
- Maeda, I., Hirose, N., Yamashiro, H., Ichibori, A., Ohshima, S., Fujimoto, T., Kawase, M., and Yagi, K. (2003). Comparative study of the N-terminal hydrophilic region in *Streptomyces lividans* and *E. coli* FtsY. *Current microbiology*, 47(1), 0022-0025.

- Maki, H., and Sekiguchi, M. (1992). MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, 355(6357), 273-275.
- Malmberg, L. H., Hu, W. S., and Sherman, D. H. (1993). Precursor flux control through targeted chromosomal insertion of the lysine epsilon-aminotransferase (*lat*) gene in cephamycin C biosynthesis. *Journal of bacteriology*, 175(21), 6916-6924.
- Manteca, A., Alvarez, R., Salazar, N., Yagüe, P., and Sanchez, J. (2008). Mycelium differentiation and antibiotic production in submerged cultures of *Streptomyces coelicolor*. *Applied and environmental microbiology*, 74(12), 3877-3886.
- Mao, Y., Varoglu, M., and Sherman, D. H. (1999). Molecular characterization and analysis of the biosynthetic gene cluster for the antitumor antibiotic mitomycin C from *Streptomyces lavendulae* NRRL 2564. *Chemistry and biology*, 6(4), 251-263.
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, 17(1), 10-12.
- Martínez-Burgo, Y., Álvarez-Álvarez, R., Rodríguez-García, A., and Liras, P. (2015). The pathway-specific regulator ClaR of *Streptomyces clavuligerus* has a global effect on the expression of genes for secondary metabolism and differentiation. *Applied and environmental microbiology*, 81(19), 6637-6648.
- Martínez-Burgo, Y., Santos-Aberturas, J., Rodríguez-García, A., Barreales, E. G., Tormo, J. R., Truman, A. W., Reyes, F., Aparicio, J. F., and Liras, P. (2019). Activation of secondary metabolite gene clusters in *Streptomyces*

- clavuligerus* by the PimM regulator of *Streptomyces natalensis*. *Frontiers in microbiology*, 10, 580.
- Medema, M. H., Alam, M. T., Heijne, W. H., Van Den Berg, M. A., Müller, U., Trefzer, A., Bovenberg, R. A. L., Breitling, R., and Takano, E. (2011). Genome- wide gene expression changes in an industrial clavulanic acid overproduction strain of *Streptomyces clavuligerus*. *Microbial biotechnology*, 4(2), 300-305.
- Medema, M. H., Trefzer, A., Kovalchuk, A., van den Berg, M., Müller, U., Heijne, W., Wu, L., Alam, M. T., Ronning, C. M., Niermen, W. C., Bovenberg, R. A. L., Breitling, R., and Takano, E. (2010). The sequence of a 1.8-Mb bacterial linear plasmid reveals a rich evolutionary reservoir of secondary metabolic pathways. *Genome biology and evolution*, 2, 212-224.
- Mellado, E., Lorenzana, L. M., Rodríguez-Sáiz, M., Díez, B., Liras, P., and Barredo, J. L. (2002). The clavulanic acid biosynthetic cluster of *Streptomyces clavuligerus*: genetic organization of the region upstream of the *car* gene. *Microbiology*, 148(Pt 5), 1427-38.
- Méndez, C., and Salas, J. A. (2001). The role of ABC transporters in antibiotic-producing organisms: drug secretion and resistance mechanisms. *Research in microbiology*, 152(3-4), 341-350.
- Ming-St, K., Yurek, D. A., Coats, J. H., and Li, G. P. (1989). Isolation and identification of 7, 8-didemethyl-8-hydroxy-5-deazariboflavin, an unusual cosynthetic factor in *Streptomyces*, from *Streptomyces lincolnensis*. *The Journal of antibiotics*, 42(3), 475-478.
- Moran, G. R. (2005). 4-Hydroxyphenylpyruvate dioxygenase. *Archives of biochemistry and biophysics*, 433(1), 117-128.

- Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., and Snyder, M. (2008). The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science*, 320(5881), 1344-1349.
- Nárdiz, N., Santamarta, I., Lorenzana, L. M., Martín, J. F., and Liras, P. (2011). A rhodanese- like protein is highly overrepresented in the mutant *S. clavuligerus oppA2::aph*: effect on holomycin and other secondary metabolites production. *Microbial biotechnology*, 4(2), 216-225.
- Nazari, B., Kobayashi, M., Saito, A., Hassaninasab, A., Miyashita, K., and Fujii, T. (2013). Chitin-induced gene expression in secondary metabolic pathways of *Streptomyces coelicolor* A3 (2) grown in soil. *Applied and environmental microbiology*, 79(2), 707-713.
- Nicholson, N. H., Baggaley, K. H., Cassels, R., Davison, M., Elson, S. W., Fulston, M., Tyler, J. W., and Woroniecki, S. R. (1994). Evidence that the immediate biosynthetic precursor of clavulanic acid is its N-aldehyde analogue. *Journal of the chemical society, chemical communications*, (11), 1281-1282.
- Nitta, K., Carratore, F. D., Breitling, R., Takano, E., Putri, S. P., and Fukusaki, E. (2020). Multi-omics analysis of the effect of cAMP on actinorhodin production in *Streptomyces coelicolor*. *Frontiers in bioengineering and biotechnology*, 8, 1267.
- Noinaj, N., Guillier, M., Barnard, T. J., and Buchanan, S. K. (2010). TonB-dependent transporters: regulation, structure, and function. *Annual review of microbiology*, 64, 43-60.
- O'Connor, T. J., and Nodwell, J. R. (2005). Pivotal roles for the receiver domain in the mechanism of action of the response regulator RamR of *Streptomyces coelicolor*. *Journal of molecular biology*, 351(5), 1030-1047.

- Okamura, K., Soga, K., Shimauchi, Y., Ishikura, T., and Lein, J. (1977). Holomycin and N-propionylholothin, antibiotics produced by a cephamycin C producer. *The journal of antibiotics*, 30:334-336.
- Oppermann, U., Filling, C., Hult, M., Shafqat, N., Wu, X., Lindh, M., Shafqat, J., Nordling, E., Kallberg, Y., Persson, B., and Jörnvall, H. (2003). Short-chain dehydrogenases/reductases (SDR): the 2002 update. *Chemico-biological interactions*, 143, 247-253.
- Ostash, B., Saghatelian, A., and Walker, S. (2007). A streamlined metabolic pathway for the biosynthesis of moenomycin A. *Chemistry and biology*, 14(3), 257-267.
- Özcengiz, G., and Demain, A. L. (2013). Recent advances in the biosynthesis of penicillins, cephalosporins and clavams and its regulation. *Biotechnology advances*, 31(2), 287-311.
- Pandey, S., and Sumant, O. (2019, August). Beta-lactam and beta-lactamase inhibitors market size and share - 2028. Retrieved from <https://www.alliedmarketresearch.com/beta-lactam-and-beta-lactamase-inhibitors-market>.
- Pao, S. S., Paulsen, I. T., and Saier Jr, M. H. (1998). Major facilitator superfamily. *Microbiology and molecular biology reviews*, 62(1), 1-34.
- Paradkar, A. S., and Jensen, S. E. (1995). Functional analysis of the gene encoding the clavamate synthase 2 isoenzyme involved in clavulanic acid biosynthesis in *Streptomyces clavuligerus*. *Journal of bacteriology*, 177(5), 1307-1314.

- Paradkar, A. S., Aidoo, K. A., and Jensen, S. E. (1998). A pathway-specific transcriptional activator regulates late steps of clavulanic acid biosynthesis in *Streptomyces clavuligerus*. *Molecular microbiology*, 27(4), 831-43.
- Perez-Redondo, R., Rodriguez-Garcia, A., Martin, J. F., and Liras, P. (1998). The *claR* gene of *Streptomyces clavuligerus*, encoding a LysR-type regulatory protein controlling clavulanic acid biosynthesis, is linked to the clavulanate-9-aldehyde reductase (*car*) gene. *Gene*, 211, 311-321.
- Pinilla, L., Toro, L. F., Laing, E., Alzate, J. F., and Ríos-Esteva, R. (2019). Comparative transcriptome analysis of *Streptomyces clavuligerus* in response to favorable and restrictive nutritional conditions. *Antibiotics*, 8(3), 96.
- Rana, P., Ghouse, S. M., Akunuri, R., Madhavi, Y. V., Chopra, S., and Nanduri, S. (2020). FabI (enoyl acyl carrier protein reductase)-A potential broad spectrum therapeutic target and its inhibitors. *European journal of medicinal chemistry*, 112757.
- Rana, R. R., Zhang, M., Spear, A. M., Atkins, H. S., and Byrne, B. (2013). Bacterial TIR-containing proteins and host innate immune system evasion. *Medical microbiology and immunology*, 202(1), 1-10.
- Reading, C., and Cole, M. (1977). Clavulanic acid: a beta-lactamase-inhibiting beta-lactam from *Streptomyces clavuligerus*. *Antimicrobial agents and chemotherapy*, 11(5), 852-857.
- Reid, M. F., and Fewson, C. A. (1994). Molecular characterization of microbial alcohol dehydrogenases. *Critical reviews in microbiology*, 20(1), 13-56.

- Rodríguez, E., and Gramajo, H. (1999). Genetic and biochemical characterization of the α and β components of a propionyl-CoA carboxylase complex of *Streptomyces coelicolor* A3 (2). *Microbiology*, 145(11), 3109-3119.
- Rodríguez, E., Banchio, C., Diacovich, L., Bibb, M. J., and Gramajo, H. (2001). Role of an essential acyl coenzyme A carboxylase in the primary and secondary metabolism of *Streptomyces coelicolor* A3 (2). *Applied and environmental microbiology*. 67(9), 4166-4176.
- Ryu, Y. G., Jin, W., Kim, J. Y., Kim, J. Y., Lee, S. H., and Lee, K. J. (2004). Stringent factor regulates antibiotics production and morphological differentiation of *Streptomyces clavuligerus*. *Journal of microbiology and biotechnology*, 14(6), 1170-1175.
- Santamarta, I., López- García, M. T., Kurt, A., Nárdiz, N., Álvarez- Álvarez, R., Pérez- Redondo, R., Martín, C. F., and Liras, P. (2011). Characterization of DNA- binding sequences for CcaR in the cephamycin–clavulanic acid supercluster of *Streptomyces clavuligerus*. *Molecular microbiology*, 81(4), 968-981.
- Santamarta, I., López- García, M. T., Pérez- Redondo, R., Koekman, B., Martín, J. F., and Liras, P. (2007). Connecting primary and secondary metabolism: AreB, an IclR- like protein, binds the ARE_{ccaR} sequence of *S. clavuligerus* and modulates leucine biosynthesis and cephamycin C and clavulanic acid production. *Molecular microbiology*, 66(2), 511-524.
- Saudagar, P. S., and Singhal, R. S. (2007). Optimization of nutritional requirements and feeding strategies for clavulanic acid production by *Streptomyces clavuligerus*. *Bioresource technology*, 98(10), 2010-2017.

- Saudagar, P. S., Survase, S. A., and Singhal, R. S. (2008). Clavulanic acid: a review. *Biotechnology advances*, 26(4), 335-351.
- Schröder, H., Langer, T., Hartl, F. U., and Bukau, B. (1993). DnaK, DnaJ and GrpE form a cellular chaperone machinery capable of repairing heat- induced protein damage. *The EMBO journal*, 12(11), 4137-4144.
- Shen, X. L., Dong, H. J., Hou, X. P., Guan, W. J., and Li, Y. Q. (2008). FtsY affects sporulation and antibiotic production by *whiH* in *Streptomyces coelicolor*. *Current microbiology*, 56(1), 61-65.
- Sofia, H. J., Chen, G., Hetzler, B. G., Reyes-Spindola, J. F., and Miller, N. E. (2001). Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. *Nucleic acids research*, 29(5), 1097-1106.
- Song, J. Y., Jeong, H., Yu, D. S., Fischbach, M. A., Park, H. S., Kim, J. J., Seo, J. J., Jensen, S. E., Oh, T. K., Lee, K. J., and Kim, J. F. (2010). Draft genome sequence of *Streptomyces clavuligerus* NRRL 3585, a producer of diverse secondary metabolites. *Journal of bacteriology*, 192(23), 6317-6318.
- Song, J. Y., Kim, E. S., Kim, D. W., Jensen, S. E. and Lee, K. J. (2009). A gene located downstream of the clavulanic acid gene cluster in *Streptomyces clavuligerus* ATCC 27064 encodes a putative response regulator that affects clavulanic acid production. *Journal of industrial microbiology and biotechnology*, 36(2):301-311.
- Song, L., and Florea, L. (2015). Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. *GigaScience*, 4(1), s13742-015.

- Sorek, R., Lawrence, C. M., and Wiedenheft, B. (2013). CRISPR-mediated adaptive immune systems in bacteria and archaea. *Annual review of biochemistry*, 82, 237-266.
- Stavrinides, A., Tatsis, E. C., Foureau, E., Caputi, L., Kellner, F., Courdavault, V., and O'Connor, S. E. (2015). Unlocking the diversity of alkaloids in *Catharanthus roseus*: nuclear localization suggests metabolic channeling in secondary metabolism. *Chemistry and biology*, 22(3), 336-341.
- Sun, D., Liu, C., Zhu, J., and Liu, W. (2017). Connecting metabolic pathways: sigma factors in *Streptomyces* spp. *Frontiers in microbiology*, 8, 2546.
- Taddei, F., Hayakawa, H., Bouton, M. F., Cirinesi, A. M., Matic, I., Sekiguchi, M., and Radman, M. (1997). Counteraction by MutT protein of transcriptional errors caused by oxidative damage. *Science*, 278(5335), 128-130.
- Tahlan, K., Anders, C., and Jensen, S. E. (2004). The paralogous pairs of genes involved in clavulanic acid and clavam metabolite biosynthesis are differently regulated in *Streptomyces clavuligerus*. *Journal of bacteriology*, 186(18), 6286-6297.
- Tahlan, K., Anders, C., Wong, A., Mosher, R. H., Beatty, P. H., Brumlik, M. J., Griffin, A., Hughes, C., Griffin, J., Barton, B., and Jensen, S. E. (2007). 5S clavam biosynthetic genes are located in both the clavam and paralog gene clusters in *Streptomyces clavuligerus*. *Chemistry & biology*, 14(2), 131-142.
- Tehrani, K. H., and Martin, N. I. (2018). β -lactam/ β -lactamase inhibitor combinations: an update. *Medical chemistry community*, 9(9), 1439-1456.

- Thai, W., Paradkar, A. S., and Jensen, S. E. (2001). Construction and analysis of β -lactamase-inhibitory protein (BLIP) non-producer mutants of *Streptomyces clavuligerus*. *Microbiology*, 147:325-335
- Thorvaldsdóttir, H., Robinson, J. T., and Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in bioinformatics*, 14(2), 178-192.
- Tipper, D. J., and Strominger, J. L. (1965). Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. *Proceedings of the National Academy of Sciences of the United States of America*, 54(4), 1133.
- Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H., Takebayashi, Y., and Spencer, J. (2019). β -lactamases and β -lactamase inhibitors in the 21st century. *Journal of molecular biology*, 431(18), 3472-3500.
- Trapnell, C., Pachter, L., and Salzberg, S. L. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, 25(9), 1105-1111.
- Ueda, K., Takano, H., Nishimoto, M., Inaba, H., and Beppu, T. (2005). Dual transcriptional control of *amfTSBA*, which regulates the onset of cellular differentiation in *Streptomyces griseus*. *Journal of bacteriology*, 187(1), 135-142.
- Ünsaldı, E., Kurt-Kızıldoğan, A., Voigt, B., Becher, D., and Özcengiz, G. (2017). Proteome-wide alterations in an industrial clavulanic acid producing strain of *Streptomyces clavuligerus*. *Synthetic and systems biotechnology*, 2(1), 39-48.

- Valton, J., Filisetti, L., Fontecave, M., and Nivière, V. (2004). A two-component flavin-dependent monooxygenase involved in actinorhodin biosynthesis in *Streptomyces coelicolor*. *Journal of biological chemistry*, 279(43), 44362-44369.
- Vitreschak, A. G., Rodionov, D. A., Mironov, A. A., and Gelfand, M. S. (2002). Regulation of riboflavin biosynthesis and transport genes in bacteria by transcriptional and translational attenuation. *Nucleic acids research*, 30(14), 3141-3151.
- Wang, L., Yu, Y., He, X., Zhou, X., Deng, Z., Chater, K. F., and Tao, M. (2007). Role of an FtsK-like protein in genetic stability in *Streptomyces coelicolor* A3 (2). *Journal of bacteriology*, 189(6), 2310-2318.
- Wang, X. W., and Hong, X. (1999). C-1027: antineoplastic antibiotic. *Drugs of the future*, 24(8), 847-852.
- Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics*, 10(1), 57-63.
- Ward, J. M., and Hodgson, J. E. (1993). The biosynthetic genes for clavulanic acid and cephamycin production occur as a 'super-cluster' in three *Streptomyces*. *FEMS microbiology letters*, 110(2), 239-242.
- Weber, T., Welzel, K., Pelzer, S., Vente, A., and Wohlleben, W. (2003). Exploiting the genetic potential of polyketide producing *Streptomyces*. *Journal of biotechnology*, 106(2-3), 221-232.
- Wietzorrek, A., and Bibb, M. (1997). A novel family of proteins that regulates antibiotic production in *Streptomyces* appears to contain an OmpR- like DNA- binding fold. *Molecular microbiology*, 25(6), 1181-1184.

- Wilson, D. J., Patton, S., Florova, G., Hale, V., and Reynolds, K. A. (1998). The shikimic acid pathway and polyketide biosynthesis. *Journal of industrial microbiology and biotechnology*, 20(5), 299-303.
- Wu, P., Wan, D., Xu, G., Wang, G., Ma, H., Wang, T., Gao, Y., Qi, J., Chen, X., Zhu, J., Li, Y. Q., Deng, Z., and Chen, W. (2017). An unusual protector-protégé strategy for the biosynthesis of purine nucleoside antibiotics. *Cell chemical biology*, 24(2), 171-181.
- Wu, T., Busby, R., and Houston, T. (1995). Identification, cloning, sequencing, and overexpression of the gene encoding proclavamate amidino hydrolase and characterization of protein function in clavulanic acid biosynthesis. *Journal of bacteriology*, 177(13), 3714-3720.
- Wu, Y., Kang, Q., Zhang, L. L., and Bai, L. (2020). Subtilisin-Involved morphology engineering for improved antibiotic production in actinomycetes. *Biomolecules*, 10(6), 851.
- Yamazaki, H., Takano, Y., Ohnishi, Y., and Horinouchi, S. (2003). *amfR*, an essential gene for aerial mycelium formation, is a member of the AdpA regulon in the A- factor regulatory cascade in *Streptomyces griseus*. *Molecular microbiology*, 50(4), 1173-1187.
- Yura, T., and Nakahigashi, K. (1999). Regulation of the heat-shock response. *Current opinion in microbiology*, 2(2), 153-158.
- Zazopoulos, E., Huang, K., Staffa, A., Liu, W., Bachmann, B. O., Nonaka, K., Ahlert, J., Thorson, B. S., and Farnet, C. M. (2003). A genomics-guided approach for discovering and expressing cryptic metabolic pathways. *Nature biotechnology*, 21(2), 187-190.

- Zhang, C., Sheng, C., Wang, W., Hu, H., Peng, H., and Zhang, X. (2015). Identification of the lomofungin biosynthesis gene cluster and associated flavin-dependent monooxygenase gene in *Streptomyces lomondensis* S015. *PloS one*, 10(8), e0136228.
- Li, B., and Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics*, 12(1), 1-16.
- Rees, D. C., Johnson, E., and Lewinson, O. (2009). ABC transporters: the power to change. *Nature reviews Molecular cell biology*, 10(3), 218-227.
- Rodríguez, H., Rico, S., Díaz, M., and Santamaría, R. I. (2013). Two-component systems in *Streptomyces*: key regulators of antibiotic complex pathways. *Microbial cell factories*, 12(1), 1-10.

APPENDICES

A. Compositions and Preparation of Culture Media/Buffers/Solutions

A.1. Trypticase Soy Broth (TSB)

Dissolve following in 1L dH₂O

TSB 21g

Autoclave at 121°C for 15 min

A.2. Starch-Asparagine (SA) Medium

600 ml of dH₂O was boiled and the following components were added to the water by continuous stirring:

Starch 10g

MOPS 21g

K₂HPO₄ 4.4g

Solution cooled to room temperature, completed to 800 ml with dH₂O.

Autoclave at 121 °C for 15 min.

Add following components:

L-Asparagine (10g/L) 200mL

MgSO₄·7H₂O (0.6 g/mL) 2mL

Trace element solution* 1mL

A.3. *Trace element solution (1L)

FeSO ₄ .7H ₂ O	1g
MnCl ₂ .4H ₂ O	1g
ZnSO ₄ .7H ₂ O	1g
CaCl ₂ .3H ₂ O	1.3g

A.4. TE Buffer (1X)

Tris 10 mM

EDTA 1 mM

pH is adjusted to 8.0 with HCl.

Autoclave for 15 minutes for sterilization if needed.

A.5. TE Buffer with lysozyme (1X)

Tris 10 mM

EDTA 1 mM

Lysozyme (lyophilized) 2 mg/mL

pH is adjusted to 8.0 with HCl.

A.6. TAE Buffer (50X)

Tris base	242 g
Glacial acetic acid	57.1 mL
0.5 M EDTA (pH 8.0)	100 mL

The final volume is adjusted to 1000 mL with distilled water.

A.7. Low Melting Agarose Gel (0.8%)

Agarose	0.8 g
TAE buffer (1X)	100 mL

Before pouring the gel into tray, add 1.5µl EtBr (final concentration: 0.5 µg/mL).

A.8. Chemicals/Enzymes and Suppliers

<u>Chemicals</u>	<u>Supplier</u>
Agarose	Prona
CaCl ₂ .3H ₂ O	Merck
EDTA	AppliChem
FeSO ₄ .7H ₂ O	Merck
Glacial acetic acid	Merck
K ₂ HPO ₄	Merck
L-Asparagine	Sigma
MgSO ₄ .7H ₂ O	Merck
MnCl ₂ .4H ₂ O	Merck
MOPS	Merck
RNAstable	Biomatrica
Starch	Merck
Tris base	Merck
Trypticase Soy Broth	Oxoid
ZnSO ₄ .7H ₂ O	Sigma
<u>Enzymes</u>	<u>Supplier</u>
Lysozyme	Sigma

B. Original Clean Data from Genome-wide Comparative RNA-seq

<https://www.ncbi.nlm.nih.gov/sra/?term=SRP276705>

