

GENOMIC AND PHYSIOLOGICAL CHARACTERIZATION OF MARINE BACTERIA *SHEWANELLA* ALGAE ISOLATED FROM AQUATIC ENVIRONMENT

Submitted in partial fulfillment of the requirements for the award of Bachelor
of Technology degree in Biotechnology

by

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**SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade “A” by NAAC
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of S. Keerthana (39230022) who carried out the project entitled "GENOMIC AND PHYSIOLOGICAL CHARACTERIZATION OF MARINE BACTERIA SHEWANELLA ALGAE ISOLATED FROM AQUATIC ENVIRONMENT" under our supervision from September 2022 to April 2023.

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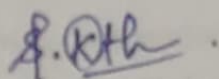
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DECLARATION

I, KEERTHANA.S (39230022) hereby declare that the Project Report entitled "GENOMIC AND PHYSIOLOGICAL CHARACTERIZATION OF MARINE BACTERIA SHEWANELLA ALGAE ISOLATED FROM AQUATIC ENVIRONMENT" done by me under the guidance of Dr. JAYSHREE NELLORE (Internal guide) and Dr. R. THIRUGNANASAMBANDAM (External guide) at Centre for Ocean Research, in Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Shewanella is a genus of Gram-negative bacteria that is widely dispersed in the aquatic environment. While the members of this genus have quickly proliferated within the past decade, numerous species have become emergent diseases worldwide, grabbing the attention of the medical community. The present study, the microorganisms were isolated from tissues of infected shrimp obtained from diseased aquaculture ponds. The isolates were obtained following sub-culturing on tryptic soy agar (TSA) plates for the selection of *Shewanella algae*. The Illumina (Novaseq) 6000 platform was used for whole genome sequencing (WGS), and the genome was assembled using SPAdes and the Velvet optimiser. Furthermore, Prokka, a quick prokaryotic genome tool, was used to predict and annotate genes. The genome of *Shewanella algae* RT-3 strain has one circular chromosome with 4.9 MB long. *Shewanella algae* RT-3 strain contains 4478 predicted genes with an average of 52.93% GC content. Kyoto Encyclopedia of Genes and Genomes (KEGG) databases- 1192 functional groups were annotated. Moreover, GC-MS analyze review the 21 metabolites from *Shewanella algae* strain. Also, Fourier transform infrared (FTIR) spectroscopy was also applied to analyze the functional composition of the representative bacterial strain. The potential of bacterial whole-genome sequencing (WGS) to supplement the existing investigative genomic architecture and to understand the phenotype of bacterial strain.

Keywords:

Whole genome sequencing; Illumina (Novaseq) 6000; *Shewanella algae*; functional annotation; Gene prediction

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ABBREVIATIONS

A	Adenine
Bp	Base Pair
C	Cytosine
DNA	Deoxyribonucleic acid
EPS	Exo polymeric substances
GO	Gene Ontology
IS	Insertion-sequence
KAAS	KEGG Automatic Annotation Server
Kbp	Kilo Base Pairs
KEGG	Kyoto Encyclopedia of Genes and Genomes
LB	Luria-Bertani
Mbp	Mega base pairs
misc-RNA	Miscellaneous ribonucleic acid
NaCl	Sodium chloride
PBS	Phosphate-Buffered Saline
Rpm	Rotation per minute
rRNA	ribosomal RNA
RT	Room temperature
T	Thymine
TSA	Tryptic Soy Agar
tRNA	Transfer ribonucleic acid
tmRNA	Transfer-messenger RNA
VF _s	Virulence Factors
VFDB	Virulence Factors Database
WGS	Whole genome sequencing
µg	Microgram
mL	Milliliter

CHAPTER-1

1. INTRODUCTION

1.1 SHEWANELLA ALGAE

Shewanella is a widespread genus of facultative anaerobes found in aquatic environments. Several *Shewanella* species are known to cause human and animal illness, with *Shewanella* algae being the most typically reported. *S. algae* infections in humans include bacteremia, soft tissue infection, and intra-abdominal infection. A number of studies have found a link between *S. algae* infection and exposure to maritime environments. *S. algae* have been isolated from coastal water, sediments, and a variety of seafoods and are often found in a wide range of marine systems and foods. Furthermore, numerous *S. algae* strains have been employed as probiotics in aquaculture. Some species of *Shewanella* have been found to have potential applications in bioremediation, biocorrosion, and bioelectrochemistry. For example, *Shewanella oneidensis* has been studied extensively for its ability to reduce metals like uranium and technetium, which can help to clean up contaminated environments. In addition to their environmental importance, *Shewanella* have also been studied for their potential in biotechnology, including the production of biofuels and bioplastics. They have also been studied for their ability to create enzymes and other industrially useful compounds. Overall, *Shewanella* are an interesting bacterial community with essential ecological and biotechnological roles.

Gram-negative, facultatively anaerobic, oxidase-positive, and motile bacteria compose the genus *Shewanella* (Lemaire, Méjean, and Iobbi-Nivol 2020)(Mukherjee *et al.* 2020). The genus *Shewanella* contains approximately 70 species of Gram-negative, facultative anaerobic bacteria that live in a variety of settings ranging from marine and freshwater ecosystems to deep sea sediments to spoiled foods(Kasai *et al.* 2019). *Shewanella algae* can survive in a variety of ecological niches due to their unique physiological and respiratory versatility (for example, suboptimal environmental conditions with extreme salinity and high barometric pressure, spoiled foods, and clinical specimens) and have been used in environmental protection and industrial development(Lemaire, Méjean, and Iobbi-

Nivol 2020). Since the discovery of *Shewanella putrefaciens* in 1931(Yousfi *et al.* 2017), Several *Shewanella* species are now recognised as global diseases, capturing the medical community's attention(Ng *et al.* 2022). *Shewanella algae*, *Shewanella putrefaciens*, and *Shewanella xiamenensis*(Prachumwat *et al.* 2021)(Zong 2011)(Antonelli *et al.* 2015) have been linked to human(Ng *et al.* 2022)(Janda and Abbott 2014) and aquatic animals diseases(Cai *et al.* 2006)(Gram and Huss 1996)(Sterniša *et al.* 2020)(Prachumwat *et al.* 2020). *Shewanella algae* is also discovered in food processing and storage(Janda and Abbott 2014)

1.2 SPECIES CHARACTERIZATION

The first *Shewanellae* were identified using phenotypic features including as morphology, gramme stain, conventional biochemical reactions, and growth under various physiological settings. Molecular analyses, on the other hand, have simplified the examination, comparison, and classification of phenotypically diverse bacteria into the genus *Shewanella* based mostly on DNA: DNA hybridization and 16S rRNA sequences. As a result, several phenotypically diverse bacteria have been reclassified as *Shewanella*, and existing *Shewanella* species have been improved or given new names.

Despite evidence that the group was more diverse, most novel *Shewanella* strains were historically classed as *S. putrefaciens*(Owen, Legros, and Lapage 1978). Owen *et al.* provided conclusive evidence using DNA: DNA hybridization tests that classified various strains of *P. putrefaciens* into four distinct groups based on DNA homology. The phenotypic traits of the *Shewanellae* are as different as the settings in which they are found, as the number of species increases(Nealson and Scott 2006)(Venkateswaran *et al.* 1999).

1.3 CLINICAL MANEFESTATIONS

Skin and soft tissue infections, sepsis, hepatobiliary illness, and otitis media are all clinical symptoms of *Shewanella* species, particularly *S. algae* and *S. putrefaciens*(Janda and Abbott 2014). They are comparable to situations induced by marine halophilic bacteria like *Vibrio* and *Aeromonas*(Myung *et al.* 2009). Otitis media (29.2%), cellulitis (abscesses, ulcers, wound infections; 24.9%),

gastroenteritis (17.6%), septicaemia (11.6%), and bacteraemia (4.3%) were the most common infectious presentations associated with *S. algae*, accounting for 199 of the 239 cases.

1.4 MECHANISM OF PATHOGENICITY

The processes underlying *Shewanella* species' pathogenicity to people and animals are mainly unclear. While some *S. algae* species produce virulence factors like haemolysins, tetrodotoxin (TTX), siderophores, and exoenzymes, their exact role in disease is unknown. Recent research has identified some of the genes in *S. algae* strain MARS 14 that encode putative virulence factors (Cimmino, Olaitan, and Rolain 2016). In addition to multiple genes for chemotaxis, genes encoding a number of virulence factors were identified: six putative haemolysins; mannose-sensitive haemagglutinin (*msh*) proteins that are involved in bacterial adhesion; a bacteriocin that is an antimicrobial peptide known to have a role in bacterial interspecies competition; and genes for the types I, IV, VI and VII secretion systems that are all involved in the translocation of virulence factors from the bacterial cell (Bhattacharjee and Srivastava 1978) (Nagayama *et al.* 1994) (Zhou *et al.* 2012).

1.5 COMPUTATIONAL APPROACH

The bioinformatic tools used for whole genome sequencing are BLAST and FASTA. The Basic Local Alignment Search Tool (BLAST) is a search program for sequence similarity that can be used as a web interface or as a standalone tool (Altschul *et al.* 1990) (Feder and Bujnicki 2005). It may be used to identify species, locate domains, establish phylogeny, and compare DNA. BLAST allows a researcher to compare a query sequence to a library or database of sequences to find library sequences that resemble the query sequence beyond a specified threshold.

WGS data analysis tools were classified into five categories: assembly, genome characterization, comparative genomics, phylogeny, and whole outbreak analysis software suites. The sequencing technology, computing requirements, speed, and assembly quality of assembly tools were all evaluated. Computational

requirements were based on reported RAM usage for different benchmarking data sets, speed on stated run time for different benchmarking data sets, and assembly quality on reported N50 values and percentages for identity for various benchmarking data sets. The N50 value defines the base pair length of the smallest contig in an assembly, such that the sum of all longer or identical length contigs results in a minimum of half of the total base pair length of all contigs in the original assembly. The input/output types of genome tools for characterization were primarily evaluated. Input/output type, run duration, and topology score/accuracy were used to evaluate tools for comparative genomics and phylogeny estimation. The RAM compatibility, number of schemes, pricing, and run time of the comprehensive outbreak analysis software suites were all considered.

CHAPTER-2

2.REVIEW OF LITERATURE

2.1 SHRIMP AQUACULTURE

Shrimps (*Litopenaeus vannamei*) are small swimming shrimp with a long body that are often found in the sea and are often used for commercial purposes. They are also known as Pinkish Gold of the Ocean due to its enormous popularity, distinct flavour, and growing demand on the global market (Doan 2011). Shrimp are the most important item in India's fish send away container, accounting for almost two-thirds of the total value of fish sales(Kumar and Birthal 2004).

2.2 EPIDEMIOLOGY

In direct contrast to *Shewanella*'s favourable effects, the two most common routes of infection are occupational or recreational exposure(Janda and Abbott 2014). The physiological versatility of the genus *Shewanella* permits for its widespread distribution. As previously stated, *S. putrefaciens* was isolated from rotting butter, and it has since been discovered as a food spoiler in a variety of foods, including poultry, beef, and shellfish(Janda and Abbott 2014). Other *Shewanella* species are primarily found in marine environments(Holt, Gahrn-Hansen, and Bruun 2005), with *S. algae*, *S. putrefaciens*, and *S. xiamenensis* emerging as opportunistic infections. *Shewanella* infections are recorded seldom, yet numerous instances are documented. All published case reports of *Shewanella* infection have been extensively evaluated in order to summarise demographic profile and clinical characteristics(Yousfi et al. 2017)(Janda and Abbott 2014)(Vignier et al. 2013)(Chen et al. 2016)(Brink, Van Straten, and Van Rensburg 1995)(Rajchgot, Glicksman, and Bogoch 2016). For the final analysis, 125 studies including 273 patients were considered.

2.3 PATHOGENICITY

S. algae appears to be more responsible for human illnesses than *S. putrefaciens* (Sharma and Kalawat 2010). The pathomechanisms that cause the

disease are not completely understood. According to current knowledge, human pathogenic *Shewanella*-sub species colonize suitable organs and cause major and eventually invasive infection in people who are predisposed(Yohe, Fishbain, and Andrews 1997)(Sharma and Kalawat 2010). Exposure to seawater has been highly linked to *Shewanella* infections(Vogel et al. 2000)(Holt, Gahrn-Hansen, and Bruun 2005). It has also been proposed that raw seafood eating may be a source of these diseases(Byun, Park, and Kim 2017). However, the sources of *Shewanella* diseases remain unknown in several documented cases. Non-human infections are also caused by members of the genus *Shewanella*. *S. algae*, for example, is a pathogen that causes abalone infection, whereas *Shewanella marisflavi* causes sea cucumber infection(Cai et al. 2006)(Li et al. 2010). *S. algae*, *S. putrefaciens*, and *Shewanella haliotis* are the *Shewanella* species implicated in human infections. Furthermore, two reported instances of *S. xiamenensis* have been documented: the first from a possible peripancreatic infection and the second from intestinal carriage(Zong 2011)(Antonelli et al. 2015). Some *Shewanella* species have been misidentified. A recent study found that current phenotypic approaches are insufficient to distinguish between *S. algae* and *S. haliotis*, and both species were linked to hepatobiliary tract and pancreatic disorders.

The process of pathogenicity of *Shewanella* remains unknown. It is hypothesised that -hemolysins involved in hemolytic action are responsible for *S. algae* being more virulent than *S. putrefaciens*(Khashe and Janda 1998). Siderophores, tetrodotoxin, and pufferfish toxin are all virulence agents found in *Shewanella algae*. Some *Shewanella* strains generate enzymes such as chitinase, lipase, protease, elastase, and alkylsulfatase, which may be involved in the pathogenicity of these bacteria(Janda and Abbott 2014). *Shewanella* can also produce biofilms, however their involvement in the pathogenicity of this genus has yet to be established(Vogel et al. 2000)(Simidu et al. 1987). It has also been postulated that the flagellum may play a role in pathogenicity by preventing engulfment and subsequent phagocytosis by macrophages(Mita et al. 2016).

2.4 ANTIBIOTIC RESISTANCE

The antibiotic susceptibility testing panel for aerobic Gram-negative bacilli

could be used to perform the micro-broth dilution method antimicrobial susceptibility testing of *Shewanella*. Huang et al. assessed the results of susceptible (S), intermediate (I), and drug resistant (R) bacteria using the American Committee for Clinical Laboratory Standards Institute (CLSI) Enterobacteriaceae standards(Huang et al. 2022). Kang et al. employed a disk-diffusion approach to test antibiotic susceptibility(Kang et al. 2013). The diameter of the zone of inhibition around each disc was measured, and the results were interpreted in accordance with the CLSI recommendations. It should be noted that because there are no unambiguous criteria for interpreting antibiotic resistance in *Shewanella spp.*, it is impossible to compare the data obtained by different authors conclusively. Although drug susceptibility varies between species in the genus *Shewanella*, these bacteria were usually susceptible to third- and fourth-generation cephalosporins, carbapenems, -lactamase inhibitor combinations, aminoglycosides, chloramphenicol, erythromycin, aztreonam, and quinolones(Yousfi et al. 2017)(Vignier et al. 2013). *Shewanella algae* and *S. putrefaciens* are typically vulnerable to aminoglycosides, carbapenems, erythromycin, and quinolones, although penicillin is usually resistant(Yousfi et al. 2017). Antibiotic resistance is on the rise in the *Shewanella* genus. Zhao et al. looked into multidrug resistance in *S. xiamenensis* isolated from a Chinese estuary water sample(Zhao et al. 2015). The strain tested positive for ampicillin, aztreonam, cefepime, cefotaxime, chloramphenicol, ciprofloxacin, erythromycin, kanamycin, and trimethoprim-sulfamethoxazole. Multi-drug resistant *S. xiamenensis* strains in the aquatic environment may become key reservoirs for resistance genes as an important *Shewanella* pathogenic species. In humans, infection can lead to clinical therapeutic failure. This genus' bacteria may be the progenitors of several antibiotic resistance genes. The oxacillinase OXA-54 found in *S. oneidensis* MR-1 was the parent of OXA-48 found in *Klebsiella pneumoniae*(Poirel, Héritier, and Nordmann 2004).

2.5 VIRULENCE FACTORS OF SHEWANELLA ALGAE

Pathogens must overcome many natural host defenses in order to bind to and infiltrate intestinal epithelial cells. Pathogen virulence factors include the ability of pathogens to infiltrate human intestinal epithelial cells via virulence factors such as flagella, homoserine lactone signal molecules, gelatinases, proteases, and

biofilm formation, all of which are required for the first stage of infectious illnesses(Dias et al. 2019) Carla et al. studied *S. putrefaciens* attachment and internalization capabilities in post-confluent human colonic cancer (Caco-2) cells, which shared morphological and functional properties with normal small intestinal cells in vitro. The findings revealed that *S. putrefaciens* could bind to and internalize Caco-2 cells. Flagella, which enhance motility, adhesion, and biofilm formation, are found in species in the genus *Shewanella*. Mannose-sensitive haem agglutination (MSHA) and extracellular DNA (eDNA) both play critical roles in microbial adhesion(Gödeke, Binnenkade, and Thormann 2012)(Thormann et al. 2004). β -haemolysins have been discovered to be involved in haemolytic activity, which is responsible for *S. algae* becoming more virulent (Khashe and Janda 1998). Moreover, *S. putrefaciens* was able to synthesize AHL molecules, which may coordinate the expression of numerous virulence factors, including the formation of biofilms(Dias et al. 2019). Chitinase, lipase, protease, elastin, and alkyl sulphate enzymes generated by some *Shewanella* species may also associate and harm host tissues, causing disruption and depletion of the mucus barrier. The genus *Shewanella* also features O-side chains or capsules with thicknesses ranging from 20 to 130 nm depending on the species (Martín-Rodríguez et al. 2017). The polysaccharide polymers on *Shewanella*'s surface aid in its adherence to solid things and consequently infectivity(Korenevsky et al. 2002). Many virulence-specific genes, including capsular polysaccharide biosynthesis, O antigen, and lasB (vibriolysin related gene), have been discovered through genome scanning(Leangapichart et al. 2021). The genes associated to type IV pili, which are controlled by external stimulation for direct movement, and the gene vasF, which is related to VAS T6SS, shed new light on *S. algae* pathogenicity. Virtually all *S. algae* strains possess the katA gene, whose product can breakdown hydrogen peroxide, protect cells from the harmful effects of hydrogen peroxide, and improve bacterial colonization in hosts. Furthermore, the discovery of genomic islands (GIs) containing a set of virulence genes and mobile elements suggested that GIs could aid in cross-species gene transfer and promote to the independent acquisition of virulence components in *S. algae*(Huang et al. 2022).

2.6 GENOME SEQUENCING

Bacterial taxonomy research necessitates the rapid application of whole-genome sequencing. Furthermore, Whole Genome Sequencing (WGS) aids in the identification of comprehensive understanding of the accumulation of individual hereditary and phenotypic microorganisms and pathogenicity of specified harmfulness factor qualities(Zhong et al. 2006).The use of genome sequences to build new diagnostic tests for recognized infections has become widespread(Balasingham et al. 2009)(Millar, Xu, and Moore 2007). WGS is the greatest tool in epidemiological typing since it allows for the detection of single genetic differences between two isolates. With the introduction of low-cost high-throughput sequencing, it is currently fast and inexpensive enough to be routinely employed in ordinary epidemiological investigations. Following the anthrax letter attack in the United States in 2001, the first attempt to sequencing a whole creature for epidemiological study was made. Sanger sequencing was used to determine the entire genome of *Bacillus anthracis* and compare it to other genotypes, indicating that the morphological variations included in the letters were not ubiquitous in the environment(Rasko et al. 2011).

2.7 KYOTO ENCYCLOPEDIA OF GENES AND GENOMES (KEGG)

KEGG (Kyoto Encyclopedia of Genes and Genomes), a bioinformatics asset helps in understanding higher- order useful implications and utilities of the cell or the life form from its genome data (Minoru Kanehisa *et al.*, 2003). It is an attempt to connect genomic data with higher order functional data by modernizing current information on cell process and by systemizing quality comments (Minoru Kanehisa, 1999). In KEGG the functional assignment is a process of integrating a set of genes in the genome with a network of interrelated molecules in the cell, such as a mechanism or a complex representing a higher order biological function which are developed by the Kanehisa Laboratory (now part of the Bioinformatics Center) in the Institute for Chemical Research, Kyoto University. While KEGG has cross-references to numerous outside databases.

2.8 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FT-IR spectroscopy is a phenotypic approach used in chemistry to assess the chemical composition of various sample types. It is a quick, non-destructive, simple, low-cost, high-throughput analytical instrument that is based on the differential modes of vibration of different chemical bonds when subjected to an infrared beam(Griffiths and De Haseth, n.d.). It became possible because to the advancement of current interferometers and Fourier transform techniques (increased analysis time, repeatability, and sensitivity), as well as powerful hardware and software analytic tools. These and many subsequent studies paved the way for the use of this methodology for discrimination, classification, and identification of bacteria at various taxonomic levels (genera, species, serotype/serogroup, or even at the strain level), a topic that has been the subject of numerous review papers over the last decade(Wenning and Scherer 2013)(Naumann 2000)(Maquelin et al. 2002). Over the years, connections between phenotypic (FT-IR) and genotypic features have been demonstrated blindly using a wide range of genotyping technologies with varying DNA targets and resolution(Rodrigues et al. 2015)(Campos et al. 2018). However, given that (i) many of these techniques do not link genomic evolution with phenotypic behaviour or key surface cell structures, and (ii) many studies have sample limitations, it is possible that some of the associations established are simply coincidences, or that the lack of correlation is not surprising(Beekes, Lasch, and Naumann 2007)(Leclère et al. 2014)(Jaya et al. 2012)(Jin, Zhang, and Martin 2017).

2.9 GAS CHROMATOGRAPHY- MASS SPECTROMETRY(GC-MS)

Because of their strong polarity and poor volatility, free FAs appear challenging to analyze by GC/MS without derivatization(Brondz 2002). Derivatization of FAs into more volatile derivatives is required to improve detection sensitivity and separation, especially for samples containing trace quantities of FAs(Eder 1995). The most common derivatives used in GC/MS analysis include methyl esters, pyrrolidide, picoliny esters, 4,4-dimethyloxazoline, and TMS. Without a doubt, the most significant disadvantage of GC/MS analysis is the difficulty of sample extraction involving derivatization. (Eder 1995) examined the methods for

determining FAs by derivatization into methyl esters and proposed that sample introduction to GC be carefully planned to minimize discrimination between high-boiling and low-boiling samples. FAMES also stated that polar stationary phases (e.g., CP Sil-88, SP-2340, and SP-2330) had low thermal stability and long retention time for FAMES despite excellent isomer separation, whereas non-polar phases (e.g., CP-Sil 5CB, DB-5, and DB-1) had high thermal stability but poor selectivity. As a result, a column with median polarity (OV-225, DB-1701, and DB-225) can be chosen to strike a balance between thermal stability and separation.

CHAPTER-3

AIM AND SCOPE

The overall aim of this project is to sequence the complete genome analysis of *Shewanella algae* strain from infected shrimp. It is also necessary to isolate the genomic DNA (gDNA) from *Shewanella algae* strains by using standard procedure, to assess the quantity and integrity of genomic DNA (gDNA), prepare DNA library and sequence the *Shewanella algae* strains to perform *de novo* assembly and Annotation studies. Furthermore, it is necessary to analyze gene prediction and functional annotation of predicted genes in *Shewanella algae*.

The objective of the present investigation is

- To isolate the genomic DNA (gDNA) from *Shewanella algae* by using standard procedure
- To assess the quantity and integrity of genomic DNA (gDNA)
- DNA library preparation and sequencing of *Shewanella algae*
- To perform *de novo* assembly and Annotation studies
- To analyze gene prediction and functional annotation of predicted genes in *Shewanella algae*

SCOPE :

The genetic and physiological characterization of *Shewanella algae* isolated from aquatic habitats will be the emphasis of this thesis. Samples will be collected from various aquatic habitats, including freshwater bodies, marine environments, and wastewater treatment plants, for the study. Various molecular techniques and microbiological tests will be used to extensively study the genomic and physiological properties of the isolated *Shewanella algae* strains. The isolated *Shewanella algae* potential use in bioremediation and other biotechnological processes will also be investigated.

CHAPTER-4

4. MATERIALS AND METHODS

4.1 GLASSWARES

Petri plates, Test tubes, Conical flask, Absorbant and Non absorbant cotton, Beakers, Micro tips, Whatman Filter Paper, Funnel, Stirrer, Micro pipette, Lighter, Spirit lamp, Marker, Eppendorf tubes, glass beads.

4.2 CHEMICALS

TN buffer (Tris HCL–NaCl), LB broth (Luria bertani), nutrient broth, Tryptic soy agar (TSA), NaCl, sea water, 70% ethanol, distilled water, chloroform, isopropanol, chilled ethanol, Phosphate Buffered Saline (PBS).

4.3 SAMPLE COLLECTION AND ISOLATION OF *SHEWANLLA ALGAE* STRAIN

Litopenus vannamei infected samples were obtained from aquaculture ponds located in Marakkanam (12.1899°N, 79.9249°E), Chennai, Tamil Nadu, India. The shrimp tissues were homogenized with TN buffer (0.02M Tris– HCl, pH 7.2, 0.4M NaCl) before being inoculated in (Tryptic soy broth) TSB with 2% NaCl and cultured for 24 hours at 37°C for colony growth. The pure colonies were identified using standard biochemical assays and preserved for future experimental study (Bauman 1984).

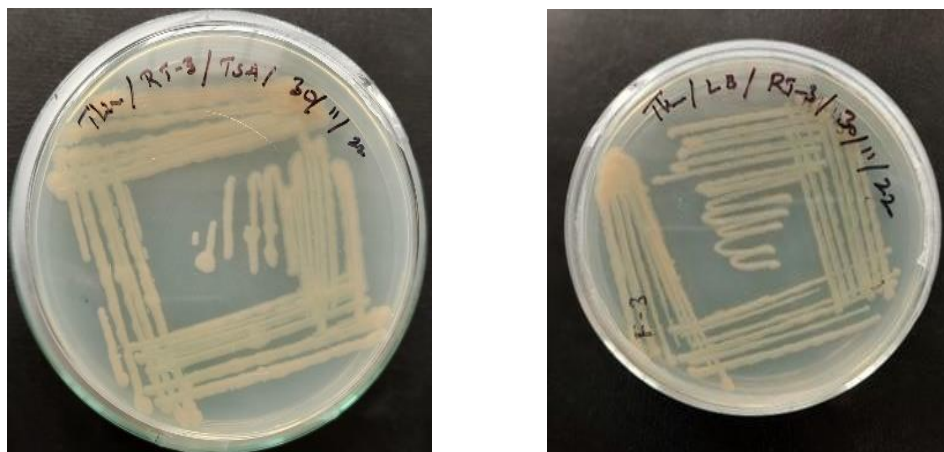
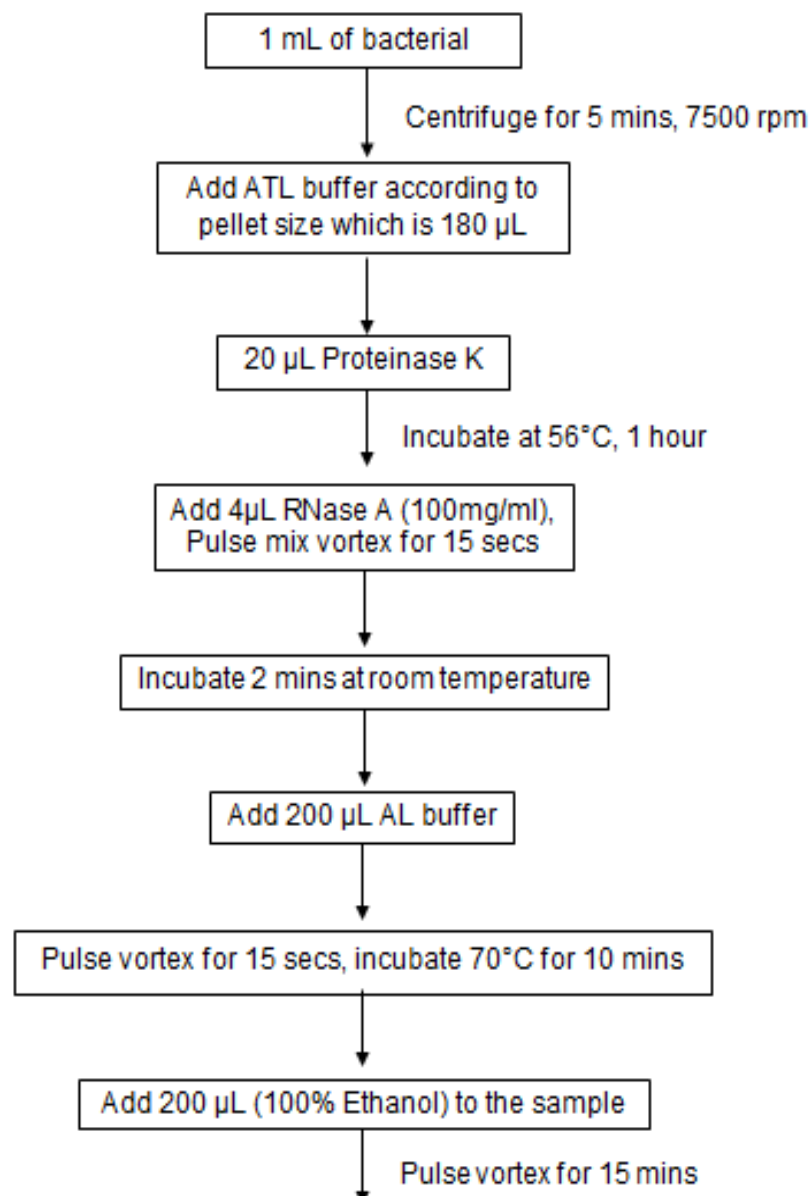


Figure 4.1 Tryptic Soy Agar (TSA) and Luria Bertani Agar Plating

4.4 DNA EXTRACTION

Total genomic DNA was extracted from RT-3 cultivated bacteria using the QIAamp DNA Mini Kit (QIAGEN) and an additional pretreatment step. The 2 mL eppendorf tubes used for the experiment were used to inoculate the results onto a TSA agar plate, which was then incubated at 37°C overnight. After that, a single colony was inoculated into 10 mL Luria Bertani (LB) broth and cultured overnight at 37°C with gentle shaking (100 rpm). The overnight culture of 10 mL was centrifuged at 7,500xg for 10 minutes before being resuspended in 180 µL ATL buffer. Proteinase (20 µL of a 10 g/liter solution, to a final concentration of 1.2 g/liter) was included to the mixture, and the cells were incubated at 56°C for 1 hour. Following incubation, 4 µL of RNase A was added to the eppendorf tubes and incubated at room temperature for 2 minutes before adding 200 µL of AL buffer and incubating the samples for 10 minutes at 70°C. 200 µL of 100% ethanol was added to the sample before it was placed to the Qi Amp small spin column. It was then centrifuged for 1 minute at 8000 rpm before adding 500 µL of AW1 buffer and centrifuging for another minute at 8000 rpm. 500 µL of AW2 buffer was added and centrifuged for 3 minutes at 14000 rpm. The tubes were then drained and centrifuged for 1 minute at 14000 rpm. 50 µL of AE buffer was added and allowed to incubate at room temperature for 5 minutes. It was centrifuged again for 1 minute at 8000 rpm before being transferred to another tube. After that, the DNA was purified using the Easy-DNA method. Its

nearly, 1476 ng of genomic DNA were used throughout the sequencing process. 1% agarose gel electrophoresis was used to analyse the extracted DNA, and the quality, amount, and integrity of the DNA were measured using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific) and an Agilent 2100 Bioanalyzer (Agilent Technologies). Sequencing was performed on DNA samples with a 260/280 nm absorbance ratio of about 1.8.



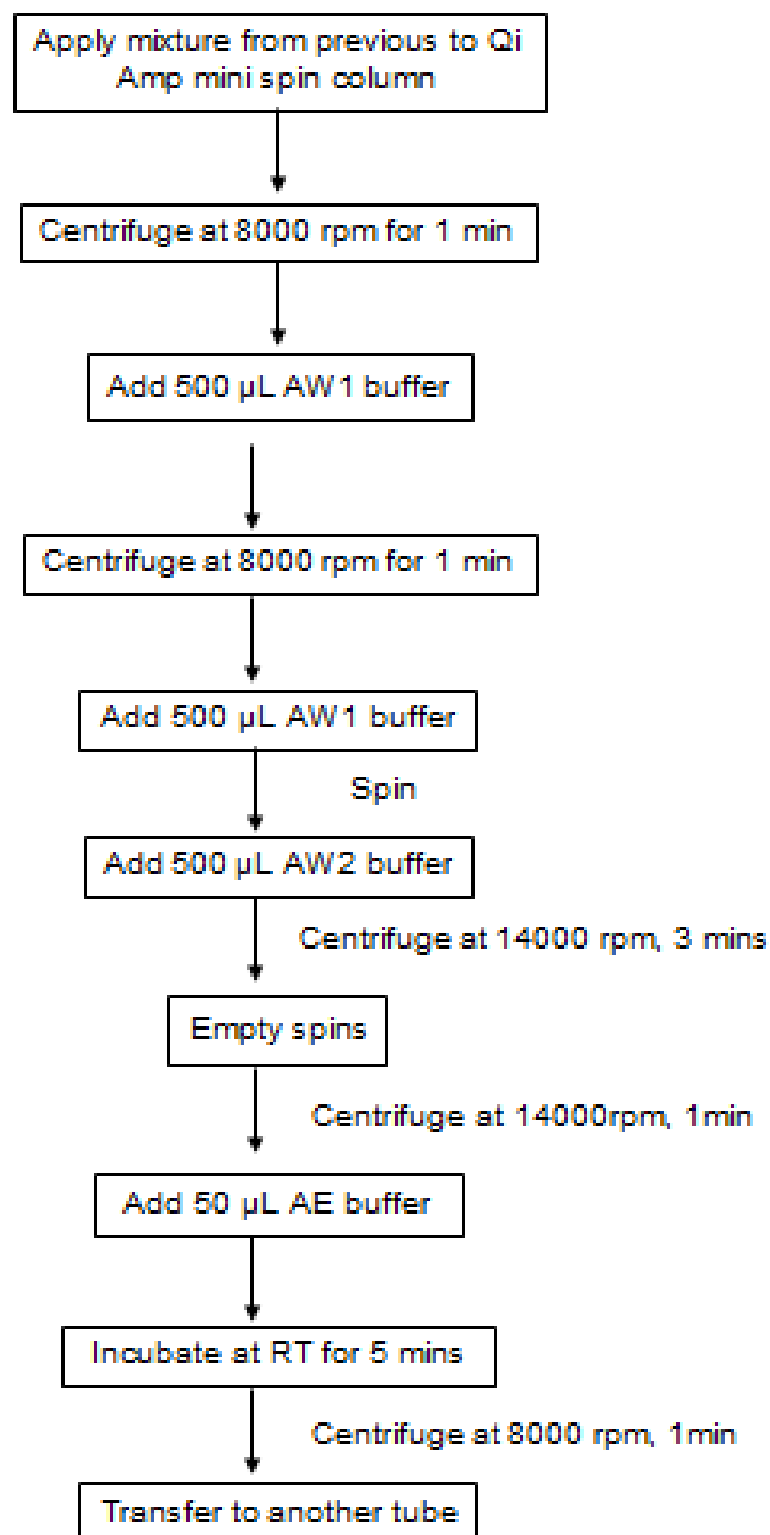


Figure 4.2 Flow chart of DNA Extraction

4.5 LIBRARY CONSTRUCTION

TrueSeq Nano DNA library preparation kit was used to create the DNA library. A total of 5 µg of genomic DNA sample was utilized to execute end repair, A-tailing, adaptor ligation, and a 400-500 bp size selection utilizing the Covaris M220 according to the manufacturer's recommendations. An Agilent Bioanalyzer DNA High Sensitivity chip was used to visualize the quality and size profiles of sheared samples. Prior to sequencing, the library quality was confirmed using Agilent DNA HS and Qubit DNA BR. The building of the genomic library was depicted in the flow chart below.

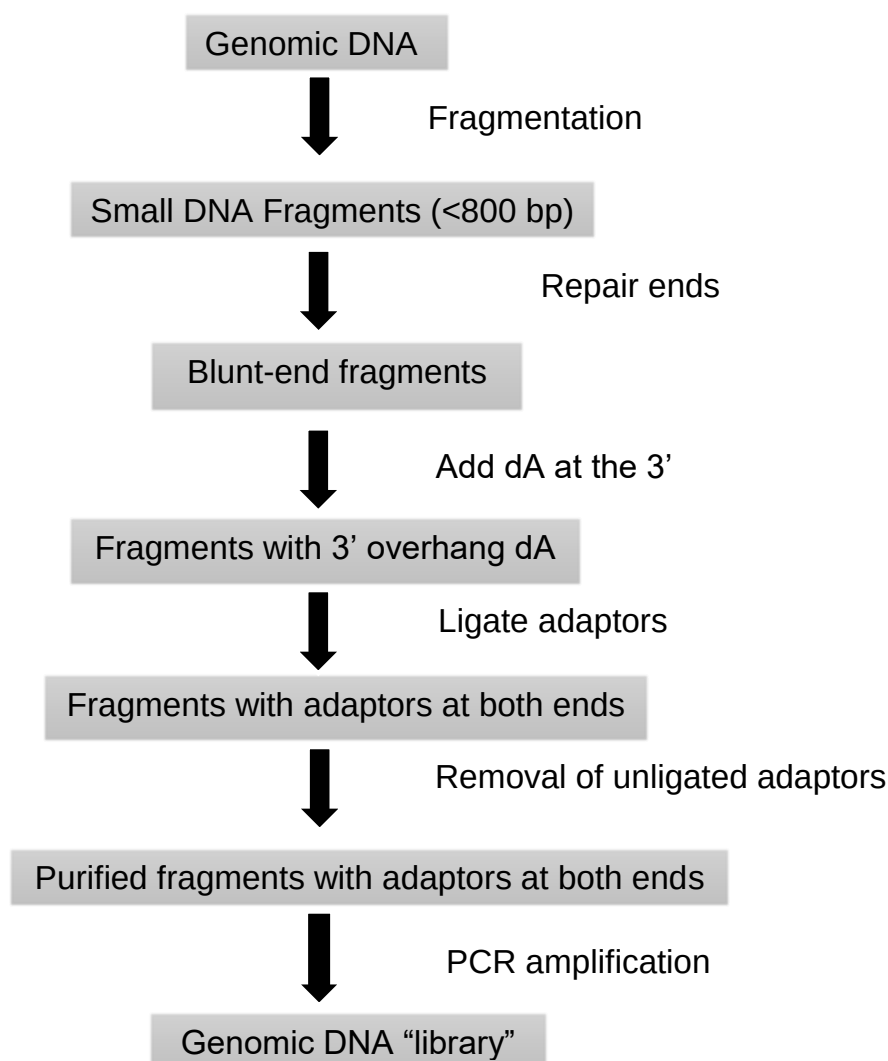


Figure 4.3 *The general procedures of library preparation for next generation sequencing*

4.6 DNA SEQUENCING

Genomic DNA was isolated from the *Shewanella algae* strain and sequenced by Sanger Sequencing on an ABI 3730 sequencer.

4.6.1 Genome Sequencing

The raw reads with PHRED quality scores <20 or shorter than 30 bp was not considered for assembling the sequence the Illumina universal adapter sequence "AGATCGGAAGAGCACACGTCTGAACTCCAGTCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT" was eliminated from all paired-end reads. The filtered paired-end readings were subjected to quality control and inventory analysis. The resulting cluster was sequenced on the Illumina Novaseq 6000 sequencing instrument using the paired end 2 × 101 bp multiplex program after library creation. The web-based Type (Strain) Genome Server (TYGS) was used to create the genome-level phylogenetic tree (Meier-Kolthoff and Göker 2019). The closest genomes were calculated using MASH algorithm and precise distance was calculated using (Genome BLAST distance phylogeny) approach under the algorithm 'GreedyWithTrimming' and distance d5. The phylogenetic tree was built with FASTME and visualized with PhyD3.

4.6.2 Data Filtering and De novo assembly

During the quality control, the raw reads were filtered by removing the following steps: (i) reads that contained adaptor contamination, (ii) reads that contained more than 5% unknown nucleotides, and (iii) low quality reads with ambiguous sequences "N" (Del Fabbro et al. 2013) (Martin 2011). Raw reads with PHRED quality scores of 20 or less than 30 bp were excluded from the building process of the sequence software Phrap. De novo assembly is a short-read assembly method. De novo genome assembly was carried out using SPAdes and the Velvet optimizer (Zerbino 2010). The assembly created by SPAdes and Velvet was integrated and minimized with minimus2 (Treangen et al. 2011).

4.6.3 Gene Prediction

Gene prediction was performed using Prokka, a quick prokaryotic genome annotation tool (Seemann 2014). The protein-coding sequences (CDS) were predicted using Prodigal (Hyatt et al. 2010). The rRNA and tRNA were predicted

using RNAmmer (Lagesen et al. 2007) and Aragorn (Laslett and Canback 2004) respectively.

4.6.4 Gene Annotation and selection of secondary metabolite clusters

Gene annotation was performed on *Shewanella algae* RT-3 strain based on homology search against the KEGG metabolic pathways were identified using KEGG Automatic Annotation Server (KAAS). Secondary metabolite gene clusters were predicted using the antiSMASH 5.0 (antibiotics and Secondary Metabolite Analysis Shell) online tool, which covers a wide range of secondary metabolite classes (Medema et al. 2011).

4.7 GAS CHROMATOGRAPHY- MASS SPECTROSCOPY (GC-MS)

GC-MS analysis of *Shewanella algae* RT-3 was performed using Shimadzu GC-MS QP2010 ultra model. The gas chromatogram interfaced to a mass spectrometer equipped with Rxi-5Sil MS (30 m, 0.2 mm, 0.25 μ m columns). The carrier gas used was helium, and the flow rate was 1 ml. The GC-MS program total running time was 50 minutes. The software used for the analysis of mass spectra and chromatograms was a Turbo mass and the relative percentage of each component was calculated by comparing its average peak area to the total area.

4.8 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

EPS was extracted by ethanol precipitation method with modifications (Chowdhury et al. 2011). Mature biofilm was grown on glass beads (2 mm diameter) in static conditions for 48 hours at 37 °C. The consumed substance was discarded after 48 hours and cleaned with 1X PBS. To disturb the biofilm, the glass beads were vortexed. The biofilm sample was collected and harvested at 4 °C for 10 minutes at 7000 rpm. By combining the supernatant with a triple volume of prechilled 100% ethanol, EPS was precipitated. The mixture was then incubated at 4 °C overnight. The precipitated EPS was separated by centrifugation at 7000 rpm for 20 minutes at 4 °C and purified through a 14 KDa molecular weight cut-off dialysis membrane (cellulose) against deionized water for 24 hours at 4 °C. The dialyzed EPS solution was then lyophilized and used for FTIR characterization studies. Using the phenol-sulphuric acid method, the extracted EPS from the ethanol precipitation extraction and the methodology established were

measured(Dubios et al. 1951). In order to determine the distribution of the functional groups, the structural characterization of the pure EPS was examined using FTIR spectroscopy between the spectral ranges 4000 and 400 cm⁻¹ utilizing IRAffinity-1s, Shimadzu, Japan(Xu et al. 2013).

CHAPTER-5

RESULTS AND DISCUSSION

5.1 GENOMIC FEATURES OF *SHEWANELLA ALGAE*

General features of strain *Shewanella algae* RT-3 and the MlxS information were summarized in Table 5.1. The Illumina Novaseq 6000 platform was generated a total of 22389016 paired end reads and with a read length of 151 bp. The raw reads were subjected to quality control, which included trimming of filtered adaptor sequences using the Cutadapt program (Martin 2011). The phylogenetic tree analysis of *Shewanella algae* RT-3 with related gene sequences using maximum likelihood and neighbor-joining algorithms has been constructed. (Figure 5.1). The processed sequences were used for functional annotation. The genome size, N50 length, and GC% content of the obtained clean reads was found to be 4.9 Mb, 173,683 bp and 52.93%, respectively. Functional annotation of the high-quality sequences generated a longest contigs of 3,97,790 with N75 of 82,251 bp, L50 length of 11 bp and L75 of 22 bp. Of the total assembled scaffolds 171 bp, were ≥ 1000 bp length. A total of 4,355 protein coding genes out of 4,478 genes were predicted using ab initio server. The strain *Shewanella algae* RT-3 contains 112 tRNAs, 10 rRNAs, and 1 tmRNA were identified in the genome (Table 5.2).

Table 5.1 Classification and General Physiological features of *Shewanella* algae RT-3

Items	Description
General features classification	Domain: Bacteria Order: Alteromonadales Family: <i>Shewanellaceae</i>
Particle Shape	rod-shaped
Gram strain	Negative
Temperature	32-37°C
Salinity	15 to 20%
pH range	5-9
Geo_loc_name	INDIA: East Coast of Chennai, Marakkanam
Lat_lon	12.1899° N, 79.9249° E
Isolation source	Aquaculture pond
Project relevance	Environmental and biotechnological
Locus Tag Prefix	B7C45
Sequencing platform	Illumina Novaseq 6000
Selection	Chip
Read type	Paired-end
Read length Fold Coverage	151 bp 100x
Assembly method	Velvet
Annotation source	GFF3, NCBI-compliant Genbank and FASTA sequences

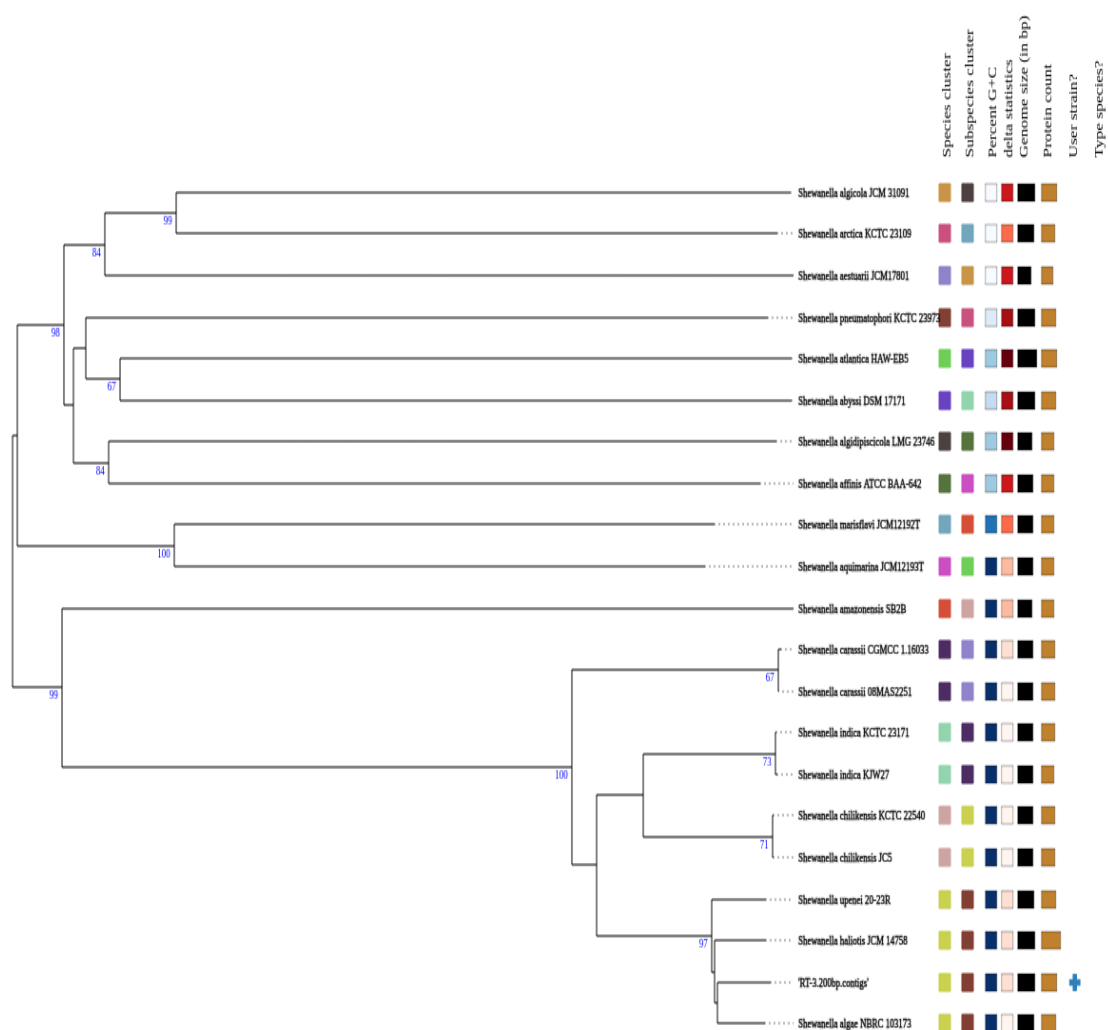


Figure 5.1 Phylogenetic tree analysis of *Shewanella algae* RT-3

Table 5.2 Genomic statistics of *Shewanella algae* RT-3

Features	<i>Shewanella algae</i> RT-3
Genome size (bp)	4,961,759
Largest Contigs	3,97,790
Number of contigs	234
N50 (bp)	173,683
N75 (bp)	82,251
L50 (bp)	11
L75 (bp)	22
G + C content (%)	52.93
Number of scaffolds	171
Protein coding genes	4,355
Predicted genes	4,478
tRNAs	112
rRNAs genes	10
tmRNA	1

5.2 KEGG PATHWAY ANNOTATION

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways annotation was helpful for biological functions of genes via interpretation of enzymes and other proteins in biochemical processes (Kanehisa and Goto 2000). The metabolic pathways annotation associated with predicted genes was identified using protein

sequences of genes through KEGG Automatic Annotation Server (KAAS)(Moriya et al. 2007). In the present study, a total of 1192 unigene sequences were classified to 210 pathways of KEGG annotated protein sequences of *Shewanella algae* RT-3. Most of the unigenes were assigned to the “amino acid metabolism” with 152 unigenes, “cell motility” with 110 unigenes and “105” with metabolism of cofactors and vitamins unigenes, forming the dominant categories. The top 20 representative pathways from KEGG pathway annotation were depicted in Table 5.5 and Figure 5.4.

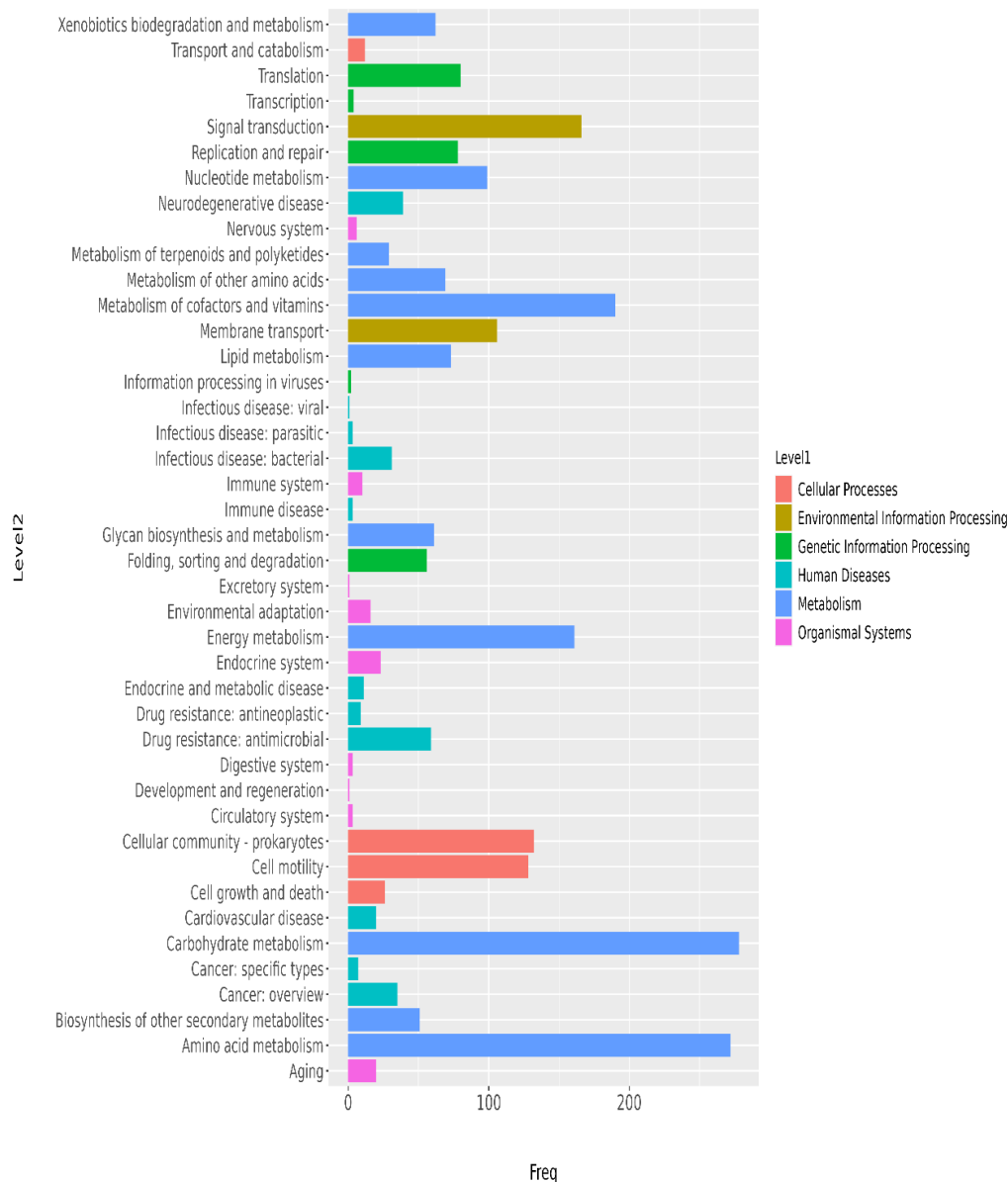


Figure 5.2 KEGG pathways associated with *Shewanella algae* RT-3 unigenes

Table 5.3 Top 20 KEGG pathways associated with *Shewanella algae* RT-3

S.No	KEGG Orthology	Pathway Description	Predicted Number of Unigenes
1	9105	Amino Acid Metabolism	152
2	9142	Cell Motility	110
3	9108	Metabolism of Co Factors and Vitamins	105
4	9101	Carbohydrate Metabolism	100
5	9145	Cellular Community - Prokaryotes	74
6	9131	Membrane Transport	73
7	9124	Replication and Repair	65
8	9132	Signal Transduction	64
9	9102	Energy Metabolism	62
10	9104	Nucleotide Metabolism	51
11	9130	Environmental Adaptation	49
12	9106	Metabolism of other amino acids	40
13	9107	Glycan Biosynthesis and Metabolism	40
14	9103	Lipid Metabolism	38
15	9110	Biosynthesis of Other Secondary Metabolites	34
16	9123	Folding, Sorting and Degradation	33
17	9175	Drug Resistance: Antimicrobial	23
18	9111	Xenobiotic Biodegradation and Metabolism	20
19	9109	Metabolism of Terpenoids and Polyketides	13
20	9143	Cell growth and Death	13

Table 5.4 Total KEGG pathway associated with *Shewanella algae* RT-3

S.No	KEGG Orthology	Pathway Description	Predicted Number of Unigenes
1	9105	Amino Acid Metabolism	152
2	9142	Cell Motility	110
3	9108	Metabolism of Co Factors and Vitamins	105

4	9101	Carbohydrate Metabolism	100
5	9145	Cellular Community - Prokaryotes	74
6	9131	Membrane Transport	73
7	9124	Replication and Repair	65
8	9132	Signal Transduction	64
9	9102	Energy Metabolism	62
10	9104	Nucleotide Metabolism	51
11	9130	Environmental Adaptation	49
12	9106	Metabolism of other amino acids	40
13	9107	Glycan Biosynthesis and Metabolism	40
14	9103	Lipid Metabolism	38
15	9110	Biosynthesis of Other Secondary Metabolites	34
16	9123	Folding, Sorting and Degradation	33
17	9175	Drug Resistance: Antimicrobial	23
18	9111	Xenobiotic Biodegradation and Metabolism	20
19	9109	Metabolism of Terpenoids and Polyketides	13
20	9143	Cell growth and Death	13
21	9171	Infectious Disease: Bacterial	8
22	9121	Transcription	4
23	9122	Translation	3
24	9174	Infectious Disease: Parasitic	3
25	9151	Immune System	3
26	9141	Transport and Catabolism	2
27	9164	Neuro Degenerative disease	2
28	9152	Endocrine System	2
29	9166	Cardio Vascular Disease	2
30	9149	Ageing	2
31	9125	Information Processing in viruses	1
32	9176	Drug Resistance: Anti Neoplastic	1
33	-	Nervous System	0
34	-	Infectious Disease: Viral	0
35	-	Immune Disease	0
36	-	Excretory System	0
37	-	Endocrine and Metabolic Disease	0
38	-	Digestive System	0
39	-	Development and Regeneration	0
40	-	Circulatory System	0

41	-	Cancer: Specific Types	
42	-	Cancer: Overview	

5.3 PREDICTION OF SECONDARY METABOLITE GENE CLUSTERS

Shewanella algae RT-3 genome includes secondary metabolite gene clusters such as non- ribosomal peptide synthesise (NRPS)- independent siderophore, hglE-KS PUFA, RiPP-like, betalactone, arylpolyene based on antiSMASH 5.0 tool analysis (Table 5.5). These clusters' biosynthetic genes are mostly made up of core biosynthetic genes, supplementary biosynthetic genes, and transport and regulatory genes. The arylpolyene biosynthetic gene cluster was revealed to be the largest and most prominent family of biosynthetic gene clusters that play a protective role in oxidative stress tolerance(Cimermancic et al. 2014). Genome mining revealed the widespread occurrence of another biologically active antimicrobial compound, RiPP, in anaerobic bacteria such as Actinobacteria, Proteobacteria, and Firmicutes(Letzel, Pidot, and Hertweck 2014). *Shewanella algae* RT-3's possession of such useful biosynthetic gene clusters allows it to adapt to harsh environments and has been utilised for pharmacological and industrial importance.

Table 5.5 Predicted secondary metabolite gene cluster of *Shewanella algae* RT-3 genome

Genomic region	Secondary metabolite	Total Length (ntd.)	Locus tag	Core Biosynthetic Gene Cluster
Region 59.1	non-ribosomal peptide synthetase (NRPS)- independent siderophore	11,893	ctg59_68	lucA_lucC
Region	hglE-KS	50,706	ctg66_50	hglE or hglD

66.1	PUFA		ctg66_52	PUFA_KS
Region 68.1	RiPP-like	7,714	ctg68_1	YcaO
Region 76.1	betalactone	29,456	ctg76_153 ctg76_159	HMGL-like and AMP-binding
Region 77.1	arylpolyene	43,630	ctg77_305 ctg77_308	APE_KS1 APE_KS2

5.4 RESISTANCE TO ANTISEPTICS AND HEAVY METALS

Genes associated with resistance to antiseptics and heavy metals included Arsenic (*arsA*, *arsR1*, *acr3*), Copper (*cusA*, *copA_1*, *copA_2*, *copA_3*, *nosL*, *actP_1*), Chromium (*chrA*), Cobalt-Zinc-Cadmium (*czcC*, *czcB*, *czcA_2*, *czcD_2*, *zntB*, *zntA*, *zur*, *veiR*), Molybdenum (*moeA_1*, *moeB*), Magnesium (*corC*, *corA*, *mgtE*), Bacteriocin (*lodA*, *lodB*, *cir A_1*, *cypA*), Enzyme biosynthesis (*menF*, *mend*, *mdh_2*, *mdh_1*), Carbohydrate metabolism (*lutC*, *lutP*, *edd*), Fatty acid metabolism (*fadR*, *fadB*, *eptA*, *atoE*, *fadJ*, *gpx1*) (Table 5.6).

Table 5.6 List of genes associated with resistance to heavy metal and other antimicrobial

Classification	Gene name	Locus tag	Description
Metal Resistance			
Arsenic	<i>arsA</i>	GNOBHPIG_01878	Arsenical pump-driving ATPase
	<i>arsR1</i>	GNOBHPIG_02469	Arsenic resistance transcriptional regulator <i>ArsR1</i>
	<i>acr3</i>	GNOBHPIG_02470	Arsenical-resistance protein <i>Acr3</i>

Copper			
	<i>cusA</i>	GNOBHPIG_02730	Cation efflux system protein <i>CusA</i>
	<i>copA_1</i>	GNOBHPIG_00561	Copper-exporting P-type ATPase
	<i>copA_2</i>	GNOBHPIG_01414	putative copper-importing P-type ATPase A
	<i>copA_3</i>	GNOBHPIG_01874	Copper-exporting P-type ATPase
	<i>nosL</i>	GNOBHPIG_02455	Copper-binding lipoprotein <i>NosL</i>
	<i>actP_1</i>	GNOBHPIG_02728	Copper-transporting P-type ATPase
Chromium			
	<i>chrA</i>	GNOBHPIG_02298	Chromate transport protein
Cobalt-zinc-cadmium			
	<i>czcC</i>	GNOBHPIG_02420	Cobalt-zinc-cadmium resistance protein <i>CzcC</i>
	<i>czcB</i>	GNOBHPIG_02421	Cobalt-zinc-cadmium resistance protein <i>CzcB</i>
	<i>czcA_2</i>	GNOBHPIG_02422	Cobalt-zinc-cadmium resistance protein <i>CzcA</i>
	<i>czcD_2</i>	GNOBHPIG_02423	Cadmium, cobalt and zinc/H(+)-K(+) antiporter
	<i>zntB</i>	GNOBHPIG_02897	Zinc transport protein <i>ZntB</i>
	<i>zntA</i>	GNOBHPIG_04346	Zinc/cadmium/lead-transporting P-type

			ATPase
	<i>zur</i>	GNOBHPIG_01111	Zinc uptake regulation protein
	<i>yeiR</i>	GNOBHPIG_01334	Zinc-binding GTPase <i>YeiR</i>
Molybdenum			
	<i>moeA_1</i>	GNOBHPIG_03806	Molybdopterin molybdenumtransferase
	<i>moeB</i>	GNOBHPIG_03945	Molybdopterin-synthase adenylyltransferase
Magnesium			
	<i>corC</i>	GNOBHPIG_00324	Magnesium and cobalt efflux protein <i>CorC</i>
	<i>corA</i>	GNOBHPIG_00351	Magnesium transport protein <i>CorA</i>
	<i>mgtE</i>	GNOBHPIG_00891	Magnesium transporter <i>MgtE</i>
Bacteriocin			
	<i>lodA</i>	GNOBHPIG_00570	L-lysine 6-oxidase
	<i>lodB</i>	GNOBHPIG_00571	Putative FAD-dependent oxidoreductase <i>LodB</i>
	<i>cirA_1</i>	GNOBHPIG_01022	Colicin I receptor
	<i>cvpA</i>	GNOBHPIG_02823	Colicin V production protein
Enzyme biosynthesis			
	<i>menF</i>	GNOBHPIG_03897	Isochorismate synthase <i>MenF</i>
	<i>menD</i>	GNOBHPIG_04000	2-succinyl-5-enolpyruvyl-

			6-hydroxy-3-cyclohexene-1-carboxylate synthase
	<i>mdh_2</i>	GNOBHPIG_02867	Malate dehydrogenase
	<i>mdh_1</i>	GNOBHPIG_02311	NAD-dependent methanol dehydrogenase
Carbohydrate metabolism			
	<i>lutC</i>	GNOBHPIG_03582	Lactate utilization protein C
	<i>lutP</i>	GNOBHPIG_03586	L-lactate permease
	<i>edd</i>	GNOBHPIG_00256	Phosphogluconate dehydratase
Fatty acid metabolism			
	<i>fadR</i>	GNOBHPIG_02171	Fatty acid metabolism regulator protein
	<i>fadB</i>	GNOBHPIG_02339	Fatty acid oxidation complex subunit alpha
	<i>eptA</i>	GNOBHPIG_01460	Phosphoethanolamine transferase EptA
	<i>atoE</i>	GNOBHPIG_00727	Putative short-chain fatty acid transporter
	<i>fadJ</i>	GNOBHPIG_01176	Fatty acid oxidation complex subunit alpha
	<i>gpx1</i>	GNOBHPIG_02655	Hydroperoxy fatty acid reductase <i>gpx1</i>

5.5 GENOMIC CIRCULAR REPRESENTATION OF *SEWANELLA* ALGAE STRAIN

The circular representations of *Shewanella algae* RT-3 genome map plotted via proksee (prokaryotic tool)(Alikhan et al. 2011)(Figure 5.3). The genomic features, GC content, ORF, and CDS of the assembly files were plotted as circular map.

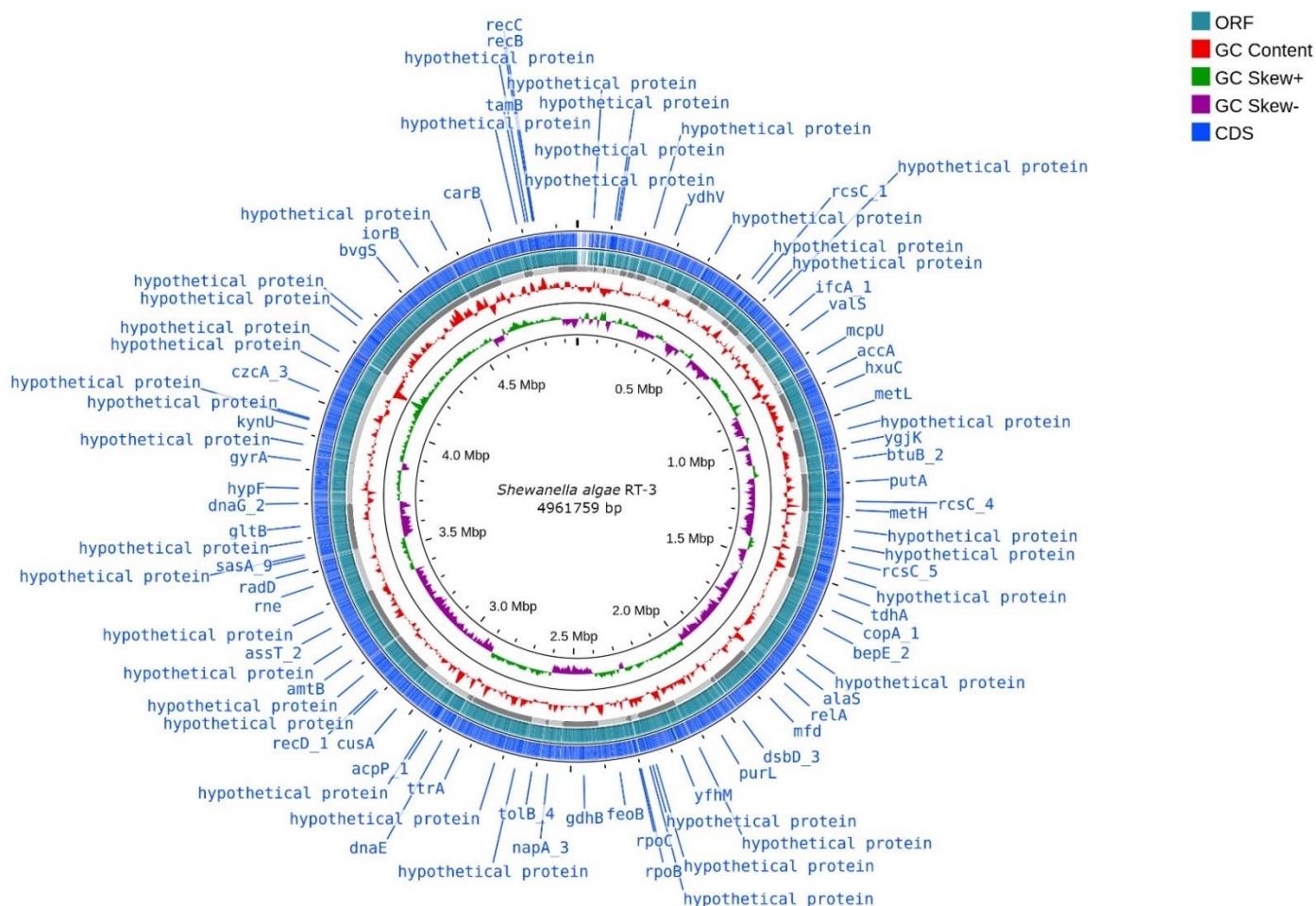


Figure 5.3 Circular representation of the genome of *Shewanella algae* RT-3 using proksee tool

5.6 GC-MS PROFILE OF *SHEWANELLA* ALGAE

The GC-MS analysis of *Shewanella algae* indicated the presence of 21 compounds respectively. The name of the compound with their retention time, and abundance (peak area in %) are presented in Table 5.3 respectively. The major compounds in *Shewanella algae* were 3-benzylhexahydropyrrolo[1,2-A]pyrazine-1,4-dione-(17.99%), formic acid, (2-fluoro-5-nitrophenyl)methyl-(14.61%), bis(2-ethylhexyl)phthalate-(10.06%), cyclo(L-prolyl-L-valine)-(8.44%), pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-(6.72%), cyclo-(l-leucyl-l-tyrosyl)-(5.85%), perhydro-htx-8-one,2-depenty-(3.87%), 4-methoxy PV8-(3.48%), 1-heneicosanol-(3.45%), 2,5-piperazinedione,3-benzyl-6-isopropyl-(2.27%).

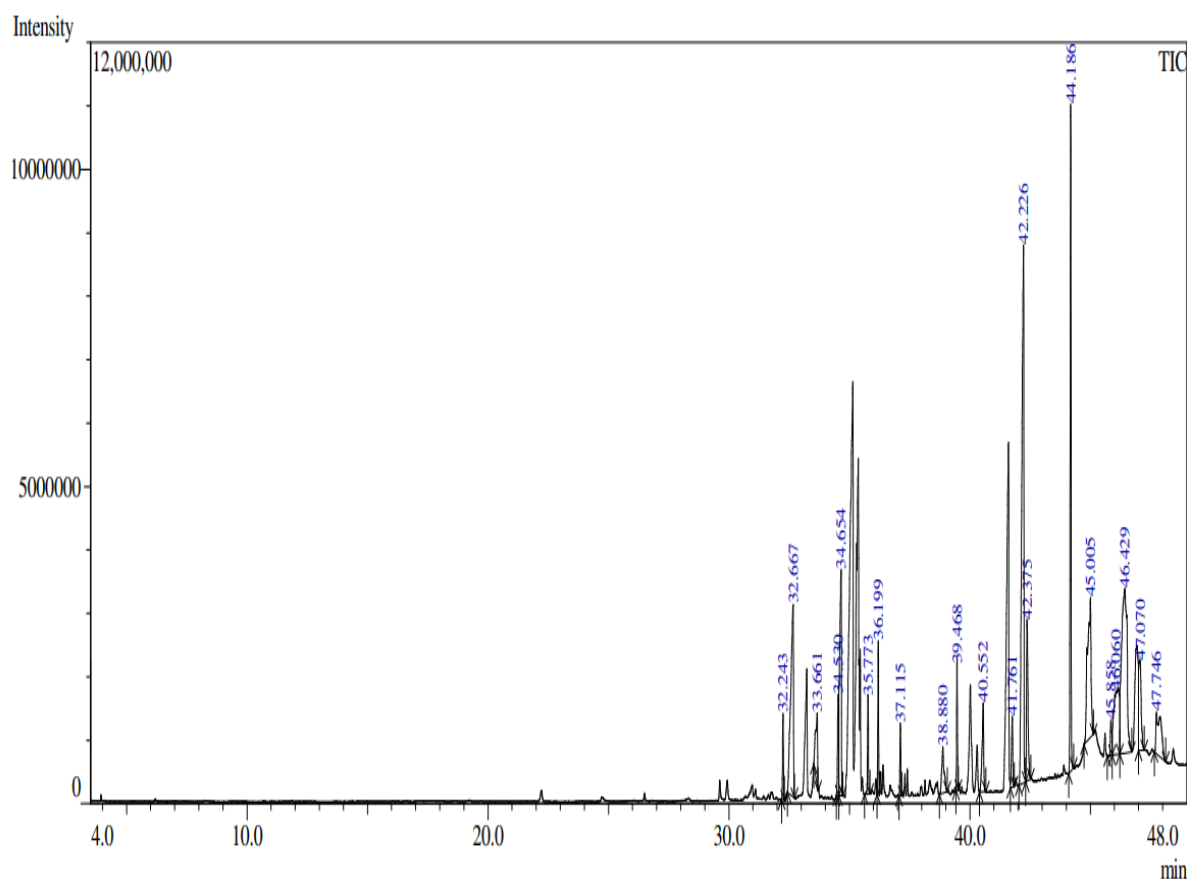


Figure 5.4 GC-MS chromatogram of *Shewanella algae* RT-3

Table 5.7 List of compounds identified from Ethylacetate extract of *Shewanella algae* by GC-MS

Peak	Retention Time	Area	Concentration	Name of the Compounds
1	32.243	3317375	1.18	n-Heptadecanol-1
2	32.667	23658628	8.44	Cyclo(L-prolyl-L-valine)
3	33.661	6315370	2.25	3,6-Diisopropylpiperazin-2,5-dione
4	34.530	5079058	1.81	Dibutyl phthalate
5	34.654	18837562	6.72	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-
6	35.773	3995898	1.42	Phthalic acid, butyl 2-pentyl ester
7	36.199	5524690	1.97	Behenic alcohol
8	37.115	2821440	1.01	Dibutyl phthalate
9	38.880	4911569	1.75	3-BENZYL-6-METHYL-2,5-PIPERAZINEDIONE
10	39.468	4638368	1.65	1-Heptacosanol
11	40.552	6379993	2.27	2,5-Piperazinedione, 3-benzyl-6-isopropyl-
12	41.761	3988177	1.42	2,5-PIPERAZINEDIONE, 3-(2-METHYLPROPYL)-6-(PHENYLMETHYL)-
13	42.226	50457126	17.99	3-BENZYLHEXAHYDROPYRROLO [1,2-A] PYRAZINE-1,4-DIONE
14	42.375	10848081	3.87	Perhydro-hx-8-one, 2-depentyl-
15	44.186	28200287	10.06	Bis(2-ethylhexyl) phthalate
16	45.005	22643536	8.07	2,5-Difluorobenzoic acid, eicosyl ester
17	45.858	1995292	0.71	3-BENZYL-6-[2-(METHYLSULFANYL) ETHYL]-2,5-PIPERAZINEDIONE
18	46.060	16402792	5.85	Cyclo-(l-leucyl-l-tyrosyl)
19	46.429	40983445	14.61	Formic acid, (2-fluoro-5-nitrophenyl) methyl
20	47.070	9765934	3.48	4-methoxy PV8
21	47.746	9689313	3.45	1-Heneicosanol

5.7 FOURIER TRANSFORM INFRARED SPECTROSCOPY

In this research study, the EPS extract from *Shewanella algae* was analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The resulting FTIR spectrum showed eight significant peaks, which were identified as specific functional groups. The first peak at 847.96936 cm^{-1} was linked to an alkene, while the second peak at 989.60820 cm^{-1} corresponded to an alkane. The third peak at 1079.06430 cm^{-1} was associated with an alkyne, and the fourth peak at 1233.74882 cm^{-1} was indicative of an amine. The fifth peak at 1543.11786 cm^{-1} was attributed to an alcohol, and the sixth peak at 1656.80167 cm^{-1} was linked to a carboxylic acid. The seventh peak at 2983.73394 cm^{-1} corresponded to a ketone, and the eighth peak at 3216.69255 cm^{-1} was associated with nitro compounds. These functional groups are known to play a significant role in biogeochemical processes, and their identification provides valuable insights into the biochemical composition of the EPS extract from *Shewanella algae*. Overall, the FTIR analysis of the EPS extract from *Shewanella algae* provided essential information about the functional groups present in the sample, which can help in understanding the microbial ecology and biogeochemistry of aquatic ecosystems.

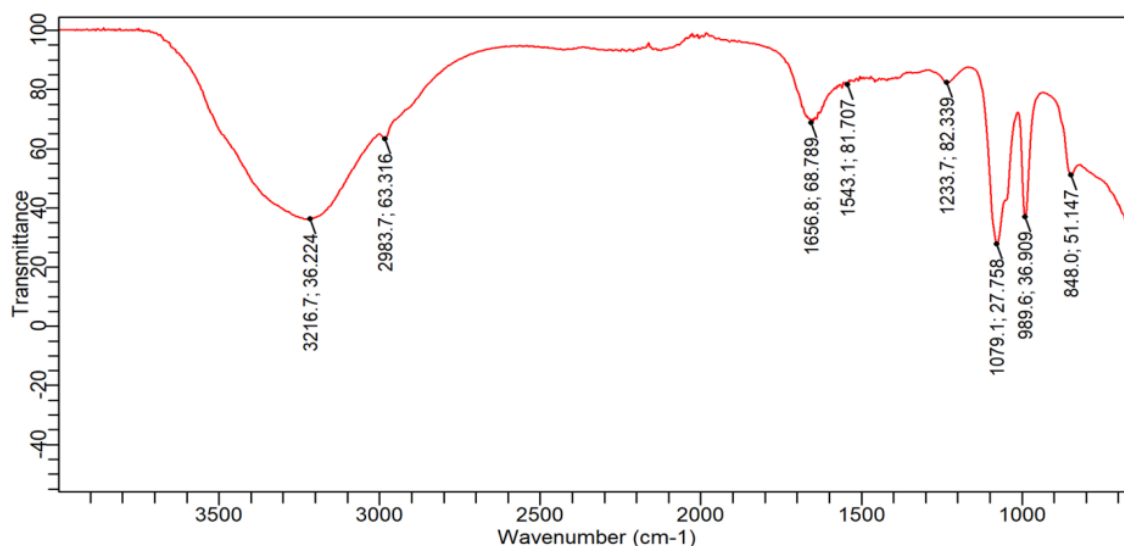


Figure 5.5 FTIR spectrum of *Shewanella algae*

Table 5.8 FTIR spectrum of *Shewanella algae* RT-3 strain indicating different functional groups

Peak number	Wavenumber (cm ⁻¹)	Intensity	Functional groups
1	847.96936	51.14684	Alkene
2	989.60820	36.90935	Alkane
3	1079.06430	27.75777	Alkyne
4	1233.74882	82.33890	Amine
5	1543.11786	81.70718	Alcohol
6	1656.80167	68.78919	Carboxylic acid
7	2983.73394	63.31638	Ketone
8	3216.69255	36.22385	Nitro compounds

CHAPTER- 6

SUMMARY&CONCLUSION

This study focused on the genetic characteristics and the phylogenetic position of *Shewanella algae* RT-3 through whole genome sequencing and bioinformatics analyses. Additionally, this information revealed a high-quality genome assembly which provides a more accurate gene annotation, genomic characterization, phylogenetic position and taxonomic classification of *Shewanella algae* RT-3 strain. Our results demonstrated that the genome was assembled into 171 scaffolds with 4,961,759 bp size and contains 4478 predicted genes with an average GC content of 52.93%. Moreover, 1192 unigenes were annotated in Kyoto Encyclopedia of Genes and Genomes (KEGG) classification. Furthermore, GC-MS analysis was performed on the 21 metabolites from the *Shewanella algae* strain. In addition, Fourier transform infrared (FTIR) spectroscopy was used to examine the functional composition of the representative bacterial strain and the intensities of various peaks from FTIR give us additional information related to the bacteria concentration. A novel and quick exopolysaccharide extraction method from the *Shewanella algae* was reported. In conclusion, these findings provide a strong platform to further understand the phenotype and pathogenic mechanism of *Shewanella algae* RT-3 strain in the shrimp *Litopenaeus vannamei*.

CHAPTER- 7

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