INTRODUCTION

Microbial biosensors are the devices that use microorganisms and enables a rapid, accurate and a sensitive detection of target analytes in different fields. For environment monitoring microbial biosensors are mainly used. Biosensors help in monitoring the contaminants present in the environment as they pose a risk to human health as well as to the environment (Chiadò et al., 2013). In the perspective of sustainable development, there has been a considerable momentum in the discharge of unplanned industrial effluents which is causing an undesirable impact on our environment. These industrial effluents have different impacts or effects on the ecosystems. The East Kolkata Wetlands has recently been declared a Ramsar site covering around 12,500 ha land area including 286 wastewater-fed fishponds. (Mukhopadhyay et al., 2007). It receives the effluents from 538 tanneries and nearly 5500 various other small-scale industrial establishments like rubber industries, electroplating industries, pigment manufacturing units, potteries, and battery manufacturing plants. Dumping of such wastes over a period of time has resulted in heavy contamination of this wetland (Chatterjee et al., 2006). Due to such anthropogenic activities, the heavy metal pollution has become a major concern today. The heavy metals are causing a major impact on soil and thus, leading to heavy metal toxicity in soil. Heavy metal toxicity hampers the ecological balance of nature resulting in risk to the survival of living beings. Heavy metal soil pollution has led to the accumulation of various heavy metals such as mercury, chromium, nickel, copper, aluminium and many more. The soil of East Kolkata wetlands contains various microorganisms such as Bacillus sp., Aspergillus sp., Penicillium sp., Fusarium sp. (Nath & Kalam, 2014) Arcella discoides and Diﬁlloqua corona (Bindu et al., 2013). The metals such as copper, nickel, chromium, cobalt, manganese are required but in trace amounts for microorganisms and therefore presence in higher concentrations leads to heavy metal toxicity and oxidative stress, which can cause damage of cellular DNA in microorganisms. To maintain a proper homeostatic balance the uptake and storage of various metals is quite important (Chen et al., 2019). Biosensor thus, acts as a sensible element and helps the microorganisms to survive and react to various metal concentrations (Chiadò et al., 2013). Siderophores play key roles of iron homeostasis in microorganisms, but there is increasing evidence implicating these chelators in the homeostasis of other metals (Schalk et al., 2011). Siderophores are produced by bacteria, fungi and plants to facilitate uptake of iron, they are low-molecular-weight chelation agents and protect from stress responses. Pyoverdines act as ﬂuorescent siderophores, plays an important role under iron-limited conditions. During the stress condition a change in the broth culture was observed where it turned green from yellow, indicating some kind of pigment release. According to some reports the pigment release happens due to the presence of Siderophore in microorganisms. This colour change can also be considered as microbial sensor for color change detection. The bacterial sample was tested for the chromium (VI) and Nickel (II) resistant capacity, presence of siderophore, DNA and protein content. The chromium (VI) and nickel (II) resistant isolates were identiﬁed as Bacillus cereus (BF2). The siderophore was detected using qualitative and quantitative assays. Using various bioinformatics softwares the siderophore produced by Bacillus cereus (Rock 3-28) was compared with the isolated culture because both of their molecular weight varied from the range of 48kDa-65kDa. National Center For Biotechnology Information (NCBI), Basic Local Alignment Search Tool (BLAST), Clustal Omega, Expert Protein Analysis System (ExPASy), Self-Optimized Prediction Method with Alignment (SOPMA) and SWISS-MODEL tools were used to study the pyoverdine protein which coded for the enzyme L-ornithine N5-oxigenase. The FASTA sequences of the query sequence were compared with other species using BLAST. The sequences of protein were used for structural and functional analysis and for phylogenetic tree construction.

Keywords: Heavy Metals, Siderophore, NCBI, SWISS-MODEL, East Kolkata Wetland
codes for the enzyme L-ornithine N5-oxgenase and produces a green fluorescent colour. Various bioinformatics tools and softwares were used for the analysis of the pyoverdine. Tools such as BLAST (to find out the sequence similarity of the known protein of the species to the other species), Clustal Omega (to find out the phylogenetic relationship between the various species), ExPasy (to study the number of amino acids, molecular weight, isoelectric point, instability index, extinction coefficient, aliphatic index and grand average of hydropathicity), SOPMA (to study the secondary structure rates of the protein) (Ishaku et al., 2019), SWISS MODEL (to study the sequence identity and GMQE) were used to study about the L-ornithine N5-oxgenase enzyme which codes for pyoverdine/siderophore A in Bacillus cereus including three-dimensional structure and Ramachandran plot analysis.

**MATERIAL AND METHODS**

**Materials and Methods**

**Sample collection**

Soil sample collection was done from different regions of East Kolkata Wetland area, Kolkata, India.

**Soil sample preparation**

The soil samples were collected from four different sites of East kolkata wetland in clean bags with the help a sterilized spatula and kept in the laboratory in room temperature for further use. East kolkata Wetland area generally have temperature ranging from 18ºC-24ºC, which is very much ideal for microbial growth. Using a mortar pestle the air dried samples were grinded. The sample was sieved properly using mesh sieve and stored for further processes. (Firdous, & Chakraborty, 2018).

**Isolation of bacteria**

Each soil sample was diluted serially in normal saline water and inoculated in Luria-Bertani agar plate which contained about 500-2000 mg/l of potassium dichromate (K2Cr2O7) and 200-800mg/l of nickel chloride (NiCl2) concentration solutions by spread plating method. All prepared 48 plates were incubated at 37ºC for 4 days. After four days of incubation single colonies were collected from Brahmi Root Soil and preserved in nutrient broth for further studies. Gram staining was performed to identify the morphology of the isolates (Ilias et al., 2011).

**Biochemical screening and sequencing**

The bacterial isolates were further tested for their biochemical characteristics and were identified using Bergey’s manual of Determinative Bacteriology (Ge et al., 2013)and further used for 16S rRNA sequencing (Bru et al., 2008).

**Protein estimation**

Protein estimation was done by using the Folin–Lowry method and then a bovine serum albumin (BSA) standard curve was made by calculating the protein concentration against optical density (OD) values at 600nm. Lowry method uses Folin–Ciocalteu as key reagent for the reaction as it involves reduction of the Folin–Ciocalteu reagent and oxidation of aromatic residues. The resultant molecule is blue in colour known as heteropolyglydulenum Blue. The concentration of the reduced Folin reagent (heteropolyglydulenum Blue) is measured by absorbance at 660 nm. As a result, the total concentration of protein in the sample can be measured. (Everette et al., 2010).

**Isolation of siderophore producing bacteria**

**Qualitative assay**

60.5 mg Chrome Azurol Sulphonate (CAS) was dissolved in 50 ml glass distilled water and then mixed with 10 ml of iron (III) solution (1Mm FeCl3•6H2O in 10mM HCl). 40ml of distilled water to which 72.9 mg of hexadecyltrimethylammoniumbromide (HDTMA) and the solution made was added. The CAS reagent (dark blue colour) was then autoclaved for 15 minutes. After incubation, the broth which was fermented was taken and centrifuged at 10,000 rpm in a cooling centrifuge at 4 ºC for 10 minutes and cell-free supernatant was mixed with 0.5 ml CAS solution. The color obtained was determined using the Spectrophotometer at absorbance of 630 nm after 20 minutes of incubation with reference containing 0.5 ml CAS solution with 0.5ml un inoculated succinate medium. The percentage of siderophore units was estimated as the proportion of CAS color shifted using the formula [(Ar - Aa) / Ar] *100.

Where, Ar is the Absorbance of reference (CAS assay solution + un inoculated media) and Aa is the Absorbance of the sample (CAS assay solution + cell-free supernatant) (Jenifer et al., 2015).

**Quantitative assay (Cas-shuttle assay)**

The quantitative estimation of siderophores, which are produced by the bacterial isolates taken from the sample can be done using CAS-shuttle assay (Schwyn, & Neilands, 1987). The strain was grown in succinate medium and incubated for 24 to 30 hrs at 28ºC with constant shaking at 120 rpm on rotator shaking incubator. After incubation, the broth which was fermented was taken and centrifuged at 10,000 rpm in a cooling centrifuge at 4 ºC for 10 minutes and cell-free supernatant was mixed with 0.5 ml CAS solution. The color obtained was determined using the Spectrophotometer at absorbance of 630 nm after 20 minutes of incubation with reference containing 0.5 ml CAS solution with 0.5ml un inoculated succinate medium. The percentage of siderophore units was estimated as the proportion of CAS color shifted using the formula [(Ar - Aa) / Ar] *100.

Where, Ar is the Absorbance of reference (CAS assay solution + un inoculated media) and Aa is the Absorbance of the sample (CAS assay solution + cell-free supernatant) (Jenifer et al., 2015).

**Determination of type of siderophore**

**Iron percolate assay**

Iron percolate assay helps in detecting the presence of hydroxamate type strain. To a 0.5ml of culture supernatant, 2.5ml of Iron percolate solution (5Mm FeCl3•6H2O in 0.1M HClO4) was added and allowed to incubate for 5min to develop the orange red colour solution and the absorbance was measured at 480nm. Desferal was used as standard (Jenifer et al., 2015).

**Arrow’s assay**

For detection of catechols, 1ml of culture supernatant was mixed with 1ml of HCl, 1ml of nitrite molydate (catechols produce yellow color),1ml of NaOH (color changes to red) followed by distilled water to make up the volume to 5ml. Absorbance was measured at 500 nm using 2,3 dihydroybenzoic acid as the standard in UV-Visible spectrophotometer (Jenifer et al., 2015).

**Statistical analysis**

Various tools like NCBI, BLAST, CLUSTAL Omega, ExPasy, SOPMA, SWISS MODEL were used. The three-dimensional structural analysis and Ramachandran plot analysis were also done (Ali, & Bala, 2016).

**RESULTS AND DISCUSSION**

**Isolation of chromium and nickel resistant bacteria**

The soil samples collected consists of a good amount of nutrients for plant nutrition (Klimiek, 2012), although presence of chromium and nickel increases the Electrical Conductivity in the soil (Firdous, & Chakraborty, 2018). Thus, the microorganisms present in this soil have both metal resistant as well as metal reducing capacity. Microorganisms having metal resistance ability is helpful in many ways including waste water treatment by decomposition of organic matter (Kavita, & Keharia, 2012). Primary study showed that all samples were having the capacity to grow by utilizing heavy metals present in their culture media (Narasinghulu et al., 2010; Nandi et al., 2012; Nandi et al., 2012; Nandi et al., 2013). According to some reports ChEA and NerB genes are responsible for chromium and nickel resistance and the molecular weight varies from 43kDa to 66kDa. As per results the strains also showed bands between 48kDa to 65kDa which confirms the presence of the genes in bacterial sample (Cervantes et al., 1990). The soil sample was spread in 500mg/l to 2000mg/l concentrations for chromium and growth was observed in 1500mg/l concentration whereas no growth was observed in 2000mg/l concentration. The same sample was spread for nickel also using 200mg/l to 1000mg/l concentration and growth was observed till 800mg/l and no growth was observed in 1000mg/l. Thus, indicating that bacteria present in this sample can tolerate chromium and Nickel up to 1500mg/l and 800mg/l concentrations respectively.

Depending on the tests the colonies were identified to be gram positive and the 16S rRNA sequencing confirmed that the strain is Bacillus cereus (BF2). Using different concentrations of heavy metal of nickel and chromium, the bacterial growth was also observed and were studied (Laila et al., 2011; Garg et al., 2012; Sevgo et al., 2010). From the soil sample a total of four single colonies (Brahmi Root Soil-5,11,17 and 19) which were isolated after growing them under proper conditions in the laboratory were later checked under the microscope for morphological and biochemical characterization. The selected bacterial isolates found to be resistant to chromium and nickel were again subjected to identification using Bergey’s manual of systemic bacteriology and found to have similar characteristics.
According to the result, bacterial isolate was gram positive, catalase positive, rod shaped, spore forming. In this study Genomic DNA was extracted from four stressed bacterial samples Brahmi Root Soil-5,11,17 and 19 including one unstressed bacteria *Bacillus cereus* (MTCC 430) and the samples were electrophoresed on 1% agarose gel against 1kb DNA ladder (Figure 1). This profiling is important to locate the gene and also to determine the genetic information. Protein profiling of the isolates was done to understand the molecular weight of the genetic material which also confirm the metal resistant genes (Masood, & Malik, 2011). 16S rRNA sequencing further confirms the strain. (Liu et al., 2006).

**Protein Estimation**

The protein analysis can be done using the BSA Standard Curve for various bacterial isolates (Ernst, & Zor, 2010). The concentration of protein was determined against the OD values at 660 nm. The colour of the broth culture changed from yellow to green when the bacterial samples were exposed to stress conditions, showing some pigment releasing activity. Due to the colour change property the protein analysis was done using BSA standard curve of the different bacterial isolates (Brahmi Root Soil 5,11,17 and 19). Thus, the protein concentration of the isolates were measured, the colour change was also observed (from yellow to green). The Table 1 depicts the concentration of protein of the bacterial strain before and after the colour change took place.

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Protein conc. before colour change of isolates (mg/l)</th>
<th>Protein conc. after colour change of isolates (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRS 5</td>
<td>0.81</td>
<td>0.84</td>
</tr>
<tr>
<td>BRS 11</td>
<td>0.83</td>
<td>0.89</td>
</tr>
<tr>
<td>BRS 17</td>
<td>0.79</td>
<td>0.86</td>
</tr>
<tr>
<td>BRS 19</td>
<td>0.87</td>
<td>0.91</td>
</tr>
</tbody>
</table>

The four stressed bacterial samples of overnight grown culture and one control unstressed sample *Bacillus cereus* (MTCC-430) were used for estimation of protein using SDS-PAGE using protein ladder (Figure 2).

**Isolation of siderophore producing bacteria**

**Qualitative and quantitative assay (CAS-Assay Shuttle)**

On CAS agar plates, the four different bacterial isolates were screened for the production of siderophore. Isolates were siderophore positive with varying intensity of yellow zones. The percentage of siderophore produced varied from 30%, 36%, 36% and 37% for the bacterial colonies Brahmi Root Soil-5,11. 17 and 19 respectively (Figure 4). For the detection of siderophore, the CAS assay is the universal colorimetric method and is based on a siderophores high affinity for ferric iron. Chrome azurol Sulphonate (CAS) dye complexes with ferric iron and the CAS assay plates are blue in color. Iron bound to CAS is easily chelated by siderophores to produce a color change from blue to orange or yellow.
The protein sequence of the enzyme L-ornithine N5-oxygenase was found to be similar to that of *Bacillus cereus* (MTCC-430). The search box was changed from all databases to protein sequence, to get the FASTA sequence (either nucleotide sequences or amino acid (protein) sequences represented in a text-based format) which the bacterial strain *Bacillus cereus* (Rock 3-28) contained. The sequence was thus retrieved using this software. The NCBI stores all the information regarding any gene, protein, and enzyme. Thus, the sequence was successfully retrieved and can consider the bacterial strain *Bacillus cereus* (Rock 3-28) a similar sequence to that of *Bacillus cereus* (MTCC-430).

The alignment score given for each colour (Figure 7). The alignment scores for the colours are below 40 depicts black, 40-50 depicts blue, 50-80 depicts green, 80-200 depicts violet and 200 or more than that means red. The alignment score of this protein sequence taken from the bacteria *Bacillus cereus* (Rock 3-28) was equal to or more than 200 since, the lines were all red in colour (McCginis, & Maddon, 2004).

**Determination of the type of siderophore**

As earlier stated, for determination of the type of siderophore we use both iron percholate assay and Arnow’s assay. Thus, after the assays were performed successfully the iron percholate assay was found to be negative while the Arnow’s assay proved to be positive. Thus, the results of the positive (Arnow’s) assay is given below:

**Arnow’s Assay**

The type of siderophore produced by the four isolates were of Phenolate type (yellow colour) and the following table represents the presence of siderophore by the OD values. Using the above OD values, the bar graph was created of the particular sequence, which was pasted in the query box from the species which was submitted and the results were displayed. From the options given in the software, a type of BLAST needs to be selected according to the sequence we choose. The types of BLAST are: BLASTn (Nucleotide BLAST), BLASTp (Protein BLAST), BLASTx (translated nucleotide to protein) and tBLASTn (protein to translated nucleotide). The protein BLAST was selected, since the query sequence which was taken from the NCBI database was a protein sequence. The sequence was submitted and the results were displayed. The tool provided results from which the sequence identity was compared of that particular sequence, which was pasted in the query box from the species which was taken from others species. The software provided with a graphic summary of the sequence and there were alignment score given for each colour (Figure 7). The alignment scores for the colours are below 40 depicts black, 40-50 depicts blue, 50-80 depicts green, 80-200 depicts violet and 200 or more than that means red. The alignment score of this protein sequence taken from the bacteria *Bacillus cereus* (Rock 3-28) was equal to or more than 200 since, the lines were all red in colour (McCginis, & Maddon, 2004).

**Figure 4** Quantitative assay for siderophore production

**Figure 5** Arnow’s assay of different bacterial strains

**Statistical analysis**

The statistical analysis were done using various bioinformatics softwares. The bacterial strain *Bacillus cereus* (Rock 3-28) was found to produce the siderophore which coded for the enzyme L-ornithine N5-oxygenase and was found to be similar to the strain *Bacillus cereus* (BF2) accession number KU955350.1.

**Sequence Analysis of the bacterial strain Bacillus cereus (Rock 3-28)**

The protein sequence of the enzyme L-ornithine N5-oxygenase (*Zwick et al., 2012*), present in the bacteria *Bacillus cereus* (Rock 3-28) which produced the siderophore A, was obtained from the database National Centre for Biotechnology Information (NCBI). A series of databases relevant to biotechnology and biomedicine which is an important resource for bioinformatics tools and services is housed by NCBI. The GenBank and PubMed (a bibliographic database) are the major databases for the getting information about
The organism *Bacillus cereus*, accession number WP_000791092.1 was found to be 100% similar to that of *Bacillus cereus* (Rock 3-28) bearing the accession number EEL31118.1.

**Phylogenetic Analysis of different strains of Bacillus cereus**

Multiple Sequence Alignment (MSA) (Jurate et al., 2013) was carried out with the rhodopsin protein sequences from different mammals using a tool, Clustal Omega. Based on the differences of physical and genetic characteristics, a phylogenetic tree is constructed to show the evolutionary relationship among various biological species. Using a multiple sequence alignment software, the phylogenetic tree is constructed. The Clustal Omega software was used for phylogenetic tree since it is a multiple sequence alignment tool. The Clustal software helps to align sequences using a heuristic approach that progressively builds a multiple sequence alignment from a series of pairwise alignments. By analyzing the sequences as a whole, the method works, then utilizes the UPGMA (Unweighted Pair Group Method With Arithmetic Mean) /Neighbour-joining method (by generating a distance matrix). The scores of the sequences in the matrix were used to build a guide tree, then subsequently the multiple sequence alignment was built by progressively aligning the sequences in order of similarity. The Clustal program accepts a wide range of input formats, including NBRF (National Biomedical Research Foundation)/PIR (Protein Information Resource), FASTA, EMBL (European Molecular Biology Laboratory) /Swiss-Prot, Clustal, GGC (Galaxy Community Conference)/MSF (Multiple Sequence Format Files), GCG/RSF, and GDE. The output format can be one or many of the following: Clustal, NBRF/PIR, GCG/MSF, PHYLIP, GDE, or NEXUS. In the query box, set it as protein or gene according to the sequence to choose. The accession number of different strains of *Bacillus cereus* were taken from BLAST software and using the NCBI database the FASTA sequences were obtained. They were pasted in the query box of the software Clustal Omega and then multiple sequence alignment was executed. This software helps to create a phylogenetic relationship between different species (Figure 8) (Sievers et al., 2011). From Table 2, the accession number can be compared given in the table for understanding the phylogenetic tree.

![Figure 8 Phylogenetic tree using the software CLUSTAL Omega](image)

**Table 2** BLAST results showing percent identity between *Bacillus cereus* (Rock3-28) and other Bacillus strain (Zwick et al., 2012)

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Organism</th>
<th>Percent Identity</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bacillus cereus</em></td>
<td>100%</td>
<td>WP_000791092.1</td>
</tr>
<tr>
<td>2.</td>
<td><em>Bacillus cereus</em></td>
<td>99.77%</td>
<td>WP_001060358.1</td>
</tr>
<tr>
<td>3.</td>
<td><em>Bacillus cereus</em></td>
<td>99.07%</td>
<td>WP_016077101.4</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacillus cereus</em></td>
<td>98.83%</td>
<td>WP_046958457.1</td>
</tr>
<tr>
<td>5.</td>
<td><em>Bacillus cereus</em></td>
<td>98.60%</td>
<td>WP_097806060.1</td>
</tr>
<tr>
<td>6.</td>
<td><em>Bacillus cereus</em></td>
<td>98.37%</td>
<td>WP_098587382.1</td>
</tr>
<tr>
<td>7.</td>
<td><em>Bacillus cereus</em></td>
<td>98.14%</td>
<td>WP_135277110.1</td>
</tr>
<tr>
<td>8.</td>
<td><em>Bacillus cereus</em></td>
<td>97.90%</td>
<td>WP_01060354.1</td>
</tr>
<tr>
<td>9.</td>
<td><em>Bacillus cereus</em></td>
<td>96.27%</td>
<td>WP_016119253.1</td>
</tr>
</tbody>
</table>

**ExPASy Analysis**

ExPASy is a bioinformatics tool for finding the physico-chemical properties of the protein, is operated by the Swiss Institute of Bioinformatics (SIB) and in particular the SIB Web Team. It is an extensible and integrative portal for accessing many scientific resources, databases and software tools in different areas of life sciences. The ExPASy software allows to find out the molecular weight, isoelectric point, extinction coefficient, aliphatic index, instability index and the grand average of hydropathicity of the protein. The UniProt KB software is used to find the accession number of the protein of the bacteria *Bacillus cereus* (Rock 3-28). The accession number is taken and pasted in the query box of the ExPasy software. The following results were obtained which are presented in Table 3. Physico-chemical properties like number of amino acids, molecular weight (M.WT) (Tran et al., 2015), isoelectric point (pI), Extinction coefficient (EC), Aliphatic index (AI), Grand Average Hydropathcy (GRAVY) and Instability index (Ii) of the sequences were analysed.

**Table 3** ExPasy results of the enzyme L-ornithine N5-oxgenase of *Bacillus cereus*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Accession No.</th>
<th>No. of Amino Acids</th>
<th>Mol.Wt</th>
<th>pI</th>
<th>EC</th>
<th>AI</th>
<th>Ii</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em> (Rock 3-28)</td>
<td>EEL31118</td>
<td>429</td>
<td>49180.50</td>
<td>5.85</td>
<td>40800</td>
<td>91.10</td>
<td>39.02</td>
<td>0.423</td>
</tr>
</tbody>
</table>

The number of amino acids in the enzyme produced is 429. The molecular weight was found to be around 49180.50 Da (or 49.180 kDa). The isoelectric point was found to be around 5.85, the extinction coefficient was 40800, aliphatic index was 91.10, instability index was 39.02 and the grand average of hydropathicity was -0.423. Thus, these following results in Table 3 were obtained using the ExPasy software (Gasteger et al., 2003).

**SOPMA Results**

SOPMA software helps in finding out the secondary structure rates of the protein that the species codes. This software helps in detecting the alpha helix, extended strand, beta turn and coil of the protein (Bhattacharya et al., 2017). Secondary structure of proteins was predicted using SOPMA with the default parameters (window width: 17; similarity threshold: 8; number of states: 4) (Table 4).

**Table 4** Secondary structure rates of the protein of the species using SOPMA software

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Alpha Helix</th>
<th>Secondary Structure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extended Strand</td>
<td>Beta Turn</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> (Rock 3-28)</td>
<td>161 is 37.53%</td>
<td>77 is 17.95%</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> (Rock 3-28)</td>
<td>170 is 39.93%</td>
<td>21 is 4.90%</td>
</tr>
</tbody>
</table>

The protein FASTA sequence is taken from the NCBI database and pasted in the query box. The sequence of protein from the bacterial strain *Bacillus cereus* (Rock 3-28) was taken and later the results were derived as given in the table below. The Table 4 detects the alpha helix, extended strand, beta turn and coil of the protein of *Bacillus cereus* (Gourjon, & Delage, 1995).

**SWISS MODEL of the protein coded by bacteria Bacillus cereus** (Rock3-28)

Swiss Model is a homology-modelling server for protein structure which is fully automated, accessible via the ExPASY web server, or from the program DeepView (Swiss-Pdb-Viewer). This tool makes protein modelling accessible to all life science researchers worldwide. The software is accessed through the linkhttps://swissmodel.expasy.org/. Swiss model is a 3D structure prediction tool. It require a viewer such as DeepView - Swiss-Pdb-Viewer, Rasmol, Cn3D v3.0 or WebMol Java PDB (Glaxo-Wellcome Experimental Research, Switzerland). Based on maximum similarity or identity with sequence the template selection is done (Roy et al., 2017; Biasini et al., 2014; Waterhouse et al., 2018). After clicking on start modelling it takes to a page where the query protein sequence is pasted and then click on build model. The following information given in Table 5 was provided by the software along with the ligands, three-dimensional structure of the protein and the Ramachandran plot results (Schweedeet al., 2003). Figure 9 shows the structure of the protein. The oligo-state of the protein is a homo-tetramer.

**Table 5** Results of the protein of *Bacillus cereus* (Rock 3-28)

<table>
<thead>
<tr>
<th>Species</th>
<th>Template</th>
<th>Seq. Identity</th>
<th>GMQE</th>
<th>QMean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em> (Rock 3-28)</td>
<td>Scnu.A</td>
<td>34.94%</td>
<td>0.70</td>
<td>-1.59</td>
</tr>
</tbody>
</table>

GMQE- Stands for Global model quality estimate
The soil sample was found to have the presence of the bacteria *Bacillus cereus* which was Chromium (VI) and Nickel (II) resistant and produces siderophores. Siderophores facilitate the iron uptake content in bacteria under iron-limiting conditions. The CAS blue agar test when performed on the bacterial strain produced a fluorescent yellow colour which later changed to green producing a pigment containing siderophores. The various assays like iron-percholate assay and Arnow’s assay were used to find out the type of siderophore the bacteria produced. The siderophore produced was found to be pyoverdine, coding for the gene *pvd A* and the enzyme L-ornithine N5-oxgenase. It was a study of transcriptional profiling of copper stressed bacteria. The experiment was done under metals nickel and chromium with a different genera group. Since, the bacteria isolated from the soil sample *Bacillus cereus* (BF2) accession number KU955350.1 was found to be similar to that of *Bacillus cereus* (Rock 3-28) bearing the accession number EEL31118.1 because of the molecular weight being within the range of 48 kDa - 65 kDa, various statistical analyses were done using the bioinformatics tools. The protein sequence was isolated, sequenced with the help of SWISS MODEL software. The results of the species *Bacillus cereus* (Rock 3-28) which were derived from the SOPMA software are given in Table 6.

### Table 6 Showing the results of the protein *Bacillus cereus* (Rock 3-28)

<table>
<thead>
<tr>
<th>Species</th>
<th>MolProbity Score</th>
<th>Ramachandran Plot (%)</th>
<th>Ramachandran Outliers (%)</th>
<th>Rotamer Outliers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>1.84</td>
<td>93.24%</td>
<td>1.33%</td>
<td>0.79%</td>
</tr>
<tr>
<td>(Rock 3-28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Zwicket al., 2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Ramachandran plot is a plot of the torsional angles - phi (φ) and psi (ψ) - of the residues (amino acids) contained in a peptide. The protein structural scientists make a Ramachandran plot to determine the torsional angles which are permitted and can obtain insight into the structure of peptides. Figure 10 shows the Ramachandran plot of the enzyme L-ornithine N5-oxgenase present in the bacteria *Bacillus cereus* (Rock 3-28).

### Figure 9 Three dimensional structure of L-ornithine N5-oxygenase of *Bacillus cereus* (Rock 3-28)

### Ramachandran Plot Analysis using the SWISS-MODEL software

Protein conformation was studied properly by Ramachandran plot analysis. Torsional angles (φ, ψ) of the Ramachandran plot describe the polypeptide as well as protein conformation (Hollingsworth et al., 2010). Ramachandran plot analysis was done with the help of SWISS MODEL software. The results of the species *Bacillus cereus* (Rock 3-28) which were derived from the SOPMA software are given in Table 6.

### Figure 10 Ramachandran plot of L-ornithine N5-oxygenase of *Bacillus cereus* (Rock 3-28)

### CONCLUSION

The various assays like iron-percholate assay and Arnow’s assay were used to find out the type of siderophore the bacteria produced. The siderophore produced was found to be pyoverdine, coding for the gene *pvd A* and the enzyme L-ornithine N5-oxgenase. It was a study of transcriptional profiling of copper stressed bacteria. The experiment was done under metals nickel and chromium with a different genera group. Since, the bacteria isolated from the soil sample *Bacillus cereus* (BF2) accession number KU955350.1 was found to be similar to that of *Bacillus cereus* (Rock 3-28) bearing the accession number EEL31118.1 because of the molecular weight being within the range of 48 kDa - 65 kDa, various statistical analyses were done using the bioinformatics tools. The protein sequence was isolated, sequenced with the help of SWISS MODEL software. The results of the species *Bacillus cereus* (Rock 3-28) which were derived from the SOPMA software are given in Table 6.

<table>
<thead>
<tr>
<th>Species</th>
<th>MolProbity Score</th>
<th>Ramachandran Plot (%)</th>
<th>Ramachandran Outliers (%)</th>
<th>Rotamer Outliers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>1.84</td>
<td>93.24%</td>
<td>1.33%</td>
<td>0.79%</td>
</tr>
<tr>
<td>(Rock 3-28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Zwicket al., 2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Ramachandran plot is a plot of the torsional angles - phi (φ) and psi (ψ) - of the residues (amino acids) contained in a peptide. The protein structural scientists make a Ramachandran plot to determine the torsional angles which are permitted and can obtain insight into the structure of peptides. Figure 10 shows the Ramachandran plot of the enzyme L-ornithine N5-oxgenase present in the bacteria *Bacillus cereus* (Rock 3-28).

### Acknowledgments

The author acknowledge Dr. Abhijit Sengupta, Director and Dr. Lopamudra Dutta Principal-in-charge at Guru Nanak Institute of Pharmaceutical Science and Technology who gave such a golden opportunity to do this wonderful project. The author would also like to extend their gratitude for the funding by the Department of Science and Technology, Government of West Bengal vide sanction No 35 (Sanc) ST/PS&T/2G-5/2015 and Department of Environment, Government of West Bengal Memo No. 1220/3K(Bio)-3/2019 dated 11/11/2019.

### REFERENCES


