Computational Analysis of Textile Dyes Decolorizing Bacteria Found in Industrial Waste Water

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Abstract

The textile industry not only plays a crucial role in our everyday lives, but it is also a major factor in developing the global economy. One of the environmental issues is the disposal of large amounts of radioactive dyes into the water, leading to significant environmental contamination. Bacillus subtilis , a bacteria with biodegradation property found in Punjab water bodies that decolorize the industrial waste water because bacteria contain laccase enzyme that is multi copper protein that can oxidize inorganic and aromatic deys such as azo dyes , syringaldazine and 2, 6-dimethoxyphenol. Bacteria is more pronounced than fungus because it contain spore coat A type protein that help them to survive in unfavourable conditions such as high temperature and ph. Our present study deals with Characterisation of Bacillus subtillus bacteria containing Spore coat protein (Uniprot id : M9Y1F2, Gene id: cotA),textile dye degrading enzymes laccase, through analyzing their structural and functional properties using standard computational tools. The spores were used for bleaching RBBR, alizarin, Congo red, methyl orange, and methyl violet. The bleaching rate was 90% in the treatment of RBBR and alizarin red. Physico-chemical characterization confirmed acidic and hydrophilic nature of both laccase enzyme . Higher aliphatic index ascertained the thermostability of laccase. Negative GRAVY value of the laccase confirmed better water interaction of the enzyme. Functional Analysis revealed that bacteria contain spore coat protein with oxidoreductase activity, with three functional domains Cu-oxidase (PF00394), , Cu-oxidase_2(PF07731), Cu-oxidase_3(PF07732) that help in survive under harsh conditions. Laccase has high substrate selectivity and more effective in dye decolorization. To know the bacterial laccase effect on dye by docking, MGL Tool and AutoDock suit are used.

Keywords: *Dye decolorization, Laccase, Textile dyes, Microbial bioremediation.*

Introduction

Synthetic dyes are poly-aromatic molecules that give a permanent colour to materials like textile fabrics. With an annual output of around 280,000 tonnes, over 100,000 commercial synthetic dyes, including several grades, have been produced worldwide. These synthetic dyes are widely used in textile, paper, food, cosmetics, and pharmaceutical industries with the textile industry as the largest consumer [1]. They are chemically and photochemically stable and are highly stable in natural environments. Textile dyes are chemically diverse in nature and are broadly divided into azo, reactive, acidic, basic, triphenylmethane, anthraquinone, based on heterocyclic, polymeric structures, etc. [2]. A huge amount of dye and water are used in the textile industry for dying. The textile industry annually discharges 30.000 to 150,000 plenty of dyes in water bodies causing severe pollution. The toxicity of dye-containing wastewater varies with the type of dye used in the textile industry. Dye impact colour to water and is thus visually identifiable in water. Colour cause hindrance in light penetration, which subsequently inhibits the method of photosynthesis. This may cause depletion of dissolved oxygen (DO) and deterioration of water quality and cause severe toxic effects on aquatic life [3]. India is the second largest exporter of dyestuffs and intermediates after China.

Especially in textile industries produced more than 70% of the total quantity of waste in India. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80% However, there are associated problems resulting from the introduction of industrial waste products into the environment [4]. Azo dyes are the most constituents of such pollution due to their wide applicability and usages, and thus, these are present majorly in textile industrial effluents. Azo bonds present in these compounds are immune to breakdown, with the potential for the persistence and accumulation within the environment. However, they can be degraded by bacteria under aerobic and anaerobic conditions [5, 6].

Microorganisms can play a very significant role in decomposition and ultimate mineralization of these dyes. Environmental biotechnology is based on the ability of a microorganism (both bacterial & fungal) to decompose larger chemical compounds, which are xeno-biotics. A large number of dyes belonging to various groups have been isolated and many microbial strains with the ability to decolorize have been studied in depth by several researchers. Biodegradation of reactive azo dyes present in textile wastewater is a complicated procedure due to versatility in the structure of dyes [7]. The general approach to bioremediation is to enhance organism's natural degradation capability. Several microorganisms have been reported by a number of investigators which have the capacity to decolourize various textile azo dyes. Degradation of azo dyes creates carcinogenic and mutagenic aromatic amines. Recently, many studies have shown that microorganisms are not only capable of decolorizing dyes, but also of detoxifying them. The process of biodegradation may be a well-established and powerful technique for treating domestic and industrial effluents. Microbial populations have a tremendous and extensive capacity to degrade a spread of organic compounds. Currently, extensive research is being focused on finding optimal microbial biomass that might be as cheap as possible for the removal of contaminating dyes from a large volume of polluted water. In this study, the screening of bacteria from dye effluent was done for adapting them for maximum removal of textile dye [8].

There are various bacteria, fungi, algae, plants and other microorganism that would help in textile dye decolorization and they have particular domains, enzymes, genes, protein for dye decolorization. Aerobic mixed bacterial culture comprised of five isolates (*Bacillus vallismortis, B. pumilus, B. cereus, B. subtilis and B. megaterium*) identified by 16srDNA analysis was developed from wastewater samples from the aeration tank of an effluent treatment plant of a textile and dyeing industry and evaluated for its ability to decolorize azo dye [9, 10]. The bacterial strain Bacillus sp. showed decolorizing activity through a degradation mechanism rather than adsorption. Sample of waste water collected from JCT Mill Phagwara, Abhishek industry, Baranala, Sheetal Industry, Jalandhar [11].

Initially twenty four bacterial isolates were screened based on their ability to decolorize a wide spectrum of dyes efficiently such as Black WNN, Blue FNR, Red FN2BL, Blue RC, TURQ Blue and Diresul RDT Black dye, by a rapid microtiter plate screening method [12]. Various microorganisms are able to metabolize azo dyes and other by biosorption and biodegradation, involving enzymatic mechanisms such as those associated with lignin peroxidases, manganese peroxidases, laccases and azoreductases [13].

Bacillus subtilis bacteria selected that is found in Punjab. Gram-positive bacterium that is commonly recovered from soil, water, air and decomposing plant**.** *Bacillus subtilis* strain exhibiting laccase activity. Laccases catalyse the removal of one hydrogen atom by electron abstraction from phenolic substrates and aromatic amines. Free radicals formed during the reaction are also able to be depolymerized, further repolymerized, demethylated or formed by quinone. Its industrial-technological and biotechnological applications suggest the low substrate specificity of Laccases and their ability to oxidise different contaminants [14]. Laccases in fungi and plants are generally distributed. Laccases are mainly present in fungi and plants. However, it has been found that Laccases are also widespread in bacteria. To date, Laccases have mostly been isolated and characterized from plants and fungi,

canonical four areas for the binding of copper. Nevertheless, overall sequences of bacterial but only fungal Laccases are currently used in biotechnology applications. In contrast, only a couple of bacterial Laccases are characterized. Bacterial Laccases have ability to oxidize syringaldazine and a couple of 6-dimethoxyphenol, which are typical substrates for Laccases and bacterial Laccases show little resemblance to fungal Laccases. Therefore the first report of bacterial laccase was from the strain *Azospirillum lipoferum,* which was isolated from the rhizosphere of rice. This enzyme has been identified as a a combination of substrates and inhibitors [15,16].

Laccase (EC 1.10.3.2) is a multicopper blue oxidase that couples the four electron reduction of oxygen with the oxidation of a broad range of organic substrates, including phenols, polyphenols, anilines, and even certain inorganic compounds by a one-electron transfer mechanism. Laccase is widely distributed in higher plants and fungi and has been found also in insects and bacteria [16,17].

The present study was therefore conducted characterize the bacterium, *Bacillus subtilis,* UniProt id-**M9Y1F2**.The spore laccase of this bacterium was characterized and used to decolorize various synthetic dyes. Spores were used to bleach RBBR, alizarin, Congo red, methyl orange, and methyl violet for the practical use of this bacterium in the treatment of waste water containing a dye. The bleaching rate was 90% in the treatment of RBBR and alizarin red, and 50 to 70% in the treatment of the other dyes. These results indicate that the spore laccase has the ability to decolorize the selected dyes without the need for redox mediators [15] .

Materials and Methods

The research methodology (tools and software) used for the present study is as follows:

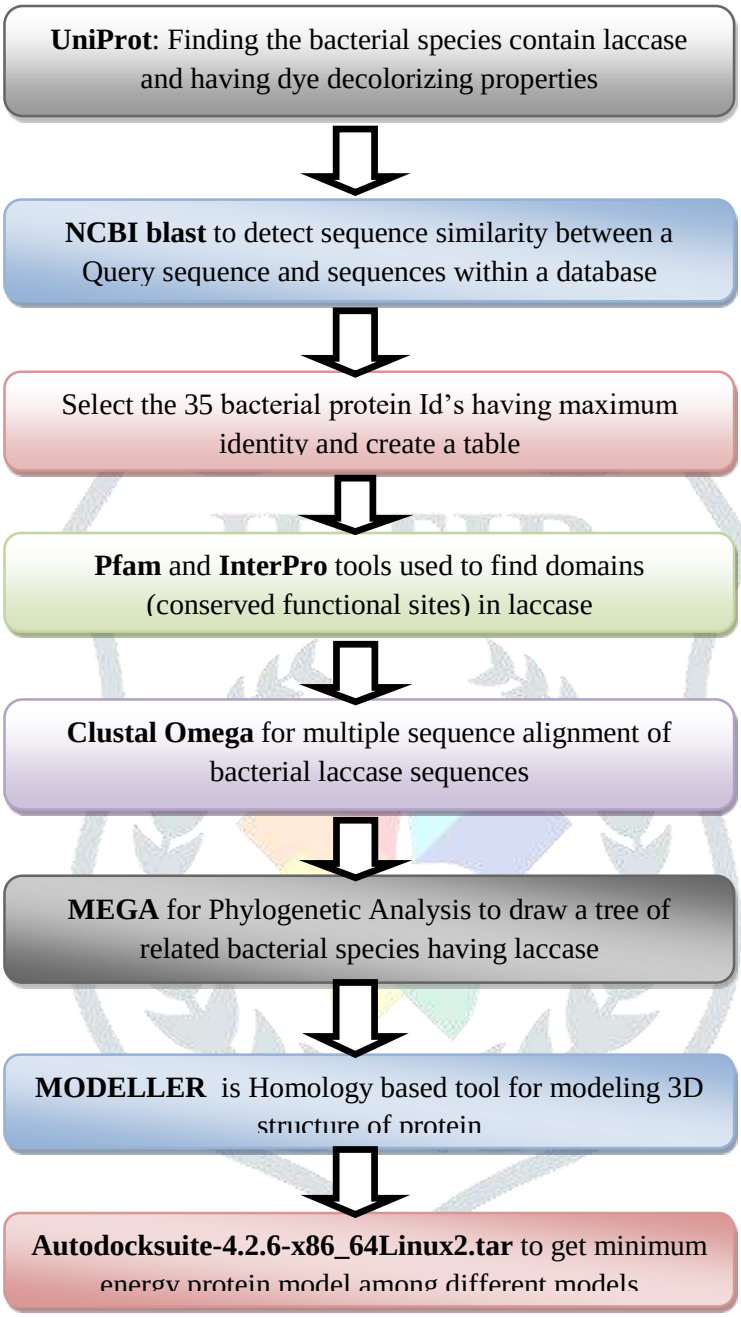

Figure1. Flowchart showing research methodology

Step 1: Sequence retrieval from UniProt

Uniprot is a knowledgebase database, search for dye decolorizing bacteria with typing keyword laccase in bracket for advance search (https://www.uniprot.org). The output shows and selects the **M9Y1F2** id that is *Bacillus subtillis* and having spore coat protein. After selecting one id do the blast search to get the similar bacterial genome sequence. The spore laccase of this bacterium was characterized and used to decolorize various synthetic dyes [18].

Step 2: To find Sequence similarity

NCBI Blast used to detect sequence similarity between a Query sequence and sequences within a database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Query sequence in FASTA format paste in input box then select the PSI

Blast and select the pdb database (https://www.rcsb.org) and then run. 3.1.2 .Table1 shows 35 bacterial proteins that is taken from the blast result of M9Y1F2 UniProt ID [19].

Step 3: Domain finder

Proteins are generally comprised of one or more functional regions, commonly termed domains Pfam and Interpro are two databases used to find domains and motifs. [http://pfam.xfam.org] and (https://www.ebi.ac.uk/interpro) [20].

Step 4: Multiple Sequence Alignment

Clustal Omega is a multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences. It shows identical and similar region. Its result shown in symbols like asterisk (*) for conserved region, dot (.) for identical residue, double dot (:) for highly or moderately conserved. It gives the phylogenetic tree from (https://www.ebi.ac.uk/Tools/msa/clustalo/) [21].

Step 5: Phylogenetic Analysis

Molecular Evolutionary Genetics Analysis (MEGA) is computer software used for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees [22].

Step 6: Protein Structure Modelling

Modeller is a computer program used for homology modeling to produce model of protein tertiary structures and quaternary structures [24, 25, 26]. It implements method inspired by nuclear magnetic resonance spectroscopy of protein (NMR), termed as satisfaction of spatial restraints, by which geometrical criteria are used to create probability density function for the location of atom in protein [27].

Step 7: Pocket finder and Ligand binding site

Meta Pocket 2.0 and Rasmol databases are used to find specific binding site for ligand in protein and get three top ranked clusters from (http://www.openrasmol.org/).

Step 8: Docking

MGL Tool: autodocksuite-4.2.6-x86_64Linux2.tar to get minimum energy protein model[28]. Docking is done with modeller 3d structured protein and dyes as ligands such as blue19, RBBR, alizarin, Congo red, methyl orange, and methyl violet[29],[30]. Structured of dyes obtained from Open Babel and Pubchem. (https://openbabel.org/docs/dev/Installation/install.html), (https://pubchem.ncbi.nlm.nih.gov/) [31,32, 33].

Results and Discussion

Using the UniProt knowledgebase database search for dye decolorizing bacteria with laccase of bacteria keyword. Type dye decolorizing in bracket for advance search. The output shows and selects the M9Y1F2 id that is *Bacillus subtilis* and having spore coat protein. After selecting one id do the blast search to get the similar bacterial genome sequence. NCBI Blast used to detect sequence similarity between a Query sequence and sequences within a database. M9Y1F2 protein fasta sequence paste in input box then select the PSI Blast and select the PDB database and then run the Blast. Select the maximum coverage and more similar 35 protein ids. After doing BLAST, find conserved domains in similar proteins with help of Pfam and InterPro tools. Most of proteins collected from different species contain all the three domains (Cu-Oxidase_1,Cu-Oxidase_2,Cu-Oxidase_3) which are responsible for dye decolourization property. Results are shown in table given below:

Sr.No	UniProt ID's	Organism	Region	Domain	Source
1	M9Y1F2	*Bacillus subtilis*	33-97 95-179	Cu-Oxidase_3	Pfam
			183-339	Cu-Oxidase_1	Pfam
			358-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-308	Cu-Oxidase_1	InterPro
			380-509	Cu-Oxidase_2	InterPro
2	L8PW18	*Bacillus subtilis subsp. inaquosorum KCTC 13429*	29-97 93-179	Cu-Oxidase_3	Pfam
			178-341	Cu-Oxidase_1	Pfam
			358-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			239-323	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
3	A0A0M0KQ12	*Jeotgalibacillus marinus*	35-97 94-179	Cu-Oxidase_3	Pfam
			188-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			242-318	Cu-Oxidase_1	InterPro
			381-509	Cu-Oxidase_2	InterPro
4	A0A0K6KH03	*Bacillus cereus*	35-97 94-179	Cu-Oxidase_3	Pfam
			188-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			242-318	Cu-Oxidase_1	InterPro
			381-509	Cu-Oxidase_2	InterPro
5	A0A136GF70	*Bacillus subtilis*	35-97 94-179	Cu-Oxidase_3	Pfam

			188-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			242-318	Cu-Oxidase_1	InterPro
			381-509	Cu-Oxidase_2	InterPro
6	U5U549	*Bacillus subtilis*	33-96 95-179	Cu-Oxidase_3	Pfam
			198-340	Cu-Oxidase_1	Pfam
			358-512	Cu-Oxidase_2	Pfam
			45-80 101-178	Cu-Oxidase_3	InterPro
			231-322	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
7	A0A1G4LN50	*Bacillus subtilis*	41-118	Cu-Oxidase_3	Pfam
			181-262	Cu-Oxidase_1	Pfam
			320-449	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			242-318	Cu-Oxidase_1	InterPro
			381-509	Cu-Oxidase_2	InterPro
8	E0TU44	*Bacillus subtilis subsp. spizizenii (strain ATCC 23059 / NRRL B-14472 / W23)*	33-96 95-179	Cu-Oxidase_3	Pfam
			198-340	Cu-Oxidase_1	Pfam
			358-512	Cu-Oxidase_2	Pfam
			45-80 101-178	Cu-Oxidase_3	InterPro
			231-322	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
9	A0A1A0GCS5	*Bacillus subtilis*	29-97 93-179	Cu-Oxidase_3	Pfam
			190-340	Cu-	Pfam

				Oxidase_1	
			358-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			242-322	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
10	S5MW00	*Bacillus vallismortis*	29-97 3-93-179	Cu-Oxidase_3	Pfam
			188-341	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			242-322	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
11	U1Z6H1	*Bacillus sp. EGD-AK10*	31-99 95-181	Cu-Oxidase_3	Pfam
			177-342	Cu-Oxidase_1	Pfam
			361-514	Cu-Oxidase_2	Pfam
			47-83 103-180	Cu-Oxidase_3	InterPro
			242-320	Cu-Oxidase_1	InterPro
			381-511	Cu-Oxidase_2	InterPro
12	M4KP30	*Bacillus subtilis XF-1*	31-99 35-181	Cu-Oxidase_3	Pfam
			177-342	Cu-Oxidase_3	Pfam
			361-514	Cu-Oxidase_1	Pfam
			47-83 103-180	Cu-Oxidase_3	InterPro
			242-320	Cu-Oxidase_2	InterPro
			381-511	Cu-Oxidase_1	InterPro
13	A0A0T8PV75	*Streptococcus pneumoniae*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-31	Cu-	InterPro

			101-178	Oxidase_3	
			240-318	Cu-Oxidase_1	InterPro
			379-509	Cu-Oxidase_2	InterPro
14	A0A125UEQ9	*Bacillus sp. LM 4-2*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			379-509	Cu-Oxidase_2	InterPro
15	A0A0D1KWM1	*Bacillus subtilis*	31-99 95-181	Cu-Oxidase_3	Pfam
			177-342	Cu-Oxidase_1	Pfam
			361-514	Cu-Oxidase_2	Pfam
			47-83 103-180	Cu-Oxidase_3	InterPro
			242-320	Cu-Oxidase_1	InterPro
			381-511	Cu-Oxidase_2	InterPro
16	D4G5Y5	*Bacillus subtilis subsp. natto (strain BEST195)*	31-99 95-181	Cu-Oxidase_3	Pfam
			177-342	Cu-Oxidase_1	Pfam
			361-514	Cu-Oxidase_2	Pfam
			47-83 103-180	Cu-Oxidase_3	InterPro
			242-320	Cu-Oxidase_3	InterPro
			381-511	Cu-Oxidase_1	InterPro
17	H8WGE2	*Bacillus sp. LS02*	29-97 93-179	Cu-Oxidase_2	Pfam
			175-340	Cu-Oxidase_3	Pfam
			359-512	Cu-Oxidase_1	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro

			379-509	Cu-Oxidase_2	InterPro
18	H8WGE6	*Bacillus sp. WN01*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			379-509	Cu-Oxidase_2	InterPro
19	H8WGE3	*Bacillus sp. LS03*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			379-509	Cu-Oxidase_2	InterPro
20	H8WGE7	*Bacillus subtilis*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-181 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			379-509	Cu-Oxidase_2	InterPro
21	A0A165A680	*Bacillus subtilis*	31-97 95-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			384-509	Cu-Oxidase_2	InterPro
22	A0A1J5XF48	*Bacillus sp. FMQ74*	41-118	Cu-Oxidase_3	Pfam

			180-262	Cu-Oxidase_1	Pfam
			318-449	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-322	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
23	I0F185	*Bacillus sp. JS*	30-97 94-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
24	A0A1D8FHP8	*Bacillus subtilis subsp. subtilis*	41-118	Cu-Oxidase_3	Pfam
			180-258	Cu-Oxidase_1	Pfam
			318-449	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
25	A0A164UCV6	*Bacillus subtilis*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
26	P07788	*Bacillus subtilis (strain 168)*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam

			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
27	A0A1Q9FIG6	*Bacillus licheniformis*	31-99 95-181	Cu-Oxidase_3	Pfam
			177-342	Cu-Oxidase_1	Pfam
			361-514	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
28	G4EZC6	*Bacillus subtilis subsp. subtilis str. SC-8*	43-120	Cu-Oxidase_3	Pfam
			182-260	Cu-Oxidase_1	Pfam
			321-451	Cu-Oxidase_2	Pfam
			47-83 103-180	Cu-Oxidase_3	InterPro
			242-320	Cu-Oxidase_1	InterPro
			381-511	Cu-Oxidase_2	InterPro
29	A0A1N7B127	*Bacillus subtilis*	41-118	Cu-Oxidase_3	Pfam
			180-258	Cu-Oxidase_1	Pfam
			319-449	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
30	A0A182CBL5	*Pseudomonas stutzeri (Pseudomonas perfectomarina)*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-341	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81	Cu-	InterPro

			101-178	Oxidase_3	
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
31	C6KEH7	*Bacillus subtilis*	29-99 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
32	A0A089YNV4	*Bacillus subtilis*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-341	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-322	Cu-Oxidase_1	InterPro
			379-509	Cu-Oxidase_2	InterPro
33	I6ZLM4	*Bacillus sp. ZW2531-1*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
34	B9W2C5	*Bacillus sp. HR03*	29-97 93-179	Cu-Oxidase_3	Pfam
			175-340	Cu-Oxidase_1	Pfam
			359-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			240-318	Cu-Oxidase_1	InterPro
			384-509	Cu-	InterPro

				Oxidase_2	
35	G1E8V8	*Bacillus subtilis*	32-98 95-179	Cu-Oxidase_3	Pfam
			193-340	Cu-Oxidase_1	Pfam
			360-512	Cu-Oxidase_2	Pfam
			45-81 101-178	Cu-Oxidase_3	InterPro
			231-318	Cu-Oxidase_1	InterPro
			378-509	Cu-Oxidase_2	InterPro
				Cu-Oxidase_3	Pfam

Table1: 35 different bacterial protein ids and species name after performing Blast result of UniProt id:M9Y1F2. It also contains similar domains present in different protein retrieved through Pfam and InterPro tools.

MEGA is used for Phylogenetic analysis of 35 different bacterial species containing laccase having same domain responsible for dye decolorization.

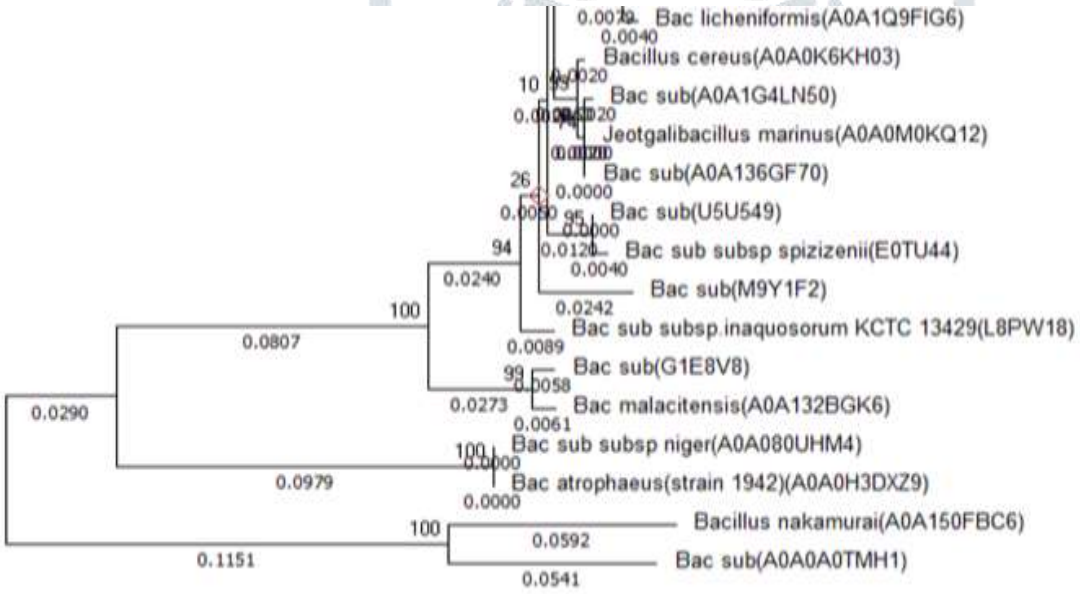

Figure 2: Maximum likelihood tree prepared in MEGA

MODELLER is used for homology or comparative modeling of protein three-dimensional structures. M9Y1F2 protein is selected as target sequence and five template sequence is selected i.e.: 1GSK A, 4A68 A, 4AkQ, 4A66 A, 2X87 A. At the end, Five protein structures build i.e. M9Y1F2-B99990001, M9Y1F2-B99990002, M9Y1F2-B99990003, M9Y1F2-B99990004, M9Y1F2-B99990005

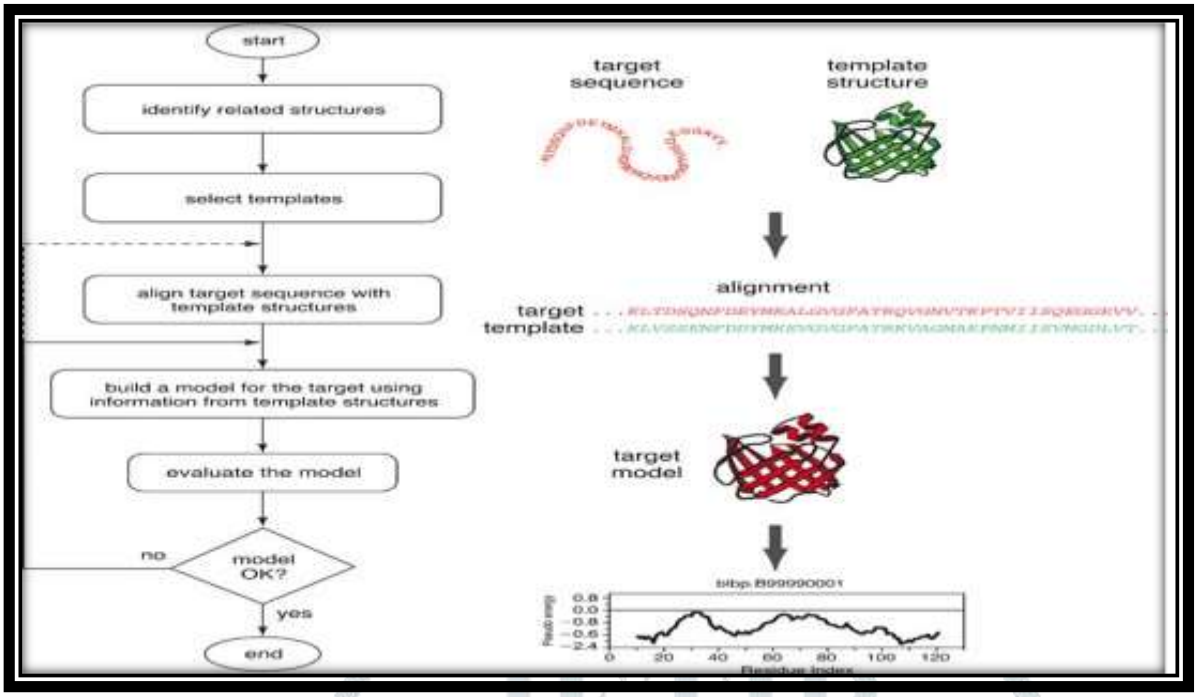

Figure 3: Steps in comparative protein structure modeling

Structures obtained through Modeller (Comparative protein structure modelling)

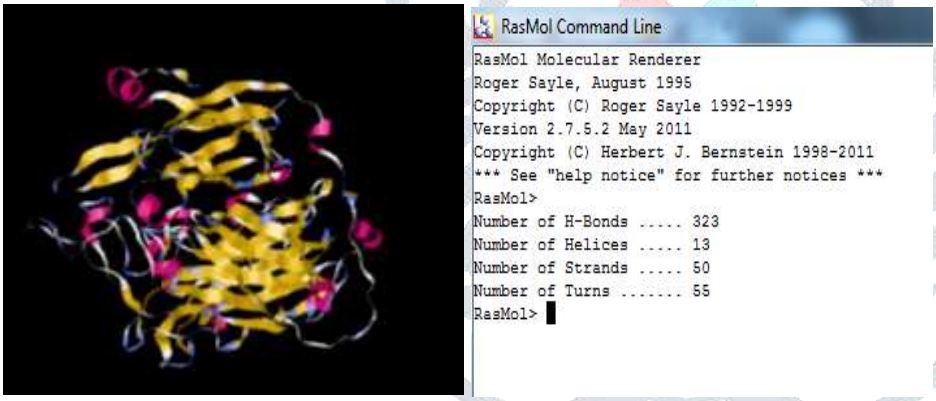

Figure 4: Protein structure 1(M9Y1F2-B99990001)

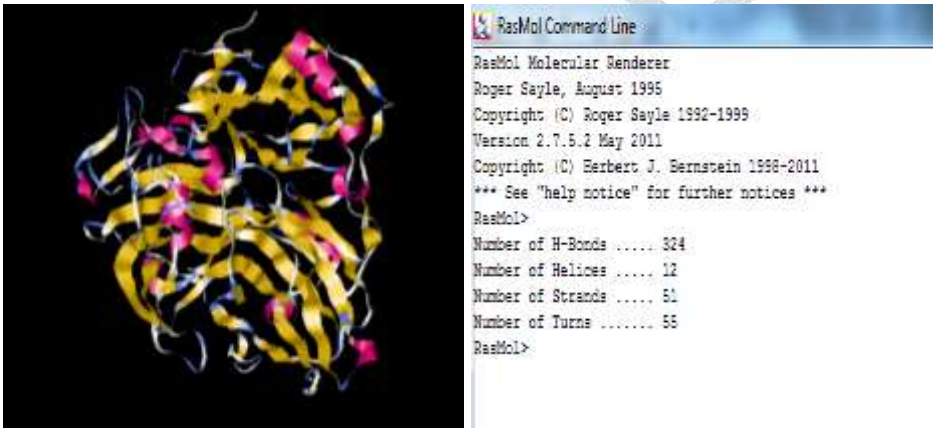

Figure 5: Protein structure 2 (M9Y1F2-B99990002)

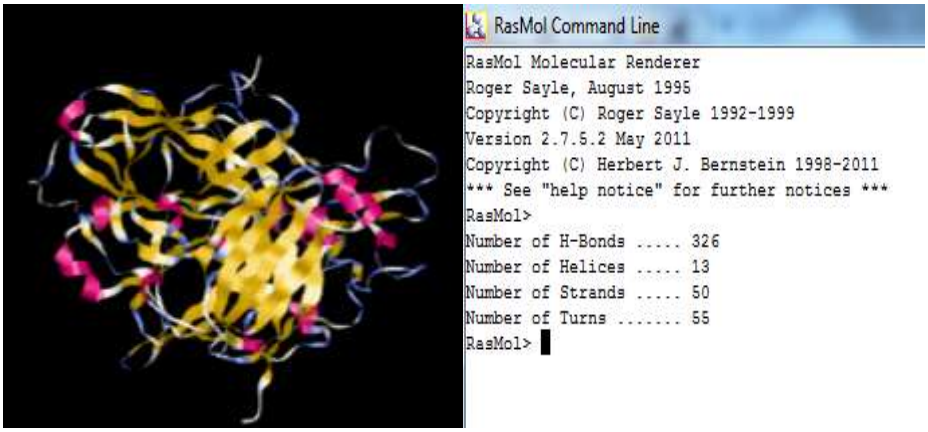

Figure 6: Protein structure 3(M9Y1F2-B99990003)

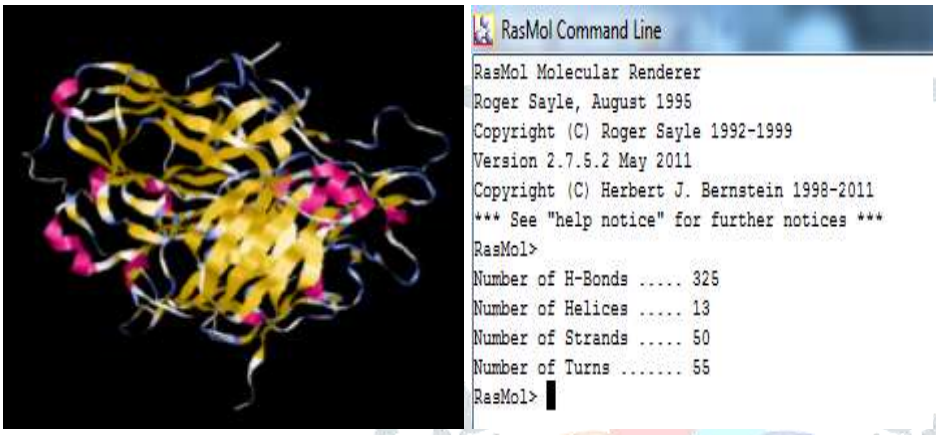

Figure 7: Protein structure 4 (M9Y1F2-B99990004)

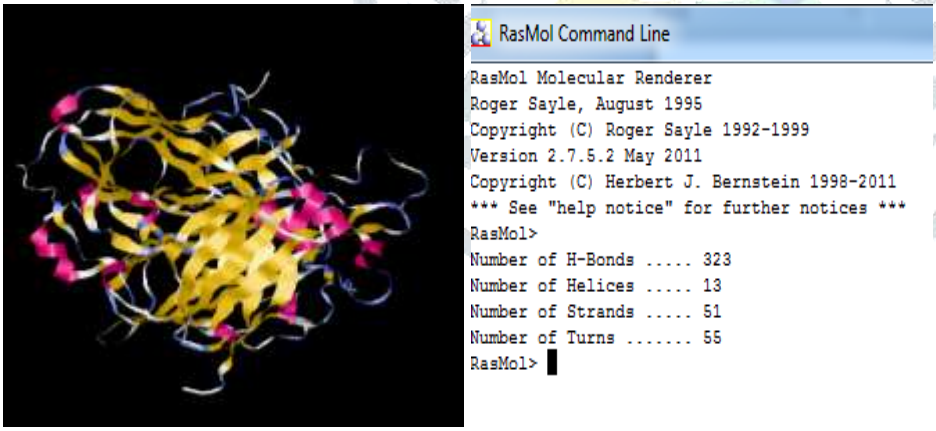

Figure 8: Protein structure 5 (M9Y1F2-B99990005)

Docking is done with 3D structured proteins obtained from Modeller and dyes as ligands such as blue19, RBBR, alizarin, Congo red, methyl orange, and methyl violet. Structures of dyes obtained from Open Babel, PubChem from (https://pubchem.ncbi.nlm.nih.gov/) and (https://openbabel.org/docs/dev/Installation/install.html) and finally got minimum binding energy for dye blue19 with Modeller protein M9Y1F2-B99990001 with 10 best clustering confirmations.Binding energy of reactive blue19 dye with receptor is -6.49.

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	6	-6.49	0.00	76.28	RANKING
2	1	5	-5.37	0.00	73.78	RANKING
3	1	9	-4.91	0.00	73.67	RANKING
4	1	4	-4.68	0.00	74.88	RANKING
5	1	3	-4.09	0.00	74.13	RANKING
5	2	7	-2.93	1.52	73.51	RANKING
6	1	2	-3.15	0.00	72.46	RANKING
7	1	10	-3.15	0.00	72.15	RANKING
8	1	8	-2.89	0.00	71.68	RANKING
9	1	1	-2.32	0.00	79.8[illegible]	RANKING

Table 2: Ten binding energy confirmations

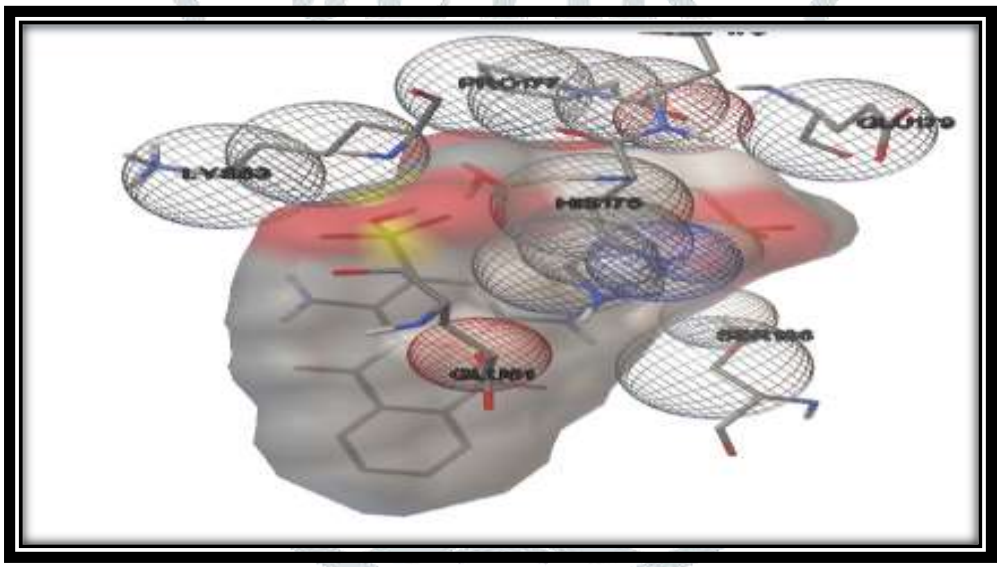

Figure 9: Confirmation of Reactive Blue 19 dye and Modeller protein

Conclusion

Textile dyes induce more water pollution. In this study, Bacterial laccase enzyme is used for dye decolorization due to its multicopper oxidase activity. Laccases found in many microorganism, But *Bacillus subtillus* having spore coat A protein and three types of functional domains copper oxidase 1,2,3 that help the bacteria to survive in harsh conditions also. Laccases have high substrate selectivity and they are more effective in dye decolorization. To see the effect of bacterial laccase on various dyes ,docking is done between modelled protein structure of laccase and dyes by using MGL Tool and AutoDock suit. From docking ,structure with minimum binding energy is selected that shows about how much bacterial Laccases decolorize the dyes present in industrial waste water.

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