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Exploring prospective forested wetland — Actinomycetes for biodeterioration of genotoxic textile azo dyes

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Disposal of synthetic textile dye effluents into waterbodies is a major cause of water pollution and undesirable toxicity on aquatic organisms affecting their metabolic and hormonal cycles. Moreover, it affects human health significantly by carcinogenic and mutagenic effects. Therefore, this study targets the biotreatment of azo dyes as a green eco-friendly tool using the tropical mangrove actinomycete, *Streptomyces coelicolor* strain SPR7, that manifests versatile decolourization of azo dyes. The highest decolourization rate (97.5%) was achieved using Methylene Blue (MB) within 72 h under the optimal conditions. Molecular docking analysis was accomplished by Autodock software, to discern docking score, binding pocket residues and interaction of azoreductase, DyP-type peroxidase and laccase with MB. The *in vitro* findings were highly correlating with *in silico* appraisals. UV-vis spectrometry, HPLC and FTIR were validating the MB biodecolourization and degradation. Interestingly, the strain detoxified MB *viz*. further validated by the *Vigna radiata* toxicity assay. Supplementarily, azo reductase was assessed to be the key biocatalyst for decolourization, while other two enzymes were involved in further mineralization of decolourized metabolites. Henceforth, a coupled in silico cum biological dye deterioration is a booming strategy for an economical, eco-friendly and time saving bioremediation to reuse textile wastewater.

Keywords: Aquatic pollution, Aerobic biotransformation, Biodecolourization, Bioremediation, Green gram, Molecular docking, Mung bean, *Streptomyces coelicolor*, *Vigna radiata*

Synthetic dyes are extensively used in the textile and other industries[1-4]. Over 100000 commercial dyes with an outranking annual production of 7×10^5 metric tonnes are commercially procurable across the globe of which the textile sector contributes two-thirds of the production[2]. Among these, azo dyes are the most extensively used dyes in the industrial arena, particularly in textile, cosmetics, leather, food and paper[3,4]. They can be ascribed to incorporate one or several azo linkages incorporated with aromatic groups, which could be categorized into distinct classes, such as direct, mordant, acidic, basic, reactive, solvent and disperse dyes. Among these wider classes, reactive dyes are leading, due to their wider applicability[2].

The conventional textile finishing industries expends large volume of dyeing wastewater. These waste water effluents carry out the coloured azo dyes, causing a harmful and toxic environment to aquatic life of the water bodies and streams. In addition, azo dyes are recalcitrant in nature exhibiting mutagenic and carcinogenic side effects on humans. Furthermore, they are known to impede the process of oxygen solubility in water, thereby causing undesirable ambience for the aquatic organisms[5] and treatment of noxious end-products is of critical importance to reuse the treated water[6]. These by-products can be eradicated or removed from wastewaters through physico-chemical treatments *viz*. coagulation, flocculation, adsorption and oxidation, respectively. However, due to an expensive nature (as chemicals are used) of these techniques, their usage is not highly encouraged[7,8].

A novel approach for the same target have come up, namely bioremediation techniques which intends to recycle wastewater as an extravagant resource and conjointly, to control pollution by eradicating the recalcitrant with anthropogenic traits permanently through natural processes[6]. As the biological approaches are more eco-friendly and economical, they have gained more attention. Recruiting microbial systems for biodegradation is preferred because of their ease of synthesis, perpetuation, with their efficacy to exist under stressful conditions and mineralize the dyes into less toxic metabolites which is effectuated by amalgamation of several oxidative and reductive enzymes for biodeterioration[9].

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In this context, actinomycetes have gathered considerable interest over the period. However, the actinobacterial resources enduring related applications are still lacking. Hence, it is necessitous to explore broadly on these bacterial systems such taxas capable of mineralizing the recalcitrant azo dyes and further research to capitalize them for biotechnological and industrial applications. These Gram positive bacterial systems are well-known to produce promising intracellular and extracellular enzymes, such as laccase, lignin peroxidase and azo reductase[10-12]. Studies have shown the ability of yeast strains to degrade dyes[13]. Meanwhile, the treatment of organic pollutants by bacteria is also reported[9]. Interestingly, azo dyes can also be mineralized by fungal cultures[14].

Though few studies are available on how actinomycetes crude culture mediate azo dyes degradation[10,15], there are only limited reports on degradation of azo dyes by actinobacteria. Hence, it is important to explore some more organisms for biotreatment of wastewater for industrial applications. The present study focuses on isolation, identification and biochemical characterization of an actinobacteria which decolourize azo dyes via aerobic biodegradation. Methylene Blue (MB) was selected as the model dye for further analyses of the effects of distinct factors on decolourization by actinobacterial culture. The major catalytic interaction profile of the accountable dye remediating enzymes were examined by molecular docking followed by the assessment of their catalytic activities. Additionally, the degradation target of MB (by the isolate) was affirmed by HPLC and FTIR techniques; the metabolite phytotoxicity was assessed through *Vigna radiata* assay system.

Material and Methods

Reagents

All the reagents and chemicals were of analytical grade (Merck, India). The azo dyes, were procured from Hi-media, India. Suppl. Table S1, illustrates the structure and other features of the azo dyes used (*All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*).

Isolation and Identification of actinobacteria

The sediment samples used for isolation of actinomycetes were collected from Jaladi Mangroves (13°39'41"N, 74°42'16"E), Karnataka, India. The microbial communities were isolated, acclimatized and cultured in starch-casein medium (SCM), containing 10 g L^{-1} starch, 0.3 g L^{-1} casein, 2 g L^{-1} K_2HPO_4, 2 g L^{-1} KNO_3, 0.05 g L^{-1} $MgSO_4$, 0.02 g L^{-1} $CaCO_3$, 0.01 g L^{-1} $FeSO_4$,15 g L^{-1} agar and 10 g L^{-1} NaCl[16] (pH: 7.0±0.1). The pure colonies were regularly subcultured on SCM plates incubated at room temperature (RT) to effectuate good sporulation and were preserved at 4°C. The stock cultures were maintained in 20% (v/v) glycerol at −80°C for long-term storage[17]. The culture was incubated in triplicates at RT at 180 rpm for 98 h and the molecular identification by 16S rRNA sequencing was accomplished; the corresponding sequence was deposited in GenBank database under the accession number (MH712067).

Morphological and Biochemical characterization

The isolate was subjected to both physiological and morphological characterizations by following the previously proposed method with slight modifications[18]. Phenotypic traits *viz.* consistency, reverse side pigments, aerial mass colour were investigated. Moreover, Gram staining, growth on starch-casein medium (SCM), international *Streptomyces* project 2 (ISP-2) medium, actinomycetes isolation agar (AIA) were accomplished. The tests for biochemical characterization included starch hydrolysis, casein hydrolysis, urea hydrolysis, nitrate reduction, citrate utilization, indole and acid production from sugars.

Assays for enzyme activities

Cells of the actinobacterial culture were collected by centrifugation (10000 ×g, 20 min and 4°C) of the fermentation medium containing, 20 mg/L of target dye for 72 h. The collected supernatant was recruited for conducting activity assays of extracellular enzymes, whereas the obtained pellet was targeted for determining the intracellular enzyme activities. After a complete wash with 10 mM potassium phosphate buffer (pH 7.2), the cells were suspended in the same buffer followed by a gentle homogenization and sonication at 4°C. The sonicated cells were centrifuged at 10000 ×g, 20 min and 4°C and the procured supernatant were further processed for estimating the activities of intracellular enzymes[13]. The enzyme activities were estimated spectrophotometrically (Evolution 300UV-VIS spectrophotometer, Thermo Scientific) at room temperature with a final reaction volume of 2 mL.

The laccase activity was measured as previously described[19]. The oxidation of 0.5 mM ABTS to $ABTS^{2+}$ was monitored at 420 nm (ε = 36 000 M^{-1} cm^{-1}) in reaction mixture consisting of 100 mM sodium acetate

buffer (pH 4) and 0.6 mL of enzyme. The azoreductase activity was monitored by a reduction in Methyl Red concentration at 440 nm, by following the method proposed earlier[20].

Dye-type peroxidase activity was estimated in 100 mM sodium acetate buffer (pH 5.5) at 30ºC as described previously using 1.0 mM of ABTS. One unit of enzymatic activity follows as described earlier[21].

Bioinformatics tools in dye biotransformation

Preparation of the receptor and ligands

The crystallographic structure of DyP-type peroxidase and laccase were retrieved from the Protein Data Bank (PDB) with the following access codes: 4GRC and 3CG8. The protein sequence of azo reductase (Q9S1U6) was retrieved from UniProt and homology modeling of its 3D structure was accomplished by Swiss model server (https://swissmodel.expasy.org/) with slight modifications as described previously[22]. The templates were searched through swiss model template library and templates of highest similarity were selected for protein modeling. The generated protein models were assessed and validated by the Q-MEAN score (composite scoring function).

The 3D structure of all the azo dyes were procured from PubChem compound databases (https://pubchem.ncbi.nlm.nih.gov/) in the form of SDF file and their SMILE notations were converted into PDB file by Online SMILES convertor and Structure file generator[23]. The minimized protein and ligand preparation were accomplished by PyMol 2.1 version.

Molecular docking

The Autodock software version 4.2 was used to carryout molecular docking, to estimate the dock score and determine best docking poses for interactions between the target dye and the distinct enzymes. The docked complexes of the ligand-receptor were appraised carefully, to identify the binding residues and their interactions[24].

Comparative analysis between docking scores and degradation

The correlative analysis of all the enzymes with target dye were exhibited by comparing their dock scores with decolourization rate obtained by *in vitro* studies. As a conjunction, HPLC and FTIR were performed to confirm the dye degradation.

Decolourization and degradation analysis of dye metabolites

The decolourization assay was performed as previously described with slight modifications[13]. The tests were conducted in 250 mL erlenmeyer flasks with a culture volume of 100 mL. To discern the colour removal efficiency of distinct azo dyes by *Streptomyces coelicolor* strain SPR7, 1% of the pure culture inoculum was used in 100 mL of sterile medium comprising of 20 mg L^{-1} of dyes, subjected to incubation at 160 rpm at room temperature for 12-72 h until the decolourization process was achieved. The dye samples in the absence of the microbial inoculum was used as the control. The completely decolourized azo dye was selected for further analysis. Effects of various factors, *viz.* concentration of starch, KNO_3, casein, NaCl, pH, temperature, target dye and speed of flasks rotation on target dye degradation by *S. coelicolor* strain SPR7 were scrutinized[13].

The target dye metabolites formed by *S. coelicolor* strain SPR7, after the decolourization and biodegradation processes were analyzed by spectral and chromatographic techniques like UV–Vis spectroscopy, HPLC and FTIR. The decolourization rate was determined spectrophotometrically (Evolution 300UV-VIS spectrophotometer, Thermo Scientific) at λmax of the dye, by following equation as follows: Decolourization (%) = (Ai-At)/Ai ×100, where A_i and A_t are the initial and final absorbance of the dye reaction system prior and to post decolourization process, respectively. All the experiments were conducted in triplicates. The culture broth was centrifuged and the metabolites were extracted with supernatants using equal volume of ethyl acetate. After extraction the samples were subjected to dryness in rotary evaporator and the resulting residue was dissolved in methanol (HPLC Grade). The operation conditions and equipments of HPLC and FTIR were performed as described previously[9].

Phytotoxicity analysis

The agricultural crops exposed to synthetic dyes and textile effluents can be subjected to major distortions in their physiological processes *viz.* photosynthesis, vernalization and photoperiodism, etc. Henceforth, phytotoxicity assays were executed, to assess the acute toxicity of the enzyme treated and untreated dye samples[25]. The degraded metabolites of target dye extracted by ethyl acetate were dried and dissolved in the sterile distilled water. The agriculturally important dicot crop Mung bean or Green gram, *Vigna radiata* (ten seeds) was treated with extracted compounds of target dye (50 mg L^{-1}) or its degradation compounds. The control assays were run simultaneously, using sterile distilled water at ambient temperature. Germination (%) in conjunction with the length of the radical and plumule were observed after

5 days. The germination (%) was estimated by the following equation:

$$\text{Germination}(\%) = \frac{\text{Number of seed germinated}}{\text{Number of seeds sowed}} \times 100 \quad (1)$$

Statistical analysis

The data were statistically analyzed by one-way ANOVA using GraphPrad Prism 7.03 software for significance at $P \leq 0.05$ and all the experiments were carried out in triplicates..

Results and Discussion

Isolation and identification of the strain

The strain isolation was accomplished using active sediments samples of southwest coastal mangroves. The mangrove ecosystems are tidal forests existing in the tropical and subtropical regions of the globe and are comprised of diverse microbial communities, among which actinomycetes are dominating and well known phylogenetic taxa for production of multifarious bioactive metabolites[26].

A full-length 16S rRNA gene sequence of the isolate was determined. The comparative analysis of the 16S rRNA gene sequences propounded that the isolate (GenBank accession number: MH712067), represented a member of *Streptomyces* taxa, showing a higher similarity (99%) with *Streptomyces coelicolor* (AB588124.1). Hence, the strain was assigned as *S. coelicolor* strain SPR7. The culture remained in the exponential phase for 48 h and lasted in the steady state phase upto 72 h. Several other *streptomyces* genera are reported predominantly for their efficacy to decolourize and biotransform textile azo dyes[10,27]. However, there are only few reports shedding light on *S. coelicolor* for their biotransformation strategies. Hence, this study dwells as an additional evidence for the same objectives.

Morphological characterization of the isolate

After 28 days of incubation timeline, the colonies were observed with whitish, cream and powdery surfaces, which are the characteristics of actinomycetes. The appearance characteristics were signifying *streptomyces* properties *viz.* greyish-white aerial mycelia with yellow pigmentation features on the reversal side of the colonies. The Gram's staining results displayed filamentous mycelium with conidia, typifying the characteristics of actinomycetes.

Biochemical characterization of the isolate

The isolate, *S. coelicolor* strain SPR7 was biochemically characterized for identification of its preliminary taxa. Table 1, enlists the result of the distinct biochemical tests.

Enzyme analysis

The dye mineralization in bacteria cells, is mediated by the collaborated activity of several biodegradation enzymes. Moreover, these enzymes are major indicators of the extent of micropollutant biodegradation[28]. The rate of decolourization is influenced by the complexity of dye structure followed by the increase or decrease in the activities of these biological enzymes. The enhancement in activity profiles (U min^{-1} mg protein^{-1}) of azo reductase, DyP-type peroxidase and laccases after the MB decolourization process (72 h) compared to the control systems is depicted in Table 2 for *S. coelicolor* strain SPR7. Hence, these results affirm the amplified activities of coupled enzyme classes *viz.* oxidative and reductive enzymes in the treated reaction system, when compared to the control samples. All the enzymes were observed to be secreted extracellularly, inferring that no intracellular production exists for these biocatalytic enzymes. The degradative role of these enzymes is well established for textile azo dyes[9,13].

Homology modeling of azoreductase

The sequence of azoreductase protein (FASTA format) was submitted to SWISS-MODEL

Table 1 — Biochemical Characterization of *Streptomyces coelicolor* strain SPR7

Biochemical Tests	Results
Starch Hydrolysis	+
Caesin hydrolysis	+
Urea hydrolysis	+
Nitrate reduction	-
Indole Production	-
Methyl Red	+
Citrate	+
Glucose	+
Lactose	+
Sucrose	-
Cellobiose	+
Fructose	+
Maltose	+
Mannitol	+
Vogesproskauer	+

Table 2 — Enzyme activities (U min^{-1} mg protein^{-1}) of *Streptomyces coelicolor* strain SPR7 control and test samples for Methylene Blue degradation with *in silico* docking analysis

Enzymes	Control	Test	Dock score
Azo reductase	0.71±0.42	1.2±0.16	–25.4 KJ/mol
DyP-type Peroxidase	0.85±0.43	1.32±0.38	–32 KJ/mol
Laccase	0.46±0.29	0.95±0.19	–18.32 KJ/mol

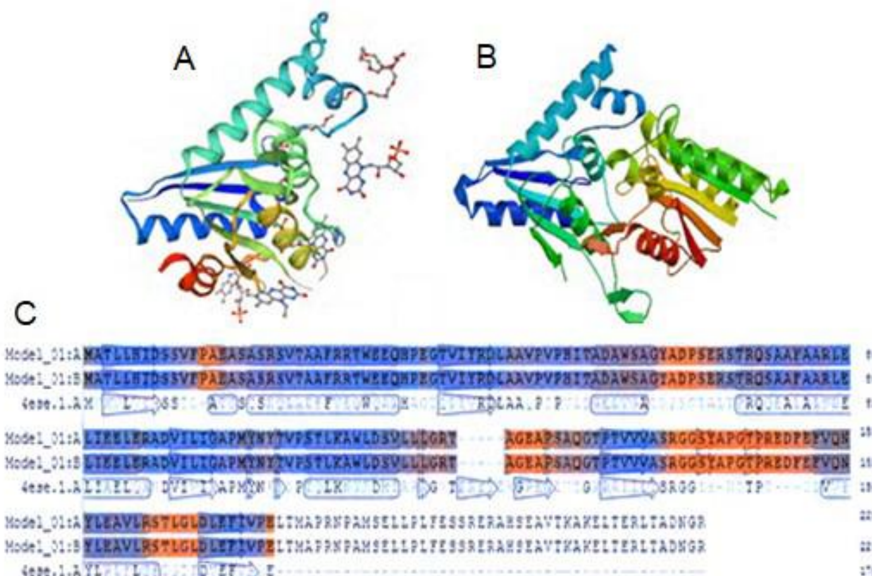

Fig. 1 — Homology modelling of the enzyme protein, azo reductase in swiss model automated workspace. 3D protein model of (A) template, 4ese.1.A; and (B) Azoreductase of *Streptomyces coelicolor*; and (C) Sequence alignment of azoreductase protein of *S. coelicolor* with template, 4ese.1.A.

WORKSPACE server in order to develop a 3D protein model by automated homology modelling. A total of 50 templates of query sequence (azoreductase protein sequence) were generated. The template 4ese.1.A (Fig. 1A) was targeted to develop the azoreductase protein model (Fig. 1B) which exhibited highest identity to the query sequence (Fig. 1C). Chen and co-workers, achieved a similar modelling of azoreductase using the Swiss Model server[29].

Molecular docking analysis

Computational docking is an outstanding option for large-scale screening of enzymes to select the most prospective decolourizer. Hence, a collaborated remediation process, comprising of computational strategies and subsequent validation through microbial degradation has been projected to support the experimental analysis. Autodock software was recruited for molecular docking for analysis of protein-ligand interactions of MB with DyP-type peroxidase, azoreductase and laccase of *S. coelicolor*. The binding site residues, docking scores and binding modes were investigated for the docked receptor ligand complexes. Molecular docking of MB with azoreductase yielded a dock score of –25.4 KJ/ mol and hydrogen bond mediated interactions were favoured between Tyr141*, Ser19* and Ala25*.The hydrophobic interactions were exhibited by His51, Arg162, Thr164, Ser140 and Phe150 (Fig. 2A).

Dyp-type peroxidase displayed a binding energy of -32 KJ/mol, which can be ascribed as the highest negative binding energy with MB. The hydrogen bonding and electrostatic interactions were contributed by Gly270*, Val296*, Ser231*, Phe298* and Glu 255*; the non bonded interactions with hydrophobicity were exhibited by Leu140, Arg137, Lys 167, Ser 178, Val193, Arg203, Gly205, His 137 and Glu303 (Fig. 2B). Ultimately, laccase of *S. coelicolor* illustrated a dock score of -18.32 KJ/ mol. The hydrogen and electrostatic bondings were produced by His137*, Ala59*, Ser111*, Cys288*, Arg244*, while the hydrophobic interactions were observed for Met53, Trp145, His104, Thr127, Leu101, Arg133 (Fig. 2C). Molecular docking appraisals and interaction studies for azoreductase and laccase have been accomplished in previous studies[9,30].

Correlation between *in silico* and *in vitro* analysis

The docking score is associated with decolourization rate and hence it follows that, the former can be directly proportional to the latter process[31]. Henceforth, a least binding energy (dock score) required by azoreductase, DyP-type peroxidase and laccase shows the higher interaction of these enzymes with MB, thereby displaying a higher correlation strategies with the *in vitro* dye biotransformation. The adaptive efficacy of the microbial systems towards conditions typical to regions comprising of textile effluents could execute

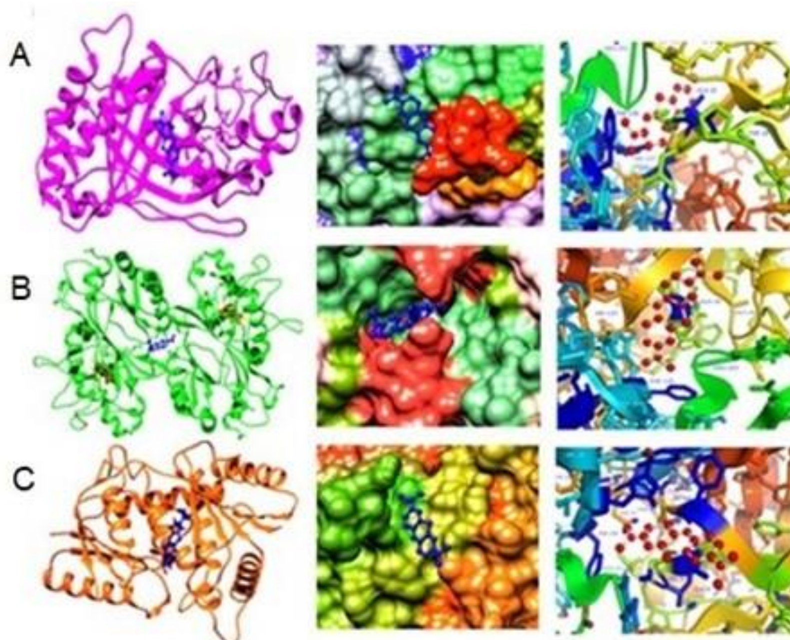

Fig. 2 — Docked pose of methylene blue dye with distinct biological enzymes shown in ribbon models, docked adducts by interactions with hydrogen bonds. Molecular interaction of methylene blue with (A) azo reductase; (B) DyP-type peroxidase; and (C) laccase

the decolourization phenomenon neglecting the origination factor of microbes[32]. A coupled systematic approach of in silico screening of recalcitrant xenobiotics with in vitro validating studies was suggested[33]. Thus, the application of preliminary computational screening prior to microbial dye biotransformation could be a channelizing route for economical and time saving bioremediatory intuitions.

Decolourization and degradation analysis of methylene blue

The growing cells of the isolate, executed aerobic decolourization of all the six azo dyes. The decolourization rates of tested azo dyes (20 mg L^{-1}) were more than 60% in 72 h of incubation time (Fig. 3). In the midst of all the six dyes, MB was completely biotransformed and hence, was selected as target dye for further appraisals. After the decolourization of MB, both of the suspension and colonies were colourless, indicating that the key mechanism involved as biodegradation; whereas, the remaining dyes were decolourized via. bioadsorption and biodegradation, as the coupled coloring strategies were observed for colonies and suspensions, respectively.

The competency of distinct biocatalytic enzymes generated by *S. coelicolor* strain SPR7 (for MB biotransformation) was scrutinized via changes in the intensity of peak absorbance by monitoring the optical density at λmax of 220 nm till 72 h. The spectral

Fig. 3 — Biodecolourization of distinct azo dyes (20 mg L^{-1}) by *Streptomyces coelicolor* strain SPR7: reaction system (i) before (control) and (ii) after decolourization. [P1-P6 (cell pellets) of strain SPR7 after centrifugation. The highest decolourization rate achieved in 72 h were A, methyl red (62 %); B, methylene blue (97.5 %); C, orange G (66.3 %); D, metanil yellow (90.1 %); E, mordant black (61.4%); and F, sandopel blue P (65.7%)]

profiles of the control sample was characterized by an absorbance peak at 220 nm for blue colour, which corresponds to azo moiety of the chromophore. In the presence of dye control as the reference, the degradative strategies were validated for biotreated dye samples. The treated samples displayed an interesting reduction in the absorbance, with concomitant increase in the incubation period, thereby signifying the azo bond cleavage and biotransformation of dye-bound chromophore promoting the decolourization process. The dye decolourization process of the dyes is based on the azo bond reduction coupled with the presence of anoxic environment by the oxidoreductive enzymes[34].

There are only few current reports, found on decolourization cum biodegradation of textile azo dyes by actinobacterial strains, representing *S. coelicolor* species to our knowledge. However, some actinomycete taxas affiliated to *Streptomyces* genera were established as potent strains for azo dye breakdown into lower toxic products. For instance, an actinobacterial strain namely, *Streptomyces cacaoi* could decolourize a red azo dye liquid broth comprising of variegated concentrations (0.005, 0.01 and 0.02 g) of dye after 96 h of incubation[27]. Besides, an actinomycete belonging to *Streptomyces* genus, had been addressed as the promising degrader of azo blue dye[10]. Supplementarily, several other actinomycete genera are exploited as active bioremediatory and eco-friendly tool for mineralization of azo dyes. With the consistent perspective, an actinobacterial strain affiliated to genera *Micrococcus* (*glutamicus*), evinced a characteristic degradation of diazo dye (50 mg L^{-1}) namely, C. I. Reactive Green 19A within 42 h[35]. However, it is obvious that the *S. coelicolor* strain SPR7 showed remarkable potency for colour removal targets of all the tested azo dyes recruited in this study.

Besides, the effect of diverse physiological and chemical parameters holds a direct influence on the decolourization strategies. Fig. 4 A-D, displayed the influencing effects of starch, caesin, KNO_3 and NaCl concentrations on decolourization and degradation of MB, respectively. As presented in Fig 4A, more than 90% of 20 mgL^{-1} MB was decolourized in 72 h in the presence of 2.0-4.0 g L^{-1} of starch concentration. On the contrary, the decolourization rate was estimated to be less than 90% within the same duration with 0.0-2.0 and 4.0-6.0 g L^{-1} of starch concentrations. Hence, it follows that neither meagre nor excessive concentrations of starch would be favouring the decolourization process of MB by biological enzymes secreted by *S. coelicolor* strain SPR7. As azo and related substances could not be consumed as carbon supplements by most of the microbial population, the sufficient and external supplementation of carbon sources is highly requisite for bio-based treatment of azo dyes[36]. Moreover, microbes could prefer external carbon compounds to dyes, when the ratio of carbon supplements to dyes exceeds a saturation range[37]. Hence, a reduced rate of decolourization was achieved at elevated starch concentration of 6 g L^{-1}. These results are consistent with previous reports[13,25].

To enhance the microbial growth rate and secretion of oxidoreductive enzymes, casein was incorporated into the fermentation media. As shown in Fig. 4B, decolourization efficiency of MB increased from 52 to 65.6% within 72 h, with a casein concentration of 0.1-0.3 g L^{-1}. Indeed, the elevation in the concentration of casein from 0.3-0.6 g L^{-1}, effectuated the lower decolourization of MB. Fig. 4C, displays the effect of potassium nitrate on decolourization of 20 mg L^{-1} by enzyme systems of *S. coelicolor* strain SPR7. Decolourization efficacy of the isolate was achieved over 68.5%. However, upgrading the concentration of potassium nitrate to 6 g L^{-1} resulted in lower decolourization outcome of 62% within 72 h. In a similar vein, effects of NaCl concentration (Fig. 4D) on

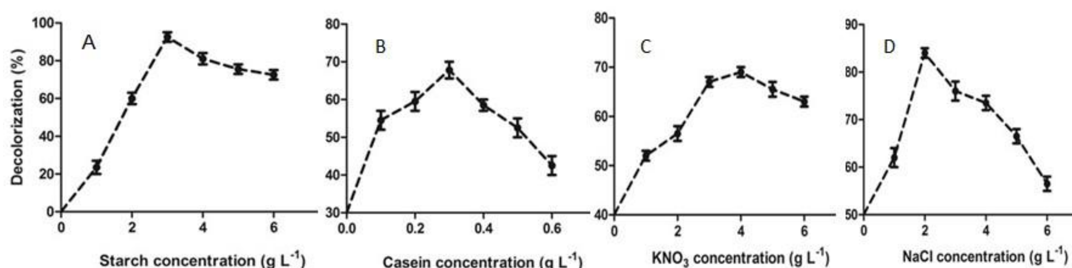

Fig. 4 — Effect of distinct parameters on methylene blue decolourization (20 mg L^{-1}) by growing cells of *Streptomyces coelicolor* strain SPR7. Concentration of (A) starch; (B) caesin; (C) potassium nitrate; and (D) sodium chloride

the dye decolourization was analyzed as an auxiliary studies with other optimization parameters. The highest decolourization activity (83%) was achieved with incorporation of 2.0 g L^{-1} NaCl, however, incrementing the concentration led to a meagre decolourization percentage of 55%. Henceforth, it is certain to propound that excess quantity of NaCl, significantly impedes the decolourization phenomenon[13].

The physiological parameters *viz.* rotation speed, dye concentration, pH and temperature were scrutinized for their influencing effects on MB (20 mg L^{-1}) decolourization and their optimal values were 150 rpm, 50 mg L^{-1} with pH 5 at 30ºC (Fig. 5 A-D).

The HPLC profiles of control sample displayed a major peak at 2.942 min of retention time (Fig. 6A). The decolourized product of MB manifested two major peaks at a retention time of 3.574 and 2.651 min; in conjunction with the minor peaks 2.337 min (Fig. 6B). These chromatographic variation supports the biomineralization and biodegradation of MB into smaller compounds by *S. coelicolor* strain SPR7, owing to the changes in peak and absorbance patterns. A similar pattern of HPLC chromatograms have been recorded previously for MB degradation by both yeast and bacterial consortium of *Saccharomyces cerevisiae* and *Bacillus* sp. STIS[38].

The comparative variations in the FTIR spectra of MB control and biotreated product streams indicate biotransformation of complex dye into low molecular weight products. The control samples displays specific peaks for various functional groups in the corresponding frequency region (Fig. 7A). Peak at 1638.63 cm^{-1} for -N=N- stretching signifies the azo nature and 3357.95 cm^{-1} for -N=N- stretching represents the amino group presence in MB. Peaks at 2510.95 cm^{-1} and 2936.95 $cm^{-1}$for C-H stretching, 2128.63 cm^{-1} for aliphatic C-H bonds, 1752.63 cm^{-1} for C-O stretching. The peaks at 1555.52 cm^{-1} for N-H bending, 1444.52 for C=C ring stretching and 1026.18 cm^{-1} for C-O stretching.

The FTIR profiles of extracted compounds after MB treatment with *S. coelicolor* strain SPR7 evinced a characteristic peak at 3360.62 cm^{-1} corresponding to the stretching vibrations of N-H and O-H groups signifying the presence of amine and hydroxyl groups (Fig. 7B). Supporting the stretching of C-H groups, peaks at 3000.95 cm^{-1} were observed. The peaks at

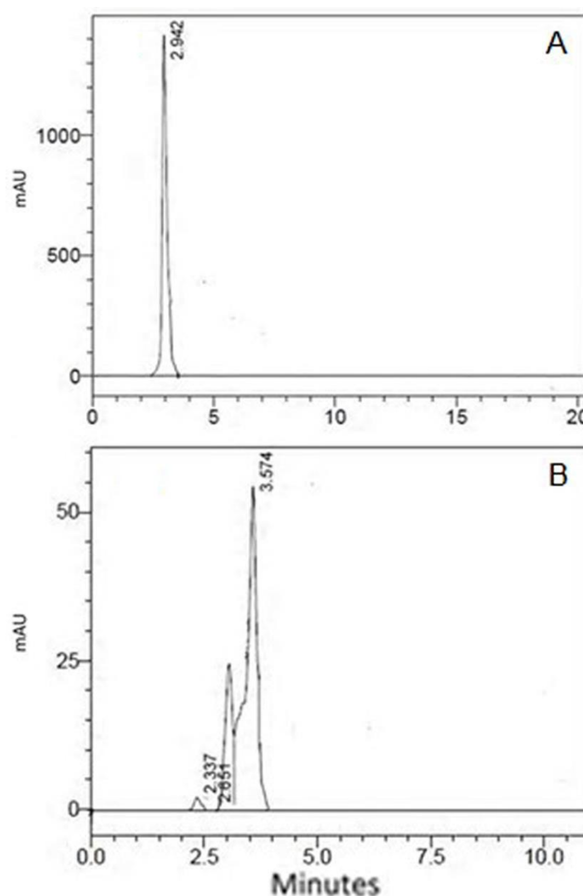

Fig. 6 — HPLC elution chromatogram of (A) methylene blue Control; and (B) metabolites formed after 72 h of degradation by *Streptomyces coelicolor* strain SPR7

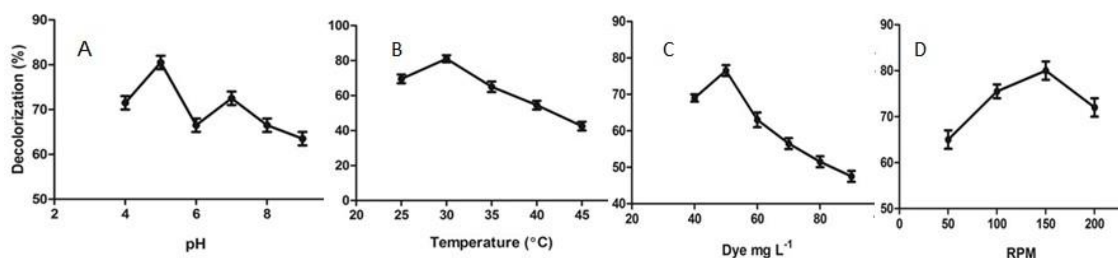

Fig. 5 — Effect of various factors influencing the methylene blue decolourization (20 mg L^{-1}) by *Streptomyces coelicolor* strain SPR7. (A) pH; (B) temperature; (C) concentration of dye; and (D) rotation speed

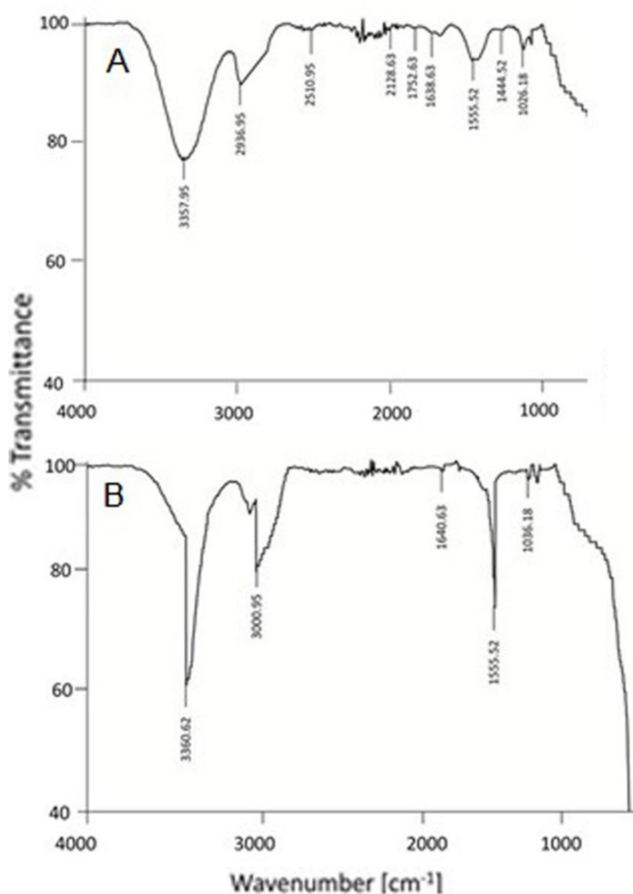

Fig. 7 — FTIR spectra of methylene blue (A) control (0 h); and (B) metabolized products of treated dye after at 72

1640.63 cm^{-1} corresponds to C=C bending, 1555.52 cm^{-1} for N-H bending, 1036.18 cm^{-1} for C-O stretching. Noraini *et al*.[39] executed the aerobic degradation of MB by *Sphingomonas paucimobilis* strain derived from industrial wastewater which showed a similar results of infra-red spectrum analysis for treated and untreated samples of the dye.

Phytotoxicity studies

Inspite of the fact that untreated textile effluents are prone to cause serious health and environmental endangerment, they are being released out into the water bodies and this is used as a source for irrigation of agricultural crops[40]. The direct usage of these untreated and treated textile effluents for agricultural process exhibits a direct impact on the soil fertility. The breakdown products of reactive dyes are ascribed to be sulfonated and unsulfonated aromatic amino compounds and are well known for their environmental toxicity[41,42]. Hence, the assessment of phytotoxicity of the dyes prior and post to the biological treatment is highly indispensible. The sensitivity towards the azo dye MB and its deteriorated metabolites by *S. coelicolor* strain SPR7 in relation to *V. radiata* was appraised. The statistical mean of plumule and radical length were 14.16±0.36 and 7.29±0.21 cm, respectively for 10 seeds using sterile distilled water as the control with 90% germination. In contrast, the germination rate (40%) and lengths of plumule and radical were drastically affected after treatment with MB (50 mg L^{-1}). However, the treated dye products were found to exhibit lower toxicity on *V. radiata* as the plumule and radical lengths were higher than the pure dye mixture i.e., 8.51±0.43 and 4.65±0.24 cm, respectively with 70% germination (Table 3). Similar to these observations, nontoxic demonstrations have been made previously using biotreated dye solutions than the control dye system on *V. radiata*[43].

Table 3 — Phytotoxicity analysis of methylene blue (50 mg L^{-1}) and its deteriorated metabolites on *Vigna radiata*

Parameter	*Vigna radiata*		
	Distilled water	Methylene blue	Extracted metabolites
Germination (%)	90%	40%	70%
Plumule (cm)	14.16±0.36	1.12±0.31	8.51±0.43
Radical (cm)	7.29±0.21	0.47±0.17	4.65±0.24

Conclusion

In the present study, an actinomycete isolate, *Streptomyces coelicolor* strain SPR7 was recruited for the biodegradation and detoxification of a distinct synthetic textile azo class of dyes. Interestingly, the docking studies were of higher corroboration with the biotransformation appraisals. The results of the present study substantiate the role of bioinformatics for exposure of the robust microbial strains encouraging the safe, low-cost and green approach of toxic azo dyes. Additionally, the phytotoxicity analysis showed the lower toxicity of degraded metabolites on *Vigna radiata.* The current study forms a basis for conversant and bioremediatory applications of the novel action-bacterial isolate. These findings may be useful for systematic degradation of textile wastewater containing toxic dyes.

Conflicts of interest

Authors have declared no conflict of interests.

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