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Degradation Potential of *Scedosporium apiospermum* SKF2 against an Azo Dye, Reactive Red 180 and Its Phytotoxicity Evaluation

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Abstract Nowadays, pollution is the biggest issue throughout the world. Dyes contaminated effluents from the textile industries are one among the massive thing which requires immediate attention. The present study focused on the biodegradation of an azo dye, Reactive Red 180 using a novel fungal strain ***Scedosporium apiospermum* SKF2.** Initially screening of a potential Reactive Red 180 degrading fungi was carried out from the effluent sediment samples collected from a textile industry located at Salem district, Tamil Nadu, India. Among the fourteen different bacterial strains isolated from it, strain no. SKF2 showed the highest dye degrading activity (78.9%). The strain was identified as *Scedosporium apiospermum* based 18S rRNA sequencing. This strain revealed dye degradation activities from the initial hours of incubation till the end of lag phase and obtained its maximum degradation activities during the trophophase. Phytotoxicity evaluation of the degraded metabolites on *Trigonella foenum graecum* seeds revealed toxicity free. In the light of above, this novel fungal isolate *S. apiospermum* SKF2 can plausibly be considered for the effective and eco-friendly biodegradation of the azo dyes like Reactive Red 180.

Keywords: *textile dye, biodegradation, reactive red 180, Scedosporium apiospermum, phytotoxicity*

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1. Introduction

Textile dyeing process requires huge amount of water, roughly 250 to 500L is required to finish one kilogram of textile product and approximately it needs 20 to 50g of dyestuffs [1] These dyes are the main sources of water pollution. Next to the textile industries, paper, wool, cotton, silk, paper printing, leather as well as pharmaceutical industries produces massive amount of dyes effluent into various water columns [2]. Among the different varieties of dyes, reactive dyes are most commonly preferred dyes especially in the textile industries. There are certain characteristics of reactive dyes which make them favourable for many industrial purposes viz., broad spectrum of colours, bright and shaded colours, brilliant pigments, high wet fastness end, minimum energy consumption, ease of application, etc [3]. Azo dyes are the most common type of reactive dyes and many of them are toxic and even carcinogenic [4]. These reactive dyes are highly toxic to the aquatic habitats and many of them are not easily biodegradable [5]. Detoxifying such reactive dyes are complex and challenging process since many of the dyes have synthetic origin and complex aromatic structures which are originally derived from the structure of naphthalene, xylene, anthracene, benzene and toluene-based compounds [6].

During the dyeing process, 10-20% of dyes remain in the processing water as wastage and 40-60% of more dyes lost through hydrolysis or washing process [7]. Disposal of such processed wastewaters into the environment have initial direct impact on photosynthetic activities and their partially breakdown products particularly reductive amines can cause serious effects on various vital organs viz., kidneys, liver, brain, reproductive and central nervous systems of aquatic organisms, animals and humans [8,9].

Removal of dyes from wastewater discharges using physical and/or chemical methods are partially successful and complete removal are costly and fairly time-consuming [10]. Therefore, biological treatment using microbial cultures of single or consortium are the recent trends for the cost effective, eco-friendly, faster and

complete mineralization of azo dyes when compared to the conventional physicochemical treatments [11]. There are many reports on the biodegradation efficiency of diversified bacterial strains against different azo dyes [12]. However, employment of fungal isolates are reported less and hence the present study focused on the isolation of textile effluent adapted fungal strains for the effective degradation of textile dyes. Biological treatment of Reactive Red 180 (RR 180) was rarely executed and only a single report is presently available proposing its biodegradation using a bacterium Citrobacter CK3 [13] and there is no exploration using a fungal isolate. So, the present study was undertaken for the screening of an appreciable fungus from textile effluent sediment and detailed its further biodegradation efficiencies.

2. Materials and Methods

2.1. Isolation of Textile Dyes Degrading Fungi

Textile effluent sediment samples were collected from an textile industry of Salem district, Tamil Nadu, India. The samples were collected using standard microbiological procedures and were transported to the lab immediately and were stored at 4°C until further analysis. One gram of effluent sediment sample was used for the serial dilution with saline followed by spread plating on Sabouraud dextrose agar (SDA) plates. After 4 days of incubation at 30°C, individual colonies with unique morphologies were isolated and pure culture was established for all the isolated. All the axenic fungal cultures were individually maintained in SDA slants.

2.2. Screening of an Appreciable RR 180 Degrading Fungi

All the isolated axenic fungal strains were screened for their degrading efficiency against a chosen azo dye, RR 180. Sabouraud dextrose broth (SDB) was prepared with 100mg/L RR 180 dye concentration and was used for the screening of potential dye degraders. After 6 days incubation at 30°C, the cultured broth using the individual fungal strains were measured for the decolorization by the separation of cell biomass involving centrifugation at 10,000 rpm for 10 min. The supernatant was subjected to the measure of decolorization through a UV-Vis spectrophotometer. The absorbance of the cell free supernatant at the λ max of the RR 180 (520 nm) was compared with that of the uninoculated broth containing the dye (control). The Percentage dye degradation rate was calculated as follows:

$$\text{Percentage dye degradation rate} = \frac{\text{Absorbance of control} - \text{Absorbance of treated broth}}{\text{Absorbance of control}} \times 10$$

2.3. Molecular Identification of RR 180 Degrading Fungus

Molecular identification of the novel strain involved the use of a universal set of fungal primers viz., ITS1 (5′-TCCG TAGG TGAA CCTG CGG-3′) and ITS4 (5′-TCCTCCGC TTAT TGAT ATGC-3′) which sequenced the internal transcribed spacer (ITS) and 5.8S rRNA regions [14]. The gene amplification was carried out by PCR and resulting product was purified using QIAGEN PCR purification kit. The sequencing of the amplified regions was performed with the help of ABI Prism 377 automatic sequencer (Applied Biosystems, FosterCity, CA, USA). The homology of the sequenced region was analysed in BLAST (2.5.0 version) of the NCBI Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The evolutionary relationships with the available sequence of NCBI GenBank were computed using MEGA 7 [15] with the help of bioinformatics tool, neighbour joining method.

2.4. Growth Kinetic Profile of the Potential Fungus as a Function of Time against the RR 180 Degradation

The RR 180 degrading efficacy of the novel isolate was investigated in this study with reference to the cell biomass growth and incubation period. These factors were studied from 0^{th} hr till the idiophase of the fungus. This study was conducted in 250ml conical flash with the working volume of 100ml SDB at 1 µg/L RR 180 concentration along with the other cultural conditions of pH 5.6, 30°C temperature and 100 rpm agitation. Further, the cell biomass and percentage of dye degradation were monitored by separating the fungal cells and cell free supernatant using centrifugation at 10,000 rpm for 10 min in which the cell biomass was used for growth estimations after drying it in hot air over at 50°C for 30min and the cell free supernatant was used for the measurement of dye decolourization as per the formula described in the earlier section.The preparation of inoculum was done with fungal spores collected from five days old culture using inoculation loop and physiological saline was used as diluent.

2.5. Evaluation of RR 180 Degradation Using HPLC

The cell free supernatant of theRR 180 dye degraded broth was separated and was extracted with ethyl acetate, dried using anhydrous Na_2SO_4, further evaporated with rotary vacuum and finally dissolved in methanol. The dye degradation was evaluated using HPLC chromatogram where the un-inoculated broth with dye (without fungus) served as a control for comparison, further, all the extraction procedures was applied as same followed in dye degraded broth. HPLC analysis was done using Shimadzu LC 40102010 in which methanol was chosen as the mobile phase with a 1.0 mL/min flow rate for 10 min in a C18 column (4.6 × 250 mm) and absorbance was taken at 470 nm using a dual absorbance detector.

2.6. Phytotoxicity Analysis

Fenugreek (*Trigonella foenum - graecum*) seeds were used in this study for the phytotoxicity evaluation of the RR 180 degraded metabolites. After biodegradation, the

metabolites was extracted with ethyl acetate and was used for the toxicity analysis. Further, untreated broth containing the RR 180 dye was used as negative control and the distilled water was used as positive control. The experiments were performed on filter paper in petri dishes dipped using the original metabolite concentration which existed in the treated broth and controls. In each experiment, ten healthy seeds of equal size were placed on the filter paper dipped in 4mL of the sample. The petri dishes were then kept in the dark at room temperature to promote the seeds germination for 72 hrs. The phytotoxicity of samples in relation to seed germination rate was estimated using the methodology described earlier by Kurade et al. [16] and Priac et al. [17]. The radicle growth length of germinated seeds was evaluated using centimetre scale.

3. Results and Discussion

3.1. Isolation and Screening of RR 180 Dye Degrading Potential Fungi

Azo dyes are widely using textile, paper, printing, rubber, cosmetics and pharmaceutical industrial colouring agent which are poorly biodegradable, so they can sustain in any environment for a longer period [18]. Hence, screening of appreciable microbes for the effective degradation of such dyes plays a challenging phase [19]. Till date, many bacterial strains have been reported for their biodegradation efficiencies, however, fungal strains have been poorly studied [20]. Hence the present study was carried out to isolate potential azo dyes degrading fungi. In this study, the collected textile effluent sediments samples (at three sites) were studied individually for the isolation of RR 180 degrading fungi. The samples were serially diluted, plated on SDA medium and morphologically unique strains were pure cultured on fresh SDA.

Totally 14 morphologically unique fungal strains have been isolated from all the three samples and they were initially named as SKF1 to SKF14. All the strains were individually screened for their potential to degrade RR 180 dye. Among the isolates, strain no. SKF2 showed highest decolourization ability of up to 78.9% followed by SKF8 strain with 64.5% and rest of the strain showed poor decolourization. Considering the above, the SKF2 strain was chosen for further biodegradation and phytotoxicity studies. To the fact, RR 180 dye degrading fungi have never been explored till date and this is the first report regarding the same. Further, only one bacterium, Citrobacter CK3 has been identified for the appreciable decolourization and degradation of RR 180 which was originally isolated from activated sludge of a textile mill by Wang et al. in 2009 [13].

3.2. Molecular Identification of SKF2 Strain

The molecular identification of the fungi, SKF2 was performed by using ITS and 5.8S rRNA regions and the amplified sequence revealed the total length of 605 base pairs. BLASTn homology was performed against the available similar sequence from NCBI GenBank nucleotide collection which revealed that this sequence has 100% similarity with seven strains of *Scedosporium apiospermum*. The sequence was submitted in the NCBI Genbank database with the accession number, MT378330. The genus *Scedosporium* belongs to the family Microascaceae and the phylum Ascomycota. The phylogenetic tree of *Scedosporium apiospermum* SKF2 was drawn against the highest similarity sequences collected from the NCBI GenBank nucleotide database (Figure 1).

3.3. Growth Kinetic Profileof the Potential Fungus

The growth kinetics of *S. apiospermum* SKF2 with reference to the RR 180 dye degradation activities as a function of time was examined in this present investigation. This strain initiates degradation activity since the 1st hr of incubation and a gradual increase of activity was recorded till the end of exponential growth phase (96hrs). Further, this strain achieved its maximum degradation during the stationary growth phase from 108hrs to 144hrs with a cell biomass concentration of 8.15 ± 0.4g/L dry weight. Furthermore, a gradual reduction of degradation was observed during the decline growth phase from 156hrs as shown in the Figure 2.

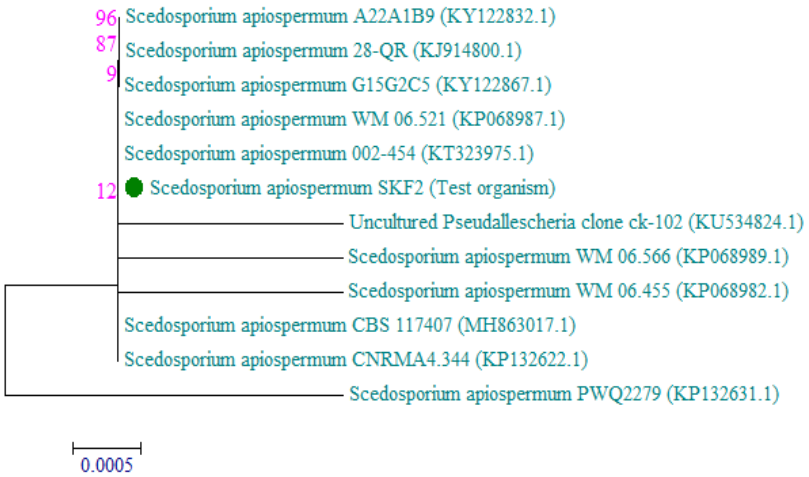

Figure 1. The evolutionary distance of *S. apiospermum* SKF2 with the highest twelve similar nucleotidesequencesof the NCBI database collection which were computed using Neighbor-Joining method

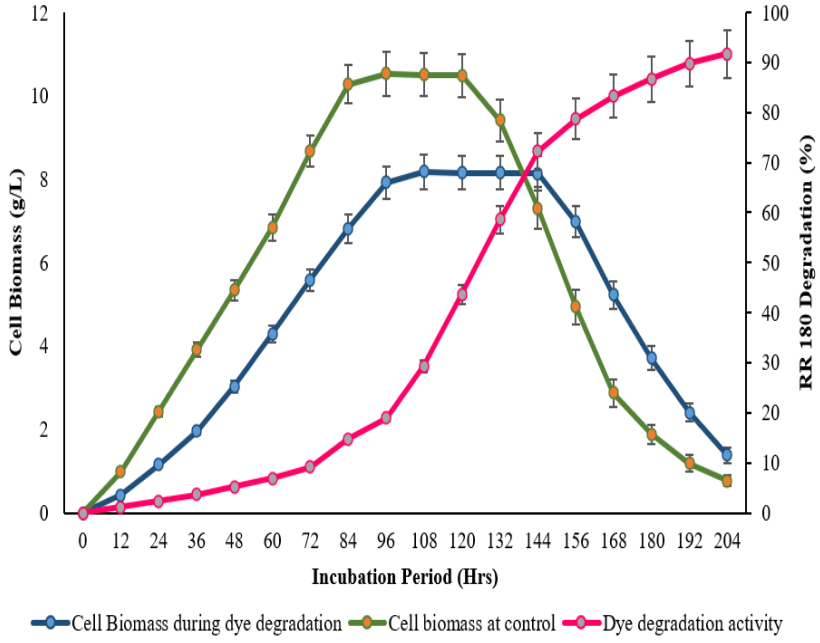

Figure 2. Growth kinetic profile of *S. apiospermum* SKF2 as a function of time against the RR 180 degradation

Figure 3. Biodegradation activity of *S. apiospermum* SKF2 against the RR 180 dye after 204hrs incubation time

At the end of this experiment, 91.7 ± 0.47% of decolorization was of RR 180 dye was observed and the difference in the decolorization of RR 180 dye during the 0hrs and 204hrs were shown in the Figure 3. This pattern of cell growth and dye degradation revealed that both primary and secondary metabolites were involved in the decolourization process. Moreover, growth retardation was observed in the dye degradation experiment when compared to the control flasks. A similar pattern of growth and biodegradation activities was observed in *Bacillus cereus* SKB 12 against an azo dye, Reactive Black 5 [21].

3.4. RR 180 Degradation Study Using HPLC Chromatogram

Degradation of RR 180 with the potential fungus, *S. Apiospermum* SKF2 was studied using HPLC chromatogram. The intensity and retention time data of the obtained peaks from control and RR 180 degraded sample revealed that the decolourization was because of biodegradation and not mere adsorption of the dye by the chosen fungus. The respective solvent extracted metabolites were used in this investigation for the evaluation of biodegradation. Decolourized samples revealed two major peaks at 3.092 and 3.686 rt and five minor peaks at 2.669, 4.846, 5.407, 7.839 and 9.482 rt in the HPLC chromatogram (Figure 4b) whereas the control RR 180 dye only one major peak at a new retention time of 3.112 and no minor peaks were recorded (Figure 4a).

The appearance of the major and minor peaks in the test sample suggests that the dye was effectively degraded by the *S. Apiospermum* SKF2 and the appearance of the major peak in the treat sample was due to the accumulation of a dye degraded metabolite during the decolourization process of the fungus. The above results are an evidence that RR 180 dye was biodegraded by the novel strain *S. Apiospermum* SKF2. A similar pattern of textile dyes biodegradation studies using HPLC analysis was carried out in earlier investigations [22,23].

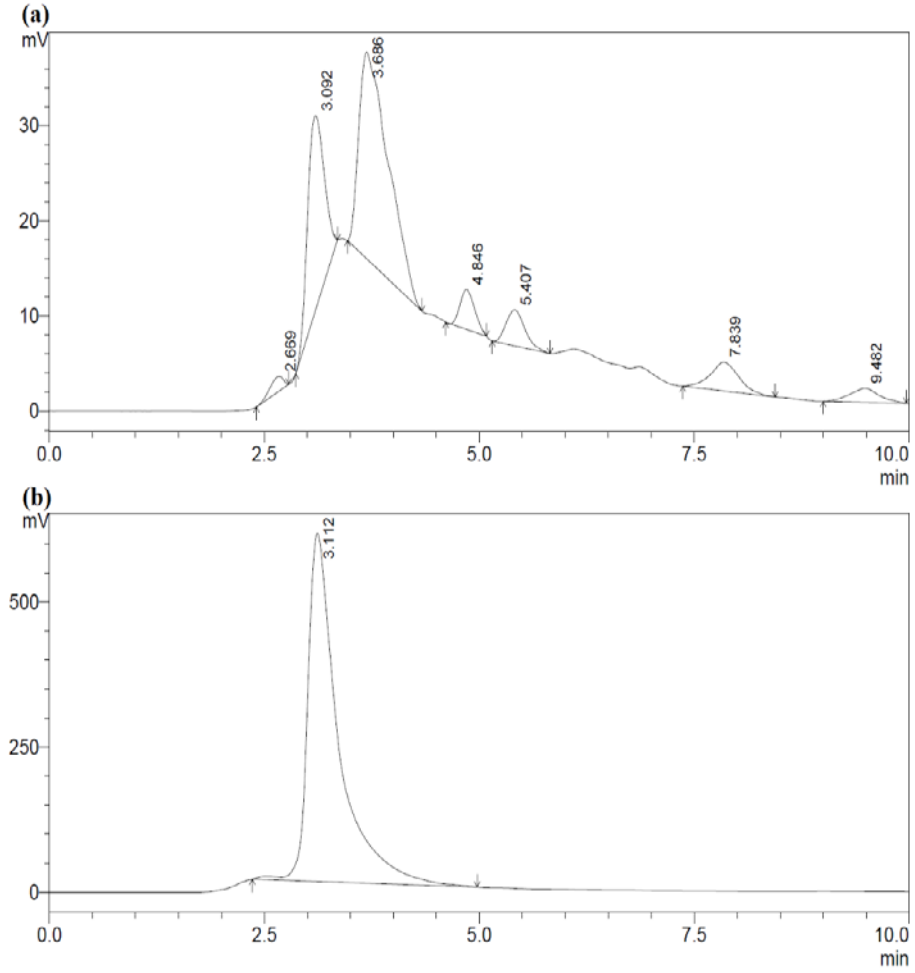

Figure 4. HPLC chromatogram of the RR 180 untreated (control) sample (b) and treated sample using *S. apiospermum* SKF2 (a)

3.5. Phytotoxicity Evaluation

The presence of phytotoxicity of the solvent extracted metabolite was examined in this study. Germination rate and radicle growth length of fenugreek seeds were used in this evaluation as it is one among the standard reference model for the study of eco/phytotoxicity experiments [24,25]. Distilled water was used as positive control and it revealed 100% germination rate with 2.8cm radicle growth of fenugreek seeds whereas RR 180 untreated broth was used as negative control and it showed poor germination rate of 16.7% with 0.7cm radicle growth. The biodegraded RR 180 treated exhibited no significant toxicity against the fenugreek seeds and it showed 100% germination rate with 2cm radicle growth.

Similarly, culture broth of *S. apiospermum* SKF2 without any added azo dye confirmed that the metabolites have no potential toxicity with fenugreek seeds with the values 100% germination rate and 2.6cm radicle growth, respectively (Figure 5). This experiment proved that the treated RR 180 sample with the *S. apiospermum* SKF2 has no toxic metabolites. Likewise, biodegradation treatment of three azo dyes namely, Reactive black 5, Reactive red 120 and Reactive blue 19 was carried out using *Acinetobacter baumannii* JC359 and the degraded metabolites were proved to be non-toxic using the same phytotoxicity testing procedure with *Vigna radiata* seeds [26]. Similarly, seeds of *Vicia faba* was also used for the phytotoxicity evaluation of treated real textile dye effluents using two fungi, *Aspergillus flavus and Fusarium oxysporium* and found that it represents no considerable toxicity [27].

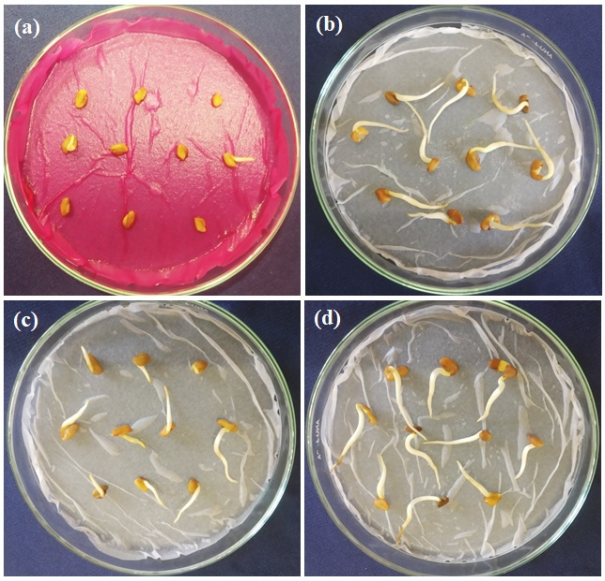

Figure 5. Phytotoxicity evaluation of *S. apiospermum* SKF2 treated RR 180 broth in Fenugreek (*Trigonella foenum-graecum*) seeds in which (a) represents the untreated broth containing the RR 180 dye (negative control), (b) illustrate the effect of distilled water (growth control), (c) shows the treated broth and (d) reveals the uninoculated broth without any added dye (positive control)

4. Conclusion

The outcome of the present study is the isolation of RR 180 dye degrading novel fungal strain *S. apiospermum* SKF2. This strain revealed degrading activities from the 1sthr of incubation till the end of log phase and achieved its peak time of degradation during the stationary growth phase. The metabolites generated on the degradation of the dyes revealed no significant toxicity when studied using Fenugreek seeds. Based on these facts, this investigation suggests the use of *S. apiospermum* SKF2 strain for the effective biodegradation of RR 180 dye in an eco-friendly manner.

Conflict of Interest

Authors declare no conflict of interest on publishing this article.

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