Decolourization of textile effluent by a thermophilic bacteria

Anoxybacillus rupiensis

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Abstract

Release of coloured textile effluents is undesirable in the aquatic environment as they reduce light penetration, thereby affecting aquatic life and limits utilization of the water media. Microbial remediation is an alternative treatment option available other than the commonly employed physico-chemical methods to treat these toxic effluents. This study investigated the potential of Anoxybacillus rupiensis, a thermophilic bacteria isolated from hot water springs of Maharashtra state of India to decolourize local textile effluent. The results showed 75% decolourization through degradation at 60°C in eight days proving the thermophilic isolate as a potential candidate for decolourizing the textile effluent.

Keywords: Textile effluent; Hot water springs; Decolourization; Thermophilic bacteria

Introduction

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrial progress has now assumed serious proportions. Management of water pollution is at present one of the major challenges for environmentalists. More than 10,000 different textile dyes with an estimated annual production of 7x10^5 metric tones are commercially available worldwide [24]. These dyes (2%) are directly discharged into the environment with consequences on aquatic life, as many of these dyes are toxic and have a high affinity for water. It is not only because of their impact on photosynthesis of aquatic plants but also due to the carcinogenic nature of many of these dyes and their breakdown products [41]. These dyes linger in the environments for longer periods if let out with-out adequate treatment. Hydrolysed Reactive Blue-19 has a half-life of about 46 years at pH 7 and 25°C [14]. Several combinations of treatment methods have been developed in order to effectively process textile wastewater; decolourization being one them. Treatment of dye wastewater involves physico-chemical methods such as coagulation, precipitation, adsorption by activated charcoal, oxidation by ozone, ionizing radiation and ultra filtration. These methods are costly, less efficient, has limited application but also generate wastes which are difficult to dispose off [7].

Decolourization of industrial effluents containing dyes can be achieved by adsorption of dyes on microbial surfaces. Live or dead biomass can be used to remove toxic synthetic dyes [11-15,18]. A large number of bacteria, fungi, yeast and algae can be used for removal of dyes by their negatively charged ligands present in cell wall components from textile effluents [20-22,35]. Immobilised Asperigillus terreus in different matrices was used for decolourization of a textile effluent by Engade and Gupta [11].

Synthetic dyes and dye residues present in various industrial effluents can be removed by enzymes produced mesophilic microorganisms [1-8,33]. Azo dyes are degraded by mesophilic and thermophilic anaerobic consortia in batches [2]. Synthetic dye Reactive Black 5 was degraded upto 80% by a thermophilic Anoxybacillus pushchinoensis, Anoxybacillus kamchatkensis, and Anoxybacillus flavithermus [32]. Microbial technologies are eco-friendly and cost-competitive alternative to chemical decomposition processes [38]. Moreover, degradation can also detoxify the effluent effectively without leaving any toxic residues. The aim of the present work was to explore the potential of the thermophilic bacteria Anoxybacillus rupiensis isolated from the hot water springs of Unhavre situated in the western coast of Maharashtra for its ability to degrade a local textile effluent containing dyes.

Materials and Methods

Enrichment and isolation of thermophilic bacteria

Water and soil samples of hot water springs of Unhavre, (Dhapol region) in Ratnagiri, Maharashtra, India. were collected under aseptic conditions. The samples were enriched and thermophilic bacteria were isolated using the streaking method on thermus agar (ATCC medium 697) containing 0.5% NaCl, 0.5% peptone, 0.4% beef extract, 0.2% yeast extract and 2% agar, pH of the medium was adjusted to 7.0 before autoclaving. Plates were incubated at 60°C for 24h and purity of the colonies was checked microscopically. The isolates were characterized according to Johansen et al. [17]. For phylogenetic analysis were performed 16S rRNA基因 sequencing and data analysis were performed according to Johansen et al. [17]. For phyloge-netic analysis were sequences for type strains in the genera Geobacillus and Anoxybacillus and the closest relatives found by BLAST downloaded from public databases and imported into the ARB program package [38]. The sequences were imported into the ARB program package [38]. The

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sequences were aligned using the ARB aligner followed by manual alignment. Phylogenetic trees were calculated using the Neighbour joining program and the Fast ML program in the ARB program package.

Collection of textile effluent

Textile industry effluent was collected from a dyeing unit in Aurangabad, Maharashtra. The colour of the effluent was reddish black and pH was 10.5. The concentrated solution from the dye bath was collected in a 10 liter plastic can for the proposed experiments.

Spectral studies

The textile effluent was filtered and the filtrate was used to determine Lambda max spectrophotometrically.

Graph to determine percent decolourization

The lambda maxima obtained for the dye containing effluent was tested to plot a standard graph considering dilutions of effluent on X-axis and their respective optical densities on Y-axis. This graph was referred to calculate percent decolourisation.

Textile effluent treatment with anoxybacillus rupiensis

Standard inoculum was developed by suspending the pure colonies of Anoxybacillus rupiensis into sterile saline and its absorbance set to 0.1 at Ab620nm to obtain uniform cell density, 5 ml of this developed inocula was inoculated into 250 ml conical flasks containing 35 ml of 0.1 at Ab620nm to obtain uniform cell density, 5 ml of this developed inocula was inoculated into 250 ml conical flasks containing 35 ml of medium. The flasks were incubated under stationary condition at 60oC.

Decolourization studies

Five ml of growth medium was removed after every 24 hours and centrifuged at 10000 rpm for 10 minutes. The Optical Density of the supernatant was recorded spectrophotometrically at the obtained lambda maxima. Decolourization was monitored at 24 h interval for 10 days. The percent decolourization was calculated from the standard graph by plotting the obtained O.D values.

| S.No. | Test          | Colony                   |
|-------|---------------|--------------------------|
| 1     | Gram staining | Gram – ve rods           |
| 2     | Indole test   | +ve                      |
| 3     | Methyl Red (MR) test | Voges-Proskauer -ve |
| 4     | (VP) test Citrate test | +ve |
| 5     | Triple Sugar Iron Agar (TSI) test | -ve Acid slant/ Acid Butt |
| 6     | Mannitol Motlity (MM) test | Urea -ve |
| 7     | Hydroxylase test | Nitrate Reduction test -ve |
| 8     | Oxidase test  | +ve                      |
| 9     | Sugar Fermentation test | +ve |
| 10    | (a) Lactose   | +ve                      |
| 11    | (b) Adonitol  | +ve                      |
| 12    | (c) Dextrose  | +ve                      |
|       | (d) Trehalose | +ve                      |
|       | (e) Melibiase | +ve                      |
|       | (f) Raffinose | +ve                      |
|       | (g) Arabinose | +ve                      |
|       | (h) Sucrose   | +ve                      |
|       | (i) Cellobiose | +ve |

Table 1: Biochemical characteristics of thermophilic bacterial isolate.

Figure 1: Phylogenetic tree of Anoxybacillus rupiensis. 16S rDNA sequence-based phylogenetic neighbour joining tree showing the phylogenetic relationship of isolated colony relative to the type strains of species in the genera Geobacillus, Anoxybacillus and sequences for the unpublished Anoxybacillus species “A. beppuensis” (identical with isolate) and “A. bogroviensis”. B. subtilis is included as outgroup. Bootstrap values (%) from 1,000 replicates are as shown. The tree topology by calculation by the FastML program was similar to the tree shown.
Results and Discussion

Yellow colored small colonies were obtained on Thermus agar medium on three days of incubation at 60°C. The colonies were confirmed as gram negative, short rods. This bacterial isolate was identified by the biochemical tests and 16s RNA ribotyping as *Anoxybacillus rupiensis*. The results of biochemical tests are computed in Table 1.

Phylogenetic tree developed in Figure 1, shows that the isolated strain is positioned between Geobacillus species from one side and *Anoxybacillus* species from the other side. The closest sequence relatives found by BLAST search was "*Anoxybacillus beppenis*" AAB243446 and *Geobacillus tepidamans* T, AY563003 (96.8% similarity), i.e., more than the level (3% distance) over which strains are generally attributed to separate taxa [36]. Except for *G. tepidamans*, the similarity between the isolated strain and other species in the genus Geobacillus was less than 91%. According to Nazina et al. [28] the observed levels of 16S rRNA gene sequence similarity in this genus are higher than 96.5%, and *Geobacillus tepidamans* T is phylogenetically close to *Anoxybacillus* species [34]. On the basis of phylogenetic similarity with *Anoxybacillus* species, our isolated strain was related to genus *Anoxybacillus*.

At wavelength 421nm maximum absorbance was recorded and hence lambda maxima was found to be at 421nm. Reduction in optical density was observed as days of incubation increased. Percent decolourization of textile effluent by *Anoxybacillus rupiensis* is shown in Table 2. Results confirmed the percent decolorization of textile effluent by *Anoxybacillus rupiensis* increased with respect to incubation period. In the first 24 hours the percent decolorization noticed was 21 and at the end of 9 days it was 83. Further extension of incubation period did not show any further increase in percent dye decolorization. This may be due to exhaustion of essential nutrients upon prolonged incubation i.e. 9 days.

In our experiment the decolorization of the textile effluent was mediated by enzymes produced by *Anoxybacillus rupiensis*. To confirm this modus operandi, growth medium was centrifuged and the obtained cell pellet was found to be colourless, this was compared to the control which was devoid of dye thus confirming decolorization of effluent is solely due to enzyme(s) mediated mechanism and not due to physical adsorption.

Conclusion

Thermophilic *Anoxybacillus rupiensis* isolated from hot water springs of Unhavre (Dhapolig region of Ratnagiri), Maharashtra showed remarkable decolourisation through degradation in stationary cultures at 60°C. Possibly maximum percent decolourisation of textile effluent could have been achieved under submerged cultures. This research work can be further extended to study exactly the types and origin of enzymes involved in decolorization process. Growth conditions can be modified to accelerate the percent decolorization. Therefore the selected thermophile appeared to be an efficient organism for the treatment of textile effluents containing mixture of dyes.

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