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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; Textile effluent; 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 as aqueous effluents and 10% are subsequently lost during textile colouration process [30]. Colour is one of the most obvious indicators of water pollution and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies [27].

The release of coloured compounds into water bodies is undesirable 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 *Aspergillus terrus* 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, Anoxibacillus kamchatkensis,and Anoxibacillus 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, (Dhapoli 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 based on Bergey's Manual of Determinative Bacteriology [16] and identified by 16S rRNA ribotyping.

Phylogenetic analysis of the Rrna Genes

16S rRNA gene sequencing and data analysis were per-formed 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

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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 decolourization.

Textile effluent treatment with anoxibacillus 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 filter sterilized textile effluent + 60 ml of filtered sterilized thermus medium. The flasks were incubated under stationary condition at 60°C. Effluent medium without bacterial inoculum was treated as control.

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	-ve Acid slant/ Acid Butt
	(TSI) test	- / +
6	Mannitol Motility (MM) test Urea	-ve
	Hydrolysis test Nitrate Reduction test	
7	Oxidase test	-ve
8	Sugar Fermentation test	+ve
9	(a) Lactose	+ve
10	(b) Adonitol	
11	(c) Dextrose	+ve
	(d) Trehalose	+ve
	(e) Melibiose	+ve
	(f) Raffinose	+ve
	(g) Arabinose	+ve
	(h) Sucrose	-ve
	(i) Cellobiose	-ve
12	Catalase	+ve
		+ve
		+ve
		+ve
Identified thermophile		*Anoxybacillus rupeinsis*

Table 1: Biochemical characteristics of thermophilic bacterial isolate.

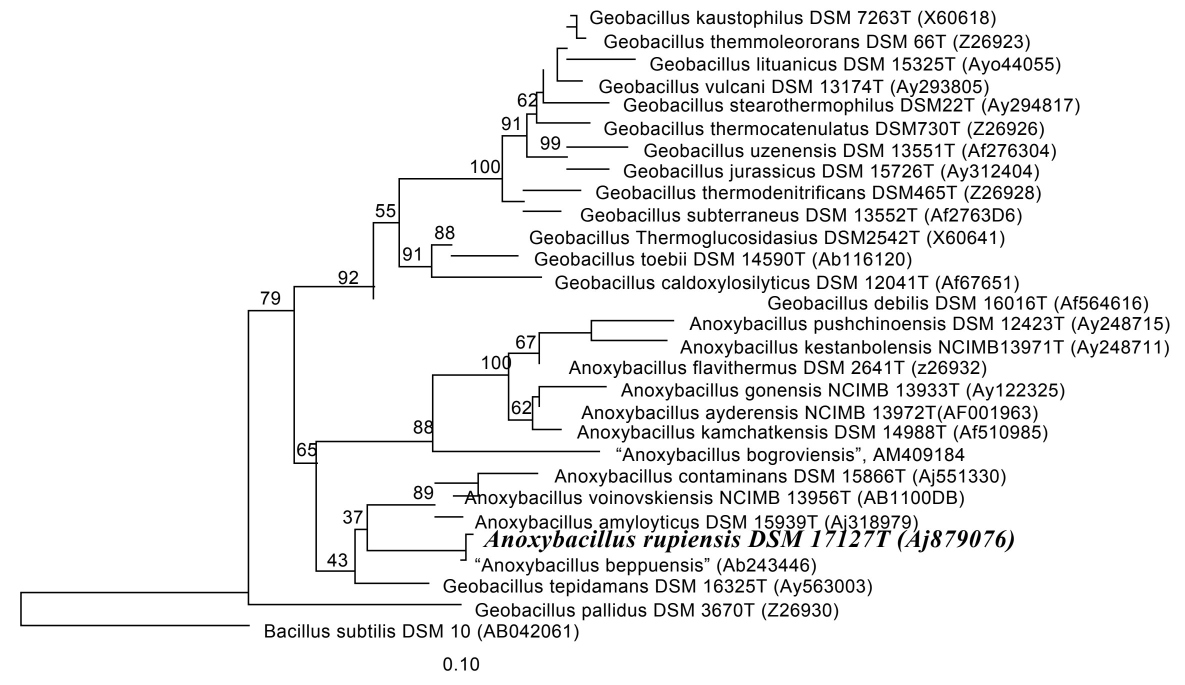

Figure 1: Phylogenetic tree of *Anoxybacillus rupeinsis*. 16S rDNA sequence-based phylogenetic neighbour joining tree showing the phylogenetic relationship of isolated colony relative to the type strains of species in the genera Geobacillus, Anoxybacillus and sequences for the unpublished Anoxybacillus 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 beppuensis*"AAB243446 and *Geobacillus tepidamansT*, 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 tepidamansT* 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 decolourization 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 decolourization. This may be due to exhaustion of essential nutrients upon prolonged incubation i.e 9 days.

In our experiment the decolourization 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 decolourization 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 (Dhapoli region of Ratnagiri), Maharashtra showed remarkable decolorisation 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 decolourization 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.

Incubation Period (Days)	*A421(620nm)*	Residual Conc (%)	Decolourization (%)
0	1.249	100	0
1	0.983	79	21
2	0.987	75	25
3	0.801	64	36
4	0.594	47	53
5	0.491	39	61
6	0.400	32	68
7	0.371	25	75
8	0.259	21	79
9	0.210	17	83

[Initial absorbance at 0 hour = 1.249 and final absorbance on Day 9 = 0.210]

Table 2: Percent decolourization of textile effluent by *Anoxybacillus rupiensis*.

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