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Biodegradation and detoxification of melanoidin from distillery effluent using an aerobic bacterial strain SAG_5 of *Alcaligenes faecalis*

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ABSTRACT

Distillery effluent retains very dark brown color even after anaerobic treatment due to presence of various water soluble, recalcitrant and coloring compounds mainly melanoidins. In laboratory conditions, melanoidin decolorizing bacteria was isolated and optimized the cultural conditions at various incubation temperatures, pH, carbon sources, nitrogen sources and combined effect of both carbon and nitrogen sources. The optimum decolorization ($72.6 \pm 0.56\%$) of melanoidins was achieved at pH 7.5 and temperature 37 °C on 5th day of cultivation. The toxicity evaluation with mung bean (*Vigna radiata*) revealed that the raw distillery effluent was environmentally highly toxic as compared to biologically treated distillery effluent, which indicated that the effluent after bacterial treatment is environmentally safe. This proves to be novel biological treatment technique for biodegradation and detoxification of melanoidin from distillery effluent using the bacterial strain SAG_5.

1. Introduction

In developing countries like India, an increase in the number of distilleries has resulted into substantial increase in industrial pollution [1]. These distilleries uses molasses as a raw material for the production of ethanol and the waste by product is discharged into the water bodies causing water pollution [2]. Untreated (raw) distillery effluent or spent wash are well known to cause pollution in the natural streams by lowering of pH value, increase in organic load, depletion of oxygen content, discoloration and destruction of aquatic life [3]. Its dark color is due to the presence of melanoidins that is the condensation product of sugar and amino acid produced by non-enzymatic browning reactions called maillard reaction [4]. This dark color hinders photosynthesis by blocking sunlight and is therefore deleterious to aquatic life [2,5]. These compounds have anti-oxidant properties, which render them toxic to many microorganisms present in wastewater treatment processes [6].

The disposal of wastes from industrial sources is becoming a serious problem throughout the world. In recent years, industrial effluents have been regarded as common source of pollution, because of lack of efficient treatment and improper mode of disposal of effluents generated by industries. Under these circumstances aquatic life suffers, resulting in loss of productivity and deterioration of water quality to such an extent that the water becomes unusable [7]. Kanimozhi and Vasudevan [8] reviewed the existing scenario on the treatment of distillery wastewater and issues requiring further research in the field of distillery wastewater treatment.

Indian distilleries employ various forms of primary, secondary and tertiary treatments of wastewater however these treatments are highly energy intensive and hence quite expensive [9]. Color can be removed by specific treatments such as coagulation–flocculation [10], adsorption on activated carbon [11] and advanced oxidation processes [12]. Wastewater treatment by conventional biological treatment leads to the large reduction in the organic load but colorants are scarcely degraded. The removal and degradation of the color contributing compounds like melanoidins, caramel, and phenolics and their metabolic products is very essential for the safe disposal of the distillery wastewater to the environmental [13].

Many researchers have tried to use biological processes for removing melanoidins from effluent. Various microorganisms like fungi [14,15], yeast [16,17] algae [18] and bacteria plays a vital role in decolorization of distillery effluent. However, in bacteria, the ability to remove melanoidins from distillery effluent was studied in *Lactobacillus plantarum* [19]; *Bacillus licheniformis*, *Bacillus* sp. and *Alcaligenes* sp. [20]; *Klebsiella oxytoca*, *Serratia mercescens* and *Citrobacter* sp. [21]; *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas maltophila*, and *Proteus mirabilis* [22];

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Pseudomonas putida, *Aeromonas* sp. [6]. Therefore, a new bacterial strain SAG_5 was screened that decolorize melanoidin rich wastewater of the distillery effluent. Hence, to achieve maximum decolorization using the isolated bacterial strain SAG_5 the efforts were directed towards the isolation, screening, optimization, toxicity test and its molecular characterization of the isolate.

2. Materials and methods

2.1. Media

Two kinds of media were used i.e. 'King's B' broth media (K_2HPO_4, 0.15%; $MgSO_4$, O.15%; Peptone, 2.0%; Glycerol, 1.0% v/v) and melanoidin pigment broth (MPB) media having the composition K_2HPO_4, 0.15%; $MgSO_4$, 0.15%; peptone, 2.0%; glycerol, 1.0% (v/v); melanoidin, 1.0% (w/v). Solid media was prepared by adding 2.0% agar in the above-mentioned composition.

2.2. Sampling and isolation of melanoidin decolorizing bacteria

Distillery effluent and soil samples were collected for the isolation of pure bacterial cultures from effluent treatment plant of Haryana distillery, Yamunanagar, Haryana, INDIA. Main site was near by the oxidation ponds, where effluent was stored after anaerobic (primary) and aerobic (secondary) treatment before disposal. The color of effluent was dark brown. The isolation of bacterial species from effluent contaminated soil was carried out using enrichment culture technique [22]. Each soil sample (10 g) was suspended in effluent amended broth media (90 ml) aseptically and incubated at 37 °C. After 8 days, 50 ml from each flask was discarded and fresh 50 ml effluent amended broth was added so that desirable microorganisms could grow and again incubated for 8 days. After 8 days, an aliquot (100 μl) was spread on 'King's B' agar plates and incubated at 37 °C.

2.3. Melanoidin preparations

Melanoidin preparation was carried out using 0.05 M of glucose (sugar, 9.00 g) and 0.05 M of glycine (amino acid, 3.75 g) dissolved in 20 ml of distilled water. This carbonyl compound–amino acid mixture was incubated at 125 °C in an oven for 2 h and was transferred to a mortar and carefully grounded to a fine powder [4].

2.4. Screening of isolates having decolorization activity

To prepare inoculum, isolated microbes were cultured in 'King's B' Broth medium (pH 7.0) for 24 h at 37 °C. The various concentrations of melanoidins (0.2–2.0%, w/v) were used for screening of isolates. The color removal activity of isolates was expressed in terms of the percent decolorization. The decolorization of effluent was measured as decrease in optical density at 475 nm of the supernatant obtained upon centrifugation (rcf of $9279 \times g$ for 15 min) against un-inoculated effluent [14].

2.5. Optimization of cultural conditions for decolorization studies

The selected isolate was optimized for various growth parameters required for the decolorization activity. These parameters included temperature (25–55 °C), pH (5.0–9.0), effect of various concentrations of distillery effluent (5–100%, v/v), supplementation of various carbon sources (maltose, sucrose, glucose, galactose, ribose, mannitol, fructose and xylose) at concentration of 2.0% (w/v), supplementation of various nitrogen sources (peptone, beef extract, ammonium nitrate, yeast extract, ammonium sulphate and sodium nitrate) at concentration of 0.5% (w/v), and supplementation of both selected carbon and nitrogen sources in various combinations. The initial bacterial concentration in each test was 1 ml/100 ml of melanoidin media.

2.6. Toxicity test

The toxicity effect of raw distillery effluent and bacterial treated effluent was studied on mung bean (*Vigna radiata*) seed germination using Petri dish method [23]. The seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min, followed by repeated washings with sterilized distilled water. Seeds were spread on sterilized Petri dishes lined with sterilized filter paper. The seeds were cultivated with equal volume (10 ml) of raw and treated distillery effluent. The Petri dishes were kept at room temperature. The germination measurements were made from the day of sowing. Criterion for germination was visible protrusion of the seed coat. The plant growth measurements included shoot length, root length, wet shoot weight, wet root weight, dry shoot weight and dry root weight were noted from 15th day old seedlings. Different seed germination parameters like % germination, germination speed, emergence index, vigour index [24] and peak value [25] were also studied.

2.7. Purification and characterization of isolates

The individual colony of bacterial isolate was picked up and subcultured to purify by streak plate method on 'King's B' agar plates. The selected isolate was further characterized on the basis of genotypic determination.

2.8. Molecular characterization

The genotype of the isolate SAG_5 was determined by using 16S rDNA sequencing and their phylogenetic relationship. The isolated bacterial culture was grown in 'King's B' broth medium for 24 h at 37 °C with shaking at 180 rpm in an incubator shaker. The culture was centrifuged by using Eppendorf Microcentrifuge Model 5415 R at rcf of $2320 \times g$ for 10 min. The standard procedure was used for genomic DNA of bacterial cultures. The standard 16S primers were used for PCR amplification of ribosomal DNA: fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) targeting ribosomal DNA according to Weisburg et al. [26]. 16S rDNA amplification was performed in 50 μL final volume containing genomic DNA (50 ng), 20 pmol of primer fD1 and rP2, a mixture of dNTPs (Sigma) (each at a concentration of 200 μM), 10× Taq polymerase buffer and 2.5 U of Taq DNA polymerase (Sigma). The reaction conditions used for PCR were: Initial denaturation step at 94 °C for 4 min, 35 amplification cycle of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and primer extension at 72 °C for 3 min; followed by a final extension at 72 °C for 10 min with MyCycler™ PCR System (BioRad). The PCR product was further purified by using Qiagen PCR purification kit following the manufacturer's instruction. The 16S rDNA nucleotide sequence was determined by PCR-direct sequencing done by Macrogen (South Korea). The nucleotide sequences thus obtained were analysed with the basic sequence alignment blastn program against the database provided on the website of National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/BLAST. These sequences were aligned using CLC workbench and a phylogenetic tree was constructed using the PHYLIP analysis programme [27].

2.9. Statistical analysis

All the experiments were performed in triplicates. The results were presented as mean ± SD (standard deviation). The standard deviation was calculated using MS Excel.

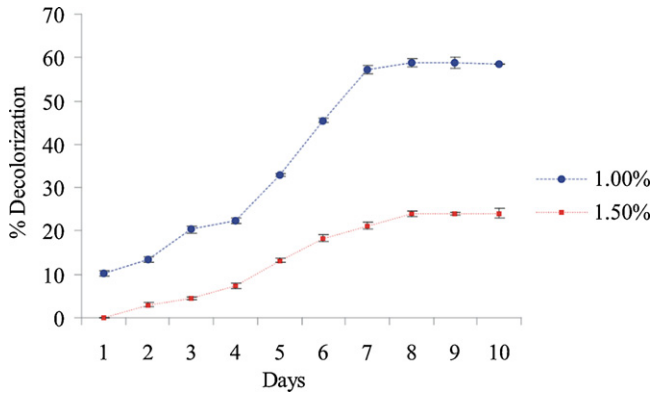

Fig. 1. Decolorization (%) by isolate SAG_5 at 1.0% and 1.5% concentration of melanoidin in 8 days.

3. Results

3.1. Isolation and screening

A total of sixty bacterial isolates were obtained and the highly efficient isolate SAG_5 was chosen after secondary screening. Various concentrations (0.2–2.0%) of melanoidins were tested to check the decolorization efficiency of the isolates. But the bacterial isolate SAG_5 showed maximum decolorization activity (i.e. $58.8 \pm 0.98\%$) at 1.0% concentration of melanoidins on 8th day (Fig. 1). This activity was decreased at 1.5% conc. level of melanoidin. The bacterial strain SAG_5 was isolated from the soil sample, where effluent was disposed off since a very long time.

3.2. Effect of the temperature and pH on decolorization activity

The effect of temperature and pH on decolorization activity was examined using 1.0% initial concentration of melanoidins. The decolorization activity of the isolate SAG_5 at various ranges of temperatures i.e. 25–55 °C was studied. The optimum temperature was found to be 37 °C for decolorization activity (Fig. 2). The further increase in temperature upto 55 °C inhibited the activity of this isolate. The effect of medium pH on decolorization was examined over the range of (6.5–9.0) and the highest decolorization activity was observed at pH 7.5 (Fig. 3).

3.3. Effect of various concentrations of distillery effluent

To see the decolorization activity, various concentrations (5.0%, 10%, 15%, 20% and 25% v/v with distilled water) of distillery effluent were studied. The order of decolorization activity in these concentration levels were found to be 5% > 10% > 15% > 25% > 20% (Fig. 4). The maximum decolorization showed by the isolate SAG_5 was $46.0 \pm 0.28\%$ at 5.0% concentration level. It was noticed that with the increase in time duration, decolorization activity of the isolate SAG_5 was increased upto 8th day and after that it was decreased (Fig. 4).

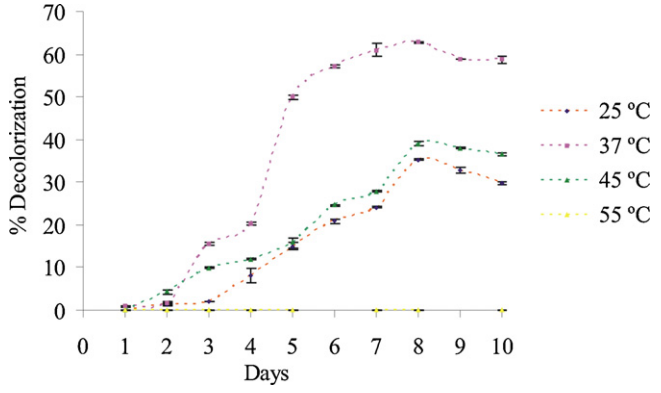

Fig. 2. Effect of temperature on the decolorization ability of isolate SAG_5.

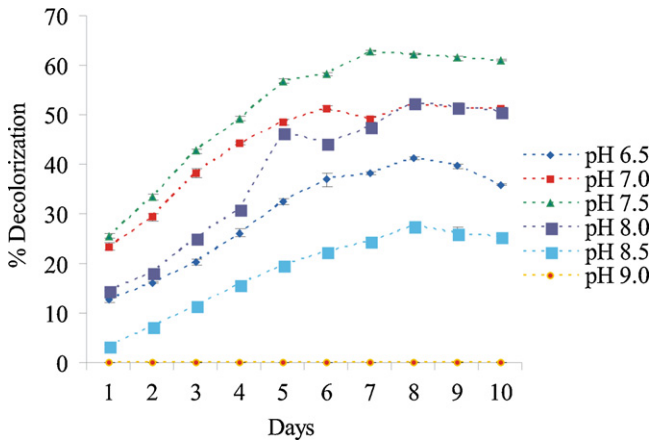

Fig. 3. Effect of medium pH on the decolorization ability of isolate SAG_5.

3.4. Effect of various carbon and nitrogen sources on decolorization activity

The presence of a readily available carbon sources was necessary for growth of isolated culture and for melanoidin decolorization. The decolorization activity of SAG_5 on the medium containing various types of sugars as a carbon source has shown in Fig. 5. The isolate sag_5 showed the high level of decolorization yields ($63.2 \pm 0.14\%$, $61.1 \pm 0.14\%$) within 5 days incubation when glucose or sucrose was used as a carbon source.

The results of the effect of various nitrogen sources (0.5%, w/v) on melanoidin biodegradation and decolorization showed that supplementation of nitrogen sources positively affected the bacterial decolorization of effluent. The highest decolorization yield ($59.2 \pm 0.28\%$) within 5 days was observed using beef extract (Fig. 6).

3.5. Effect of supplementation of both selected carbon and nitrogen sources

In another experiment supplementation of both selected carbon (2.0%, w/v) and nitrogen (0.5%, w/v) source was used in the MPB.

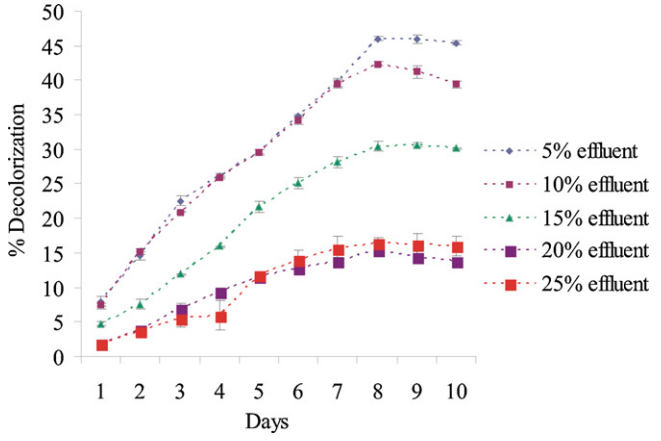

Fig. 4. Effect of different concentrations of distillery effluent.

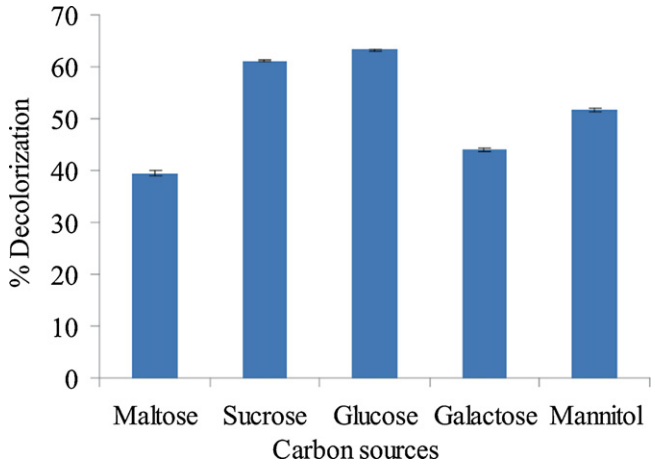

Fig. 5. Effects of carbon sources on the decolorization activity.

The media was supplemented with four different sources of sugars in combination with four different nitrogen sources viz. sucrose (+ beef extract or ammonium nitrate or yeast extract or peptone), glucose (+ beef extract or ammonium nitrate or yeast extract or peptone), maltose (+ beef extract or ammonium nitrate or yeast extract or peptone) and galactose (+ beef extract or ammonium nitrate or yeast extract or peptone) was performed. The isolate SAG_5 gave the maximum decolorization yield (72.6 ± 0.56%) within 5 days of cultivation with the supplementation of combination of glucose (2.0%) and beef extract (0.5%) (Fig. 7).

3.6. Toxicity test

Petri dish having seeds with isolate SAG_5 treated distillery effluent showed initiation of germination on 3rd day, whereas no germination was observed with non-treated raw distillery effluent. It was observed that the effluent before treatment was very toxic which inhibited the growth of seeds but after treatment the toxicity of effluent decreases (Fig. 8).

Various parameters were studied showed % germination (33.33 ± 5.77%, 90.00 ± 10.00%); germination speed (3.33 ± 0.58, 10.00 ± 1.11); emergence index (0.67 ± 0.12, 3.00 ± 0.33); and peak value (3.33 ± 0.58, 10.00 ± 1.11) with raw and treated effluent respectively. The vigor index (475.33 ± 149.43), shoot length

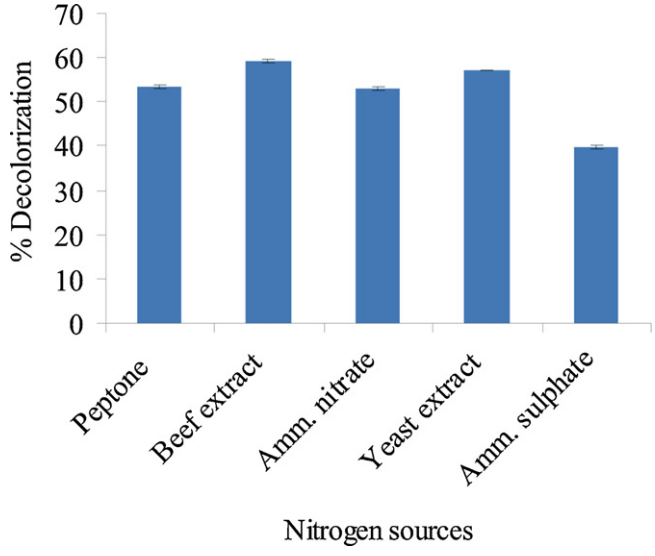

Fig. 6. Effects of nitrogen sources on the decolorization activity.

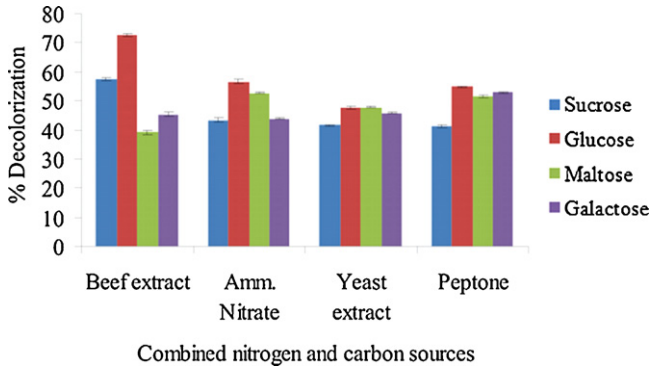

Fig. 7. Combined effects of both selected carbon and nitrogen sources on the decolorization activity.

(5.22 ± 1.30 cm), root length (3.42 ± 0.37 cm), wet root weight (93.61 ± 4.02 mg), wet shoot weight (112.40 ± 10.92 mg), dry root weight (38.51 ± 16.26 mg), dry shoot weight (133.67 ± 18.78 mg) and no. of leaves (1.97 ± 0.16) were highest in treated effluent (Table 1).

3.7. Molecular characterization of bacterial isolate

16S rDNA sequence analysis. PCR amplification of 16S rRNA gene for the melanoidin degrading bacterial isolate SAG_5 produced an amplification product of approximately 1400 bp. The partial 16S rRNA gene has been sequenced and submitted to NCBI Genbank under the accession number GQ422443 and the culture has been deposited in the Microbial Type Culture Collection and Gene Bank (MTCC) with the MTCC number MTCC 9781. The alignment of the retrieved sequences from the NCBI database with 16S rRNA gene of the melanoidin-degrading bacterial isolate SAG_5 showed sequence homology to *Alcaligenes faecalis* and *Alcaligenes* sp. Thus, the phylogenetic relationship of the SAG_5 isolate drawn using PHYLIP analysis programme resulted in close relatedness with *A. faecalis* (Fig. 9).

4. Discussion

Decolorization and degradation of distillery effluent has been a serious environmental concern, which is evident from the magnitude of research done in the last decade [15,16,22,28]. The dark brown colored wastewater after anaerobic treatment and dilution is either used for irrigation causing gradual soil darkening [29] or is disposed off into the water streams, which imparts color to the natural water bodies and hence harmful for both soil and water. Its highly colored component also leads to the reduction of sunlight penetration in rivers, lakes or lagoons which in turn decreases both

Table 1
Effect of treated and raw effluent on germination of *Vigna radiata*.

Sr. no.	Parameter (s)	Raw effluent	Treated effluent
1	% germination	33.33 ± 5.77	90.00 ± 10.00
2	Germination speed	3.33 ± 0.58	10.00 ± 1.11
3	Emergence index	0.67 ± 0.12	3.00 ± 0.33
4	Peak value	3.33 ± 0.58	10.00 ± 1.11
5	Vigor index	21.67 ± 2.89	475.33 ± 149.43
6	Shoot length (cm/seed)	0.65 ± 0.02	5.22 ± 1.30
7	Root length (cm/seed)	1.50 ± 0.33	3.42 ± 0.37
8	Wet root weight (mg/seed)	8.12 ± 0.00	93.61 ± 4.02
9	Wet shoot weight (mg/seed)	54.32 ± 0.00	112.40 ± 10.92
10	Dry root weight (mg/seed)	1.55 ± 0.00	38.51 ± 16.26
11	Dry shoot weight (mg/seed)	8.18 ± 0.00	133.67 ± 18.78
12	Number of leaves	0.50 ± 0.17	1.97 ± 0.16

All values are in Mean ± Standard deviation of three replicates.

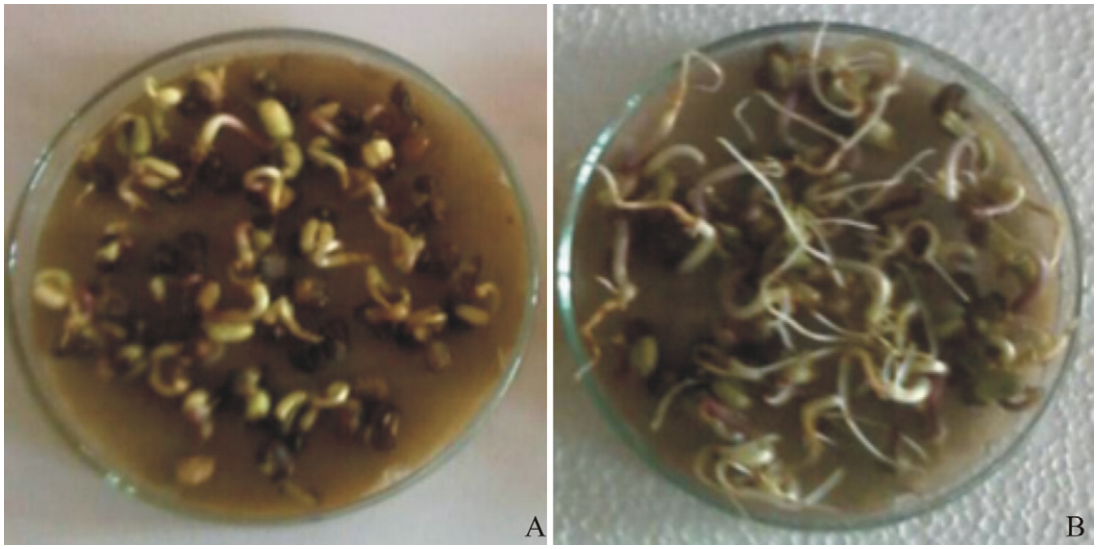

Fig. 8. Effect of toxicity of effluent on germination of seeds cultivated with raw effluent (A) and treated effluent (B).

photosynthetic activity and dissolved oxygen concentration causing hazardous to the aquatic plants and animals [2]. In the present study, the isolation of bacterial strain SAG_5 was carried out using enrichment culture technique. Similar technique has earlier been used by Mohana et al. [22] for the isolation of bacteria from the sites affected with distillery effluent.

In optimization studies, 37 °C temperature was found to be suitable for the decolorization of melanoidins. Similar temperature was found to be responsible by aerobic bacterial consortium [13]. Increase in temperature from 20 °C to 37 °C was accompanied with increase in decolorization. In another study further increase in temperature to 40 °C adversely affected the decolorization activity [22]. Earlier, reported strain no. BP103 showed higher decolorization yield of 76.4 ± 3.2% when cultivated at 30 °C [28].

The optimum pH for the melanoidin decolorization using bacterial isolate SAG_5 was found to be 7.5. On the other hand maximum decolorization yield was noted at pH range of 4.0–4.5 [30]. For *Citeromyces* sp. WR-43-6 maximum activity was at pH 6.0 [16]. However, acetogenic bacteria showed highest decolorization activity at pH range of 5.0–7.0 [28]. Aerobic bacterial consortium comprising *B. licheniformis* (DQ79010), *Bacillus* sp. (DQ779011) and *Alcaligenes* sp. (DQ779012) showed maximum activity at pH 7.0 [13].

The isolate SAG_5 showed the highest decolorization activity (63.2 ± 0.14%, 61.1 ± 0.14%) in the presence of glucose or sucrose. While in another study by Sirianuntapiboon et al. [28], glucose (3.0%) and fructose (2.0%) showed highest decolorization of 55.0 ± 1.2% and 54.8 ± 0.71% within 5 days of cultivation. Increase in concentration of glucose was essential for the growth and decolorization activity of a yeast strain (*Issatchenkia orientalis*) [17]. Ghosh et al. [6] reported the use of 1.0% glucose obligatory for growth and decolorization activity of *Pseudomonas putida.* Sirianuntapiboon et al. [16] reported that *Citeromyces* sp. WR-43-6 showed maximum decolorization with glucose at concentration of 2.0% within 8 days of incubation. According to Mohana et al. [21] the consortium DMC decolorized (67%) the anaerobically treated distillery spent wash in presence of basal salts and glucose (0.5%) and hence supplementation of glucose appeared to be necessary for decolorization. Different types of sugars such as fructose, sucrose and galactose were found to be fairly good substrates allowing 60–67% decolorization and 40–48% COD reduction. Isolated strains (BP103 and 13A) of acid forming bacteria showed maximum decolorization with maltose (87.3%) and fructose (82.5%) within 5 days of incubation [31].

In our present study, the highest decolorization yield (59.2 ± 0.28%) was observed using beef extract. Sirianuntapiboon et al. [28] reported yeast extract and peptone (0.2%) as a nitrogen source that showed highest decolorization of 55.8 ± 0.33% and 54.0 ± 0.81% within 5 days of cultivation. According to Tondee and Sirianuntapiboon [19] *Lactobacillus plantarum* No. PV71 - 1861 showed highest growth and melanoidin decolorization when yeast extract was used as nitrogen source. Sirianuntapiboon et al. [16] examined that the inorganic nitrogen source was more effective than organic nitrogen source and the maximum decolorization activity was 35.9% with $NaNO_3$ (0.1%) within 8 days of incubation. Sirianuntapiboon [31] isolated two strains that showed highest decolorization of 82.0% and 80.5% with yeast extract within 5 days. Mohana et al. [22] found inhibitory effect of both the organic and inorganic nitrogen sources on melanoidin decolorization by bacterial consortium.

The isolate SAG_5 gave the maximum decolorization yield (72.6 ± 0.56%) within 5 days of cultivation with the supplementation of combination of glucose (2.0%) and beef extract. Sirianuntapiboon [31] reported that the strain No. 13A and strain

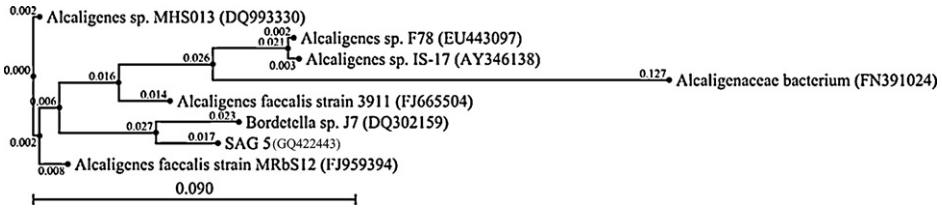

Fig. 9. Phylogenetic relationship based on 16S rDNA sequence. The melanoidin-degrading bacterial isolates SAG_5 strain with the other closely related bacterial strains resulted from Blastn search showing maximum identity with SAG_5 bacterial strain.

No. BP103 gave the maximum decolorization in the presence of fructose (2.0%) and yeast extract (0.5%). Decolorization activity of 70% was observed in presence of 1.0% glucose and 0.1% peptone [13].

It was found that the treated effluent showed less toxicity as compare to raw effluent and support the seed germination bioassay. Sharma et al. [30] reported that industrial effluent have some toxic components that inhibit the germination of seeds. According to Chandra and Sirivastava [11] the untreated effluent could not support growth. The reason for this could be high concentrations of toxicants [2] (Presence of putriciable organics like skatole, indole and other sulphur compounds), because further dilution of the effluent supported the growth and showed less harmful effects on chlorophyll content, protein and biomass of the duckweed plant. The higher concentration of dissolved salts and cations are responsible for inhibition of the seed germination and related parameters [32]. Reduction in seed germination and seedling growth treated with raw effluent may be due to higher amount of dissolved solids present in effluent. The dissolved solids may prevent the germination by contribution to salinity of the solute absorbed by the seeds before germination [33]. In another study by Ale et al. [7] higher concentrations (10% and 25%) were found completely inhibitory, whereas, lower concentrations (1% and 5%) were found stimulatory and reached up to the level of control.

The *Alcaligenes* sp. in combination with *Bacillus thuringiensis*, has also been used for the degradation of phenolic compounds as well as melanoidin from post methanated distillery effluent (PMDE) [34]. On the other hand, *Alcaligenes faecalis* in association with *Enterobacter* sp. has been used for the degradation of phenolic compounds [35].

5. Conclusion

It can be concluded that microbial decolorization of anaerobically treated effluent reduced the toxic effect which indicated that there is necessity for microbial degradation at secondary or tertiary stage prior to its disposal for environmental safety. Thus, it is suggested that the microbial decolorization can be exploited to develop a cost effective, eco-friendly biotechnology package for the treatment of distillery effluent.

Acknowledgements

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References

[1] A. Kannan, R.K. Upreti, Influence of distillery effluent on germination and growth of mung bean (*Vigna radiata*) seeds, J. Hazard. Mater. 153 (2008) 609–615.
[2] D. Pant, A. Adholeya, Biological approaches for treatment of distillery waste water: a review, Biores. Technol. 98 (2007) 2321–2334.
[3] Y. Satyawali, M. Balakrishnan, Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review, J. Environ. Manage. 86 (2008) 481–497.
[4] A. Adams, K.A. Tehrani, M. Kersiene, R. Venskutonis, N.D. Kimpe, Characterization of model melanoidins by the thermal degradation profile, J. Agric. Food Chem. 51 (2003) 4338–4343.
[5] R. Chandra, R.N. Bharagava, V. Rai, Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation, Biores. Technol. 99 (2008) 4648–4660.
[6] M. Ghosh, A. Ganguli, A.K. Tripathi, Treatment of anaerobically digested distillery spentwash in a two- stage bioreactor using *Pseudomonas putida* and *Aeromonas* sp, Process Biochem. 37 (2002) 857–862.
[7] R. Ale, P.K. Jha, N. Belbase, Effects of distillery effluent on some agricultural crops, A case of environmental injustice to local farmers in Khajura VDC, Banke, Scientific World 6 (2008) 68–75.
[8] R. Kanimozhi, N. Vasudevan, An overview of wastewater treatment in distillery industry, Int. J. Environ. Eng. 2 (2010) 159–184.
[9] Z.V.P. Murthy, L.B. Chaudhari, Treatment of distillery spent wash by combined UF and RO processes, Global NEST J. 11 (2009) 235–240.
[10] R.A. Pandey, S. Malhotra, A. Tankhiwale, S. Pandey, P.P. Pathe, S.N. Kaul, Treatment of biologically treated distillery effluent—A case study, Int. J. Environ. Stud. 60 (2003) 263–275.
[11] R. Chandra, A. Sirivastava, Toxicological evaluation of bacterial decolourised anaerobically treated distillery effluent with common duckweed (*Lemna minor*), J. Environ. Biol. 25 (2004) 93–98.
[12] C. Comninellis, A. Kapalka, M. Sixto, S.A. Parsons, I. Poulios, D. Mantzavinos, Advanced oxidation processes for water treatment: advances and trends for R&D, J. Chem. Technol. Biotechnol. 83 (2008) 769–776 (8).
[13] R.N. Bharagava, R. Chandra, Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites, Biodegradation 21 (2010) 703–711.
[14] D. Pant, A. Adholeya, Identification, ligninolytic enzyme activity and decolorization potential of two fungi isolated from a distillery effluent contaminated site, Water Air Soil Pollut. 183 (2007) 165–176.
[15] J. Dahiya, D. Singh, P. Nigam, Decolourisation of synthetic and spentwash melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40, Biores. Technol. 78 (2001) 95–98.
[16] S. Sirianuntapiboon, P. Zohsalam, S. Ohmomo, Decolourization of molasses wastewater by *Citeromyces* sp. WR-43-6, Process Biochem. 39 (2004) 917–924.
[17] T. Tondee, S. Sirianuntapiboon, Screening of melanoidin decolorization activity in yeast strain, in: International Conference on Environment, Penang, Malaysia, 13–15 November, 2006.
[18] L.T. Valderrama, C.M. Del Campo, C.M. Rodriguez, L.E. Bashan, Y. Bashan, Treatment of recalcitrant wastewater from ethanol and citric acid using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscula*, Water Res. 36 (2002) 4185–4192.
[19] T. Tondee, S. Sirianuntapiboon, Decolorization of molasses wastewater by *Lactobacillus plantarum* No. PV71-1861, Biores. Technol. 99 (2008) 6258–6265.
[20] R.N. Bharagava, R. Chandra, V. Rai, Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery effluent, World J. Microbiol. Biotechnol. 25 (2009) 737–744.
[21] S. Jirianuntipon, S. Chareonpornwattana, S. Damronglerd, C. Albasi, M.L. Delia, Decolorization of synthetic melanoidins containing wastewater by a bacterial consortium, J. Indian Microbiol. Biotechnol. 35 (2008) 1313–1321.
[22] S. Mohana, C. Desai, D. Madamwar, Biodegradation and decolourization of anaerobically treated distillery spent wash by a novel bacterial consortium, Biores. Technol. 98 (2007) 333–339.
[23] V. Sharma, R. Sharma, K.D. Sharma, Distillery effluent on seed germination, early seedling and pigment content of sugarbeet (*Beta vulgaris* Linn. Var. Mezzanau-Poly), J. Environ. Biol. 23 (2002) 77–80.
[24] M. Vanaja, P. Ratnakumar, P. Vagheera, M. Jyothi, P.R. Reddy, N.J. Lakshmi, M. Maheshwari, S.K. Yadav, Initial growth responses of blackgram (*Vigna mungo* L. Hepper) under elevated CO_2 and moisture stress, Plant Soil Environ. 52 (2006) 499–504.
[25] F.J. Czabator, Germination value: an index combining speed and completeness of pine seed germination, Forest Sci. 8 (1962) 386–396.
[26] W.G. Weisburg, S.M. Barns, D.A. Pelletier, D.J. Lane, 16S ribosomal DNA amplification for phylogenetic study, J. Bacteriol. 173 (1991) 679–703.
[27] M.H. Fulekar, S. Jaya, Bioinformatics applied in bioremediation, Innovative Rom Food Biotechnol. 2 (2008) 28–36.
[28] S. Sirianuntapiboon, P. Phothilangka, S. Ohmomo, Decolourization of molasses wastewater by a strain No. BP103 of acetogenic bacteria, Biores. Technol. 92 (2004) 31–39.
[29] F.J. Fitzgibbon, P. Nigam, D. Singh, R. Marchant, Biological treatment of distillery waste for pollution remediation, J. Basic Microbiol. 35 (2007) 293–301.
[30] S. Ohmomo, W. Daengsubha, H. Yoshikawa, M. Yui, K. Nozaki, T. Nakajima, I. Nakamura, Screening of anaerobic bacteria with the ability to decolorize molasses melanoidin, Agric. Biol. Chem. 52 (1988) 2429–2435.
[31] S. Sirianuntapiboon, Selection of acid forming bacteria having decolourization activity for removal of color substances from molasses wastewater, Thammasat. Int. J. Sci. Technol. 4 (1999) 1–12.
[32] G. Suresha, E.T. Puttaiah, Effect of distillery effluent on seed germination and seedling growth of paddy {*Oryza sativa* (L.) Var.I.R.64}, Geobios 33 (2006) 129–132.
[33] U.J. Medhi, A.K. Talukdar, S. Deka, Effect of pulp and paper mill effluent on seed germination and seedling growth of mustard (*Brassica campestris*), pea (*Pisum sativam*), and rice (*Oryza satival*) seeds, Pollut. Res. 27 (2008) 437–442.
[34] R.N. Bharagava, R. Chandra, S.K. Singh, Characterization of phenolic metabolites from post methanated distillery effluent (PMDE) after degradation with bacterial consortium, Indian J. Environ. Prot. 28 (2008) 1019–1027.
[35] S. Thomas, S. Sarfaraz, L.C. Mishra, L. Iyengar, Degradation of phenol and phenolic compounds by a defined denitrifying bacterial culture, World J. Microbiol. Biotechnol. 18 (2002) 57–63.