ISSN: 2278-5213

RESEARCH ARTICLE

Decolorization of Leather effluent by lipase producing *Bacillus* sp.

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Abstract

Biotreatment of leather effluent by selected lipase producing *Bacillus* strain was carried out with the bacterial suspension and crude enzyme extract. Bacterial strains for the study were isolated from the soil samples taken from oil mill surrounding area in Namakkal, Tamil Nadu and screened for lipase production by tributyrin agar method. The isolated bacterial strain was characterized as *Bacillus* sp. by morphological and biochemical methods. Maximum decolorization (58.06%) of leather effluent was done by the crude enzyme *Bacillus* sp. compared to bacterial suspension.

Keywords: Biotreatment, leather effluent, oil mill, *Bacillus*, lipase, tributyrin.

Introduction

The effluent discharged by industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor (Sapna *et al*., 2011). The effluent from leather industry was found to show toxic effects to various aquatic ecosystems. Some of the tanning and other industries are discharging their effluents without proper treatment to the environment and pollutants present in the effluent not only affect the color of the water but also they are toxic to aquatic and other forms of life (Subramaniam *et al.,* 2012). Bioremediation, the use of microorganisms or microbial process to detoxify and degrade the oil effluents is among the innovative technologies. Different microbes producing enzymes are used for the effluent remediation process (Mukesh Kumar *et al*., 2012).

Enzymes have a great industrial potential and are widely found in various sources like plants, animals and microbes. Microbes have undermined plants and animals as sources of enzymes due to their broad biochemical diversity, ease of mass culture and also due to the ease with which they can be genetically modified. The industrially important lipases are exploited maximally due to their various applications. Lipase enzymes are widely used in detergents, degradation of leather and dairy effluent, baking and pharmaceutical industries. Among the microbial sources, bacteria especially *Bacillus* spp. have been exploited for the production of lipases (Sangeetha *et al*., 2010). Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors such as temperature, nitrogen and carbon sources. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc. (Mukesh Kumar *et al*., 2012).

Against these backdrops, the present study was aimed to isolate bacterial species from oil contaminated soil of an oil processing plant located in Namakkal, TN and screen the best lipolytic strain for lipase production for degradation of leather effluent.

Materials and methods

Sample collection and processing: Soil samples were collected at 4-5 cm depth with the help of sterile spatula in a sterile plastic bag from oil mill surrounding area in Namakkal. After collection, the soil samples were brought to laboratory and 1 g of sample was suspended in 100 mL of sterile distilled water, agitated for 30 min on a shaker at 50ºC and kept as stock solution for further isolation of the microorganism. The enriched samples were plated in medium containing (g/L): beef extract 3.0, peptone 5.0, sodium chloride 5.0, agar 15.0, calcium chloride 0.05 and glycerol tributyrate 0.2 mL, after incubation of 24 h and the results were observed as clear zone around the colonies. The isolate which showed maximum activity was selected and maintained on tributyrin agar slant at 4ºC. The culture was examined for various morphological and biochemical characteristics according to Bergey's Manual of determinative Bacteriology (Manoj Singh *et al*., 2010).

Lipase production medium: Olive oil (2%) was added to the basal medium and it was used as the production medium for lipase production. The initial pH was adjusted to pH 6.0 using 1 M NaOH before being sterilized at 121°C. Two percent (2% v/v) of inoculums was added to 50 mL of medium in 150 mL Erlenmeyer flasks. The flasks were incubated for 120 h under orbital shaking at 150 rpm. Samples were collected after 24 hrs and centrifuged at 5000 × g for 10 min. The cell-free filtrate was used as a source of lipase (Mukesh Kumar *et al*., 2012).

Lipase assay: Lipase assay was performed according to Patil and Mali (2013). Substrate solution (27 mL) was taken in a 100 mL capacity beaker and 20 mL of enzyme solution was added to it. The mixture was stirred continuously on magnetic stirrer for 30 min at appropriate temperature. After stirring the mixture, 2-3 drops of phenolphthalein indicator was added and it was titrated against 1 N NaOH solution. The end point was colorless to pink. Units of lipases were calculated in terms of fatty acids produced per mL which were calculated in terms of acetic acid released in their reaction mixture under defined set of assay condition.

Decolorization of leather effluent: A loopful of 24 h old culture was inoculated in tubes containing Nutrient Broth (5 mL) to develop the consortium. The bacterial isolate (0.5 mL) was added to tube containing 25 mL of effluent (undiluted). The flask was incubated to observe the time required the decolorization. In case of effluent degradation with enzymes, the crude enzyme (500 mL) was added to flask containing 25 mL leather effluent (undiluted) and incubated to observe the time required for decolorization. Decolorization of the effluent was analyzed using a UV/Vis spectrophotometer at 490 nm (Subhathra *et al*., 2013). The decolorization activity was expressed in terms of percentage decolorization.

Results and discussion

In this study 7 bacterial isolates produced lipolytic zone in the initial screening process. On the basis of larger clear zone formation, isolate B4 was found to be a potent degrader of lipid by producing lipolytic enzyme (Table 1 and Fig. 1). Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc. (Mobarak *et al*., 2011). The test isolate was identified using morphological and biochemical characteristics according to Bergey's Manual of determinative Bacteriology. The isolate was gram positive, rod shaped organism and motile.

Fig. 1. Lipolytic zone produced by bacterial isolate B4.

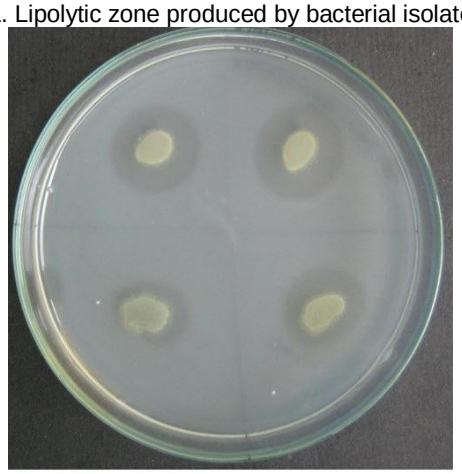


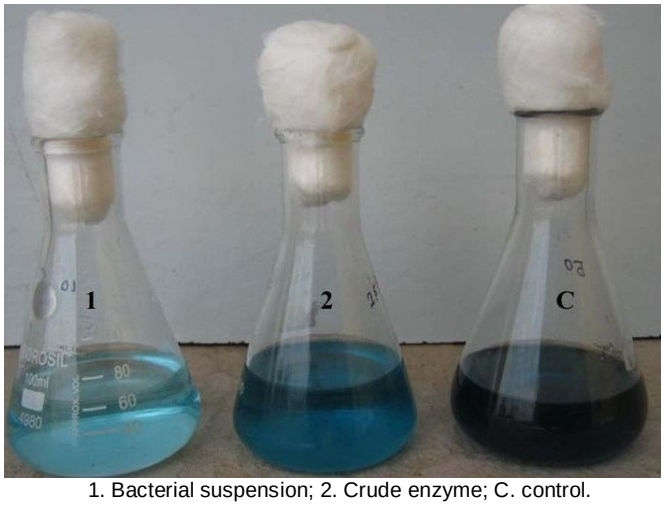
Table 1. Screening of potent lipase producing bacterial isolate.

Isolates	Lipolytic zone (mm)
B1	7
B2	8
B3	11
B4	15
B5	12
B6	12
B7	13

Table 2. Dye decolorization potential of crude enzyme and test isolate.

	Percentage of color degradation
Bacterial suspension	46.77
Crude Enzyme	58.06

Fig. 2. Dye decolorization potential of crude enzyme and test isolate on leather effluent.



1. Bacterial suspension; 2. Crude enzyme; C. control.

On solid media, the colonies were round, wavy, convex, rough and opaque. The bacterial isolate showed positive for Voges Proskauer test, Citrate utilization, nitrate and nitrite reduction, catalase, dextrose, fructose, lactose and sucrose. The following characteristics were negative for the strain: growth on indole test, methyl red, urea hydrolysis and oxidase test. Thus, based on biochemical, cultural, and morphological characteristics the isolate B4 was identified as *Bacillus* sp. Kambiz (2008) isolated lipase producing *Bacillus* from oil mill surrounding in his study. In this study, leather effluent was degraded using crude enzyme and test isolate (Table 2). Among them, the crude enzyme highly degraded (58.06%) the effluent compared to the test isolate (Fig. 2). From the treatment point of view, the degradation of dyes has received considerable attention. Nowadays, biodegradation of various dyes are getting considerable attention because of its cost-effective, environment friendly nature and does not produce large quantities of sludge (Nidhi *et al*., 2012). Numerous bacteria have been implemented in dye decolorization, either in pure cultures or in consortia (Coughlin *et al*., 2001; Pearce *et al*., 2003).

Conclusion

This little piece of investigation revealed the dye decolorization potential of crude enzyme of *Bacillus* sp. Crude enzyme highly degraded (58.06%) the leather effluent compared to the test isolate. Further studies are required to scale-up the decolorization potential of crude enzyme from *Bacillus* sp. before applying it in the industrial processes.

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