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Isolation and characterization of detergent degrading bacteria from soil

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Abstract

Detergents (surface active agents) are chemical compounds which are largely used for washing purpose. In present time the use of detergent has increased in household and industries, which causes numerous problems in sewage treatment facilities and causes toxic effect on organisms and eco system. Present study reports the isolation, morphological and estimation of detergent degrading bacteria. Soil samples collected from various detergent contaminated region (Vapi and Valsad Region). Detergent was used as a sole source of carbon to enumerate detergent degrader. Total 16 isolates (S1..... S16) were obtained from soil sample, using basal salt medium. S7 isolate as shows maximum percentage of degradation at 1% concentration of detergent. The analysis shows that isolated organism belongs to Klebsiella species.

Keywords: Detergents, Degradation, Screening, Bacteria.

1. Introduction

Detergent is organic compound that reduce surface tension in water and other liquids (Kowalska et al., 2004). Detergents contain both strong hydrophobic and hydrophilic moieties.There are many kinds of detergents, and they are classified by their use, properties and chemical structure. The detergent classification depends on water dissociation and the structure of hydrophilic group. According to the water-solubility, detergents can be classified into ionic detergents and non-ionic detergents. Ionic detergents can be divided into anionic detergents, cationic detergents and amphoteric detergent (Mozia et al., 2005).

Detergents may be from natural or synthetic sources. The first category includes naturally occurring amphiphiles such as the lipids, which are detergents based on glycerol and are vital components of the cell membrane. Soaps remained the only source of natural detergents from the seventh century till the early twentieth century, with gradually more varieties becoming available for shaving and shampooing, as well as bathing and laundering. Known today simply as detergents, synthetic detergents are washing and cleaning products obtained from a variety of raw materials (Mathew et al., 2000).

Detergents are substances used for the purpose of cleaning laundry or dishes. The main component in detergents is a detergent or soap which aids in the cleaning process.. They are most widely used in different forms, both solid and liquid, in industries, households, laundries and cosmetics. Detergents are organic substances which enhance the cleaning,

rinsing and/or fabric softening process due to their surface-active properties and are discharged into the environment by the wastewater pathway, either after treatment in a wastewater treatment plant or directly where no treatment system is available.Some examples of detergent degrading bacteria are *Pseudomonas aeruginosa, Escherichia coli ,Klebsiella liquiefaciens ,Enterobacter liquiefaciens, Klebsiella aerogenes , Bacillus sp* (Patrao et al., 2012).

Bacteria were isolated from detergent contaminated soil near household area and identified by biochemical tests. Methylene Blue Active Substance was used to determine the amount degradation by the bacteria (Shukor et al 2009). S7 strain showed better degradation for detergent. Degradation was highest during 48 hours of incubation. The objective of the current study was ti isolate and characterize detergent degrading bacteria from detergent contaminated soil and to determine the extent of biodegradation.

Biodegradation is common method for the removal (degradation and detoxification) of detergent because of its low cost and low collateral destruction of indigenous animal plant organisms. Bacterial degradation is considered to be a major factor determining the fate of detergent in the environment.

Degradation of detergent is usually a combination of a number of processes, including microbial degradation and is also influenced by some physicochemical properties such as temperature, pH and carbon and nitrogen source.

2. Materials and Methods

2.1 Screening, isolation and purification of detergent degrading bacteria

For isolation of detergent degrading bacteria, one gram of each soil sample were suspended into 9 ml of sterile distilled water and serially diluted up to 6 folds. From the last three dilution tubes take 0.1 ml and spread into the basal salt agar medium containing detergent as sole source of Carbon and Nitrogen. Plates were incubated at 30^0C for 48 hours. The isolated colonies were grown on BSM agar plate.

2.2 Storage and Maintenance of Pure Culture

The isolated organisms were streaked on BSM agar slants. The slants were incubated at 30°C for 48 hrs. The pure culture is then stored in refrigerator at 4°C and sub cultured periodically

2.3 Degradation Experiment

2.3.1 Inoculum preparation

Inoculum was prepared by transferring preserved culture and growing the cells in 100 ml Erlenmeyer flask containing 50 ml nutrient broth. The flasks were incubated at 30°C for 24 hrs. The freshly grown 24 hrs old culture with 1.0 O.D. at 600 nm is used as Inoculum to inoculate degradation medium BSM broth containing 1% detergent.

2.3.2 Media for degradation

Composition of medium for degradation [KH_2PO_4 0.8; K_2HPO_4 0.37; NH_4Cl 0.125; NaCl 0.125; Na_2SO_4 0.035; $MgSO_4$.7H2O 0.037; 100 ml of distilled water, containing 1% detergent , and medium was autoclaved].

2.3.3 Inoculation of medium for degradation

The sterilized medium was inoculated with 1% (v/v) of 24 hrs old culture. The inoculated flask was allowed to incubate at 30°C for 48 hrs. The sample was withdrawn at 24 hrs of interval and supernatant was subjected to centrifugation at 5,000 rpm for 20 min and degradation rate was determined'

2.3.4 Estimation of detergent.

Detergent degradation study by Methylene Blue Active Substance (MBAS) assay

The concentration of residual detergent was determined by measuring the intensity of Methylene blue in a chloroform extraction process. All the strains were inoculated with minimal salt medium and incubated at 30°C for "24 h". After incubation all the samples were studied for detergent degradation by using MBAS assay. Total three Erlenmeyer flasks of BSM medium were taken to test for surfactant degradation. The first flask was used as a blank and did not contain surfactant but contained 1ml inoculum and 100ml BSM. The second flask contained 100ml BSM with 1% surfactant and did not contain inoculum, thus serving as the standard for the test. The third flask contained 100ml BSM containing 1% surfactant and 1 ml of inoculum and served as the experimental flask. The flasks were incubated at 30˚C in the static condition for 24, 48 and 72 hours. To 3 ml of centrifuged cell free supernatant sample, 3 ml of methylene blue and 3ml of chloroform was mixed by shaking vigorously and incubated for 20 minutes at 30˚ C on shaker and allowed to settle down at static

condition. The chloroform layer was drawn off in to a second test tube. Absorbance was measured at 652nm. Absorbance indicates the amount of detergent degradation (Shukor et al 2009). Degradation was calculated by using the equation:

$$\text{Percentage of degradation} = \frac{At - Ai}{At} \times 100$$

Where
Ai= Blank – Experiment
At= Blank – Standard

2.3.5 Screening of isolates having degradation activity

Bacteria were screened on the basis of degradation capability using detergent. Bacteria which shows higher degradation activity is selected and further study is carried out using it. The screening of isolates degrading detergent was measured as increase in optical density using spectrophotometer. Degradation of malathion was done by promising isolate and effect of incubation period was optimized.

2.3.6 Effect of incubation period

In present study, the effect of incubation period was determined by incubating medium at different incubation periods such as 6, 12, 18, 24, 30, 36, 42, 48 hrs. In Erlenmeyer flask, 100 ml of BSM broth, 1% detergent and 1% inoculum were added and incubated at 30°C. The sample was withdrawn from medium at respective time interval and subjected to centrifugation at 5,000 rpm for 15 min and the supernatant was used for determination of degradation.

3. Results and Discussion

Total 25 isolates were screened for its capability of degrading detergent. 16 isolates capable of degrading detergent were isolated from soil samples. All these isolates were labeled as S-1 to S-16. All 16 isolates were further purified and stored after streaking on BSM agar plate. Bacterial isolate S7 showed maximum degradation (69.15%) of detergent within 48 hrs of incubation at 30°C under static condition. Followed by S7, bacterial isolate S9 & S14 shows 64.76 % & 63.34% detergent degradation, respectively. Thus, isolate S7 was selected for further study as it exhibit highest degradation of detergent.

Figure: Degradation of Isolates

3.1 Effect of Incubation Temperature on Detergent Degradation

Temperature is one of the important factors affecting the growth and activity of microorganisms. Effect of varying temperature on detergent degradation was studied by incubating the inoculated experimental flask in the temperature range of 15- 40 ° C. The sample was with drawn after 48 hrs of incubation, centrifuged at 5000 rpm for 15 min and supernatant was used to determine detergent degradation. The optimum temperature for maximum degradation of detergent was recorded at 30°C.

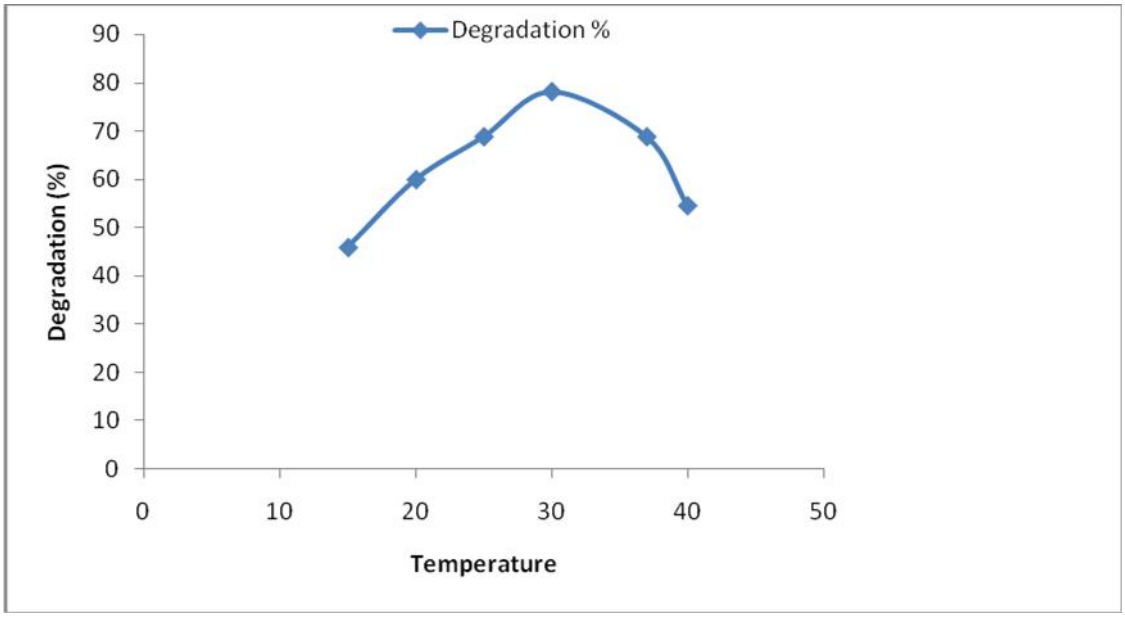

Fig Effect of Temperature on detergent degradation

4. Conclusion

The study shows that the isolated bacterium S7 is able to degrade the detergent, so that this organism is used as a biological agent for the *insitu* bioremediation of detergent contaminated soil. The present study is done only on the morphological and cultural characterisation of isolated bacteria. These eco-friendly bacteria can be used and motivate the farmers to use natural biological pesticides.

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