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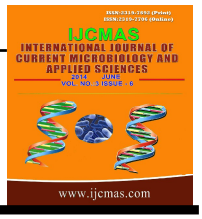

Original Research Article

Isolation and characterization of efficient poly-β-hydroxybutyrate (PHB) synthesizing bacteria from agricultural and industrial land

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A B S T R A C T

Halophiles are extremophile organisms that thrive in environments with very high concentrations of salt. In the present study, halotolerant and halophilic bacterial strains were isolated from industrial and agricultural land. These strains were grown in media with 2 molar NaCl concentrations. The growth of these strains was observed on three different growth media (nutrient agar, trypticase agar and luria agar), best growth was observed on luria agar media. Growth on selective media (DSC-97) was also observed. After optimization of different culture conditions, isolated strain was characterized biochemically.Bacterial isolates were also characterized on the basis of Gram's staining, sudan black and Simple staining etc using microscope. The most producer isolate was identified to the molecular level using 16S rRNA as *Bacillus subtilis and Pseudomonas sp.*

Keywords

Halophiles, sudan black, *Bacillus subtilis, Pseudomonas sp.*

Introduction

Plastic materials which have made entry in every sphere of human life are now causing serious environmental problems due to their non-biodegradability. The intrinsic qualities of durability and resistance to degradation, over the last two decades, have been increasingly regarded as a source of environmental and waste management problem mandating from plastic materials (Poirier et al., 1995). One option is to produce truly biodegradable polymers, which may be used in the same applications as the existing synthetic polymers. These materials, however, must be processable, impervious to water and retain their integrity during normal use but readily degradable in a biologically rich environment. Plastics produced from PHAs have been reported to be truly biodegradable in both aerobic and anaerobic environments unlike many of the "so-called "biodegradable plastics made synthetically. PHAs are composed mainly of poly beta-hydroxylbutyric acid (PHB) and poly-beta hydroxylvaleric acid (PHV), although other forms are possible. More than 80 different forms of PHAs have been detected in bacteria (Lee, 1996). The production of biodegradable plastics on a large scale is limited because of the relative expense of the substrate and low

polymer production. According to Yamane (1993), the higher production costs, especially raw material costs, make it difficult for PHA plastics to compete with conventional petroleum-based plastics in the commercial market place. Hence, alternative strategies for PHA production are being investigated. PHA production costs could be reduced by several means by using cheaper substrates such as starch, whey (Kim et al.,1994) or enhancement of product yield eg., by using recombinant *E. coli* (Lee, 1996).

There have been some investigations on the possibility of producing PHB in transgenic plants (Lee, 1996; Nawrath et al., 1994).

Materials and Methods

Sampling

Agricultural samples were collected from Agricultural land, phagwara and industrial samples were collected from Industrial land (ACC Cement manufacturing industry, Chaheru, phagwara) respectively for the isolation of efficient PHB producing bacteria.The soil samples are collected in sterile plastic zipper (polythene) bags by digging the land shore 5-10 cm deep from different sites.

Media used

The different media used for isolation & identification were Nutrient agar,trypticase agar and luria agar,NaCl concentration was maintained as 2 molar/100 ml.Growth on selective (DSC-97) media was also observed.The isolated strains were sub-cultured several times under same conditions to obtain pure cultures of morphologically different bacteria. The purified strains were further characterized and stored in refrigerator. The best growth results of bacterial growth were observed on luria agar media. Further subculture was carried on luria agar to obtain purified colonies. These purified Colonies were stored in refrigerator as stock.

Screening for PHB production

Sudan black staining

Staining of cells with Sudan black B (Murray et al. 1994) Smears of cells deposited on a glass slide were heat- fixed and stained with a 3% (w/v in 70% ethanol) solution of Sudan Black B (Sigma) for 10 min, followed by immersion of the slide in xylene until it was completely decolorized. The sample was counter stained with safranin (Sigma; 5% w/v in deionized water) for 10 s, washed with water and dried. A few drops of immersion oil were added directly on the completely dry slide, and the cells were examined by contrast microscopy. In this staining, lipid inclusion granules are stained blue-black or blue grey, while the bacterial cytoplasm is stained light pink.

Simple staining

Twenty four hour old culture was smeared on a clean glass slide and heat fixed. It was then kept on the staining tray and five drops of methylene blue was applied for few seconds. Stain was poured off and smear was washed gently with slow running water. The slide was air dried and observed under oil immersion.

Identification of isolates

On the basis of PHB screening method isolate from agricultural samples was selected for further identification and

optimization of culture condition. Along with this isolates, of industrial samples was taken as a positive standard. identification of isolate. This was done by following staining procedure:

Biochemical characterization

Citrate utilization test

The test was performed with both Simmon's citrate agar and Koser's citrate medium. After preparation of slant or, the isolate was aseptically inoculated and incubated for 2 days at 35 ± 37°C. A positive reaction is indicated by growth with an intense blue color in the slant. A negative reaction is evidenced by no growth or no change in color.

Voges-Proskauer test

The test organisms were inoculated in the culture tubes containing sterile glucose phosphate peptone water. The uninoculated culture tube containing sterile glucose phosphate peptone water served as control. Both inoculated and uninoculated (control) tubes were incubated at 37°C for overnight. After incubation, 12 drops of VP-1 reagent and 2-3 drops of reagent VP-2 were added in each tube. The tubes were agitated gently for 30 sec for aeration and then kept for 30 min at room temperature. The development of crimson to pink color in the medium was recorded

Catalase test

The test was performed with hydrogen peroxide adding to trypticase soya agar slants. After preparation of slant the isolates was incubated for 48 hr at 37°C .hydrogen peroxide drops was added after 48 hr. the result was observed on the appearance of bubbles.

Molecular characterization of bacterial isolates

The selected strains was identified by royal life sciences Amadhabad. The 16S rRNA gene was selectively with the 16S rRNA gene universal primer.

Results and Discussion

Isolation and screening of bacterial isolates for PHA production

Several bacteria was isolated from agricultural and industrial samples,but on the basic of Sudan Black staining one potential PHB producers were screened from industrial and agricultural sample each.

Characterization of PHA producing bacterial isolates

Screened bacterial strains were characterized biochemical tests (table -1)

Table.1 Microbiological Biochemical characteristics

Tests	Agricultural strains	Industrial strains
Citrate utilization test	negative	Positive
voges-Proskauer test	negative	Positive
Catalase test	positive	negative

Steps for 16s r-RNA analysis were as Follows

1.PCR amplification of genomic DNA with universal primers specific for 16S rRNA amplification

2.The PCR product was bi-directionally sequenced using 16S specific primers

3.Sequence data was aligned and analyzed for finding the cosest omologs for the sample. Based n nucleotides homology and phylogenetic analysis thee agricultural sample was detected to be *Bacillus subtilis* (accession number-KJ676546) and the industrial sample was detected as *Pseudomonas sp* (accession number-KJ680320).

Acknowledgement

No work of significance can be claimed on a result of an individual Effort and same holds true further for this project as well, for through it carries my name the energy of many have contributed in no small measure in completion of this project. I am very thankful to my parents for their support and help.

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