Research Article

Isolation and Screening of Biodegradable Polymer PHB Producing *Azospirillum* sp

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ABSTRACT

Plastic pollution is fueling the grave natural dangers right now facing people, the creature kingdom, and the planet. Among the foremost promising materials being created and assessed is poly hydroxyl butyrate (PHB), a microbial bio prepared polyester having a place to the poly hydroxyl alkanoate (PHA) family. Bacterial PHB may be a green plastic that's totally biodegradable and clears out behind no foam. In the present study, high PHB creating strain *Azospirillum* was isolated from soil sample. After the screening of PHA by Sudan black straining. *Azospirillum* appears PHA greatest production under the different carbon and nitrogen condition. Finally, characterization and confirmation of PHB was done by utilizing HPLC analysis.

Keywords: *Azospirillum*, Bioplastics, Sudan black B strain, Polyhydroxybutrate, HPLC Analysis.

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INTRODUCTION

Poly hydroxyl butyrate (PHB) is a poly hydroxyl alkanoates (PHA), a polymer having a place to the polyesters class that zone of intrigued as bio-derived and biodegradable plastics[1]. PHB materials can be delivered by numerous distinctive bacterial strains, with reports expressing that more than 300 different bacterial strains are known PHB producers[2]. In this case, we are choosing *Azospirillum* sp., for the further study.

Since the *Azospirillum* involved in biological nitrogen fixation (BNF) speaks to a cheap and economical elective to N-fertilizers and can be advanced by seed immunization with first class diazotrophic bacteria, contributing to plant's N nutrition[3]. The gram-negative nitrogen-fixing rhizobacterium *Azospirillum* spp., lives in near affiliation with plant roots, where it has advantageous impacts on plant development and the yields of numerous crops of agronomic significance4. Enzymes included within the synthesis, aggregation, and degradation of PHAs in *Azospirillum* spp., have been inspected in detail[5]. It has been appeared that in differentiate to other bacterial species, *Azospirillum* spp., does not create copolymers of hydroxyalkanoates; or may be, it produces as it were homopolymers of PHB[6].

Understanding the part played by PHAs as inside capacity polymers is of essential significance in microbial environment[7]. Most of the bacteria which deliver PHB are nitrogen fixing microorganisms. The *Azospirillum* spp., fix the molecular nitrogen and have the capacity construct to poly β hydroxybutyrates when they are developed on different carbon sources. Hence, the centered on the creating of polyhydroxybutyrate (PHB) granules by strains confined from soil. There is screening and the confinement of the bacteria by utilizing standard methods. It was taken note that most of the soil had the PHB producing strains.

The PHB collection has been recommended as vital phenotype for adjustment and perseverance of bacteria to distinctive stress conditions. A few stress conditions may be show during plant bacteria association or in capacity of inoculants for long times. Hence, microbes creating high substance of PHB may have an improved wellness for competition and/or to stand up to for long periods of capacity. In this way, PHB screening and evaluation strategies may be valuable for characterization of unused separates of *Azospirillum* and other PGPRs. This chapter aims to bring to the readers common conventions to screen for PHB producing microbes and measurement of PHB production.

MATERIALS AND METHODS

Collection of soil samples and isolation of *Azospirillum*

The soil was collected from the garden in our college, Nadar saraswathi college of Arts and Science, Theni District, Tamilnaidu. The soil was taken in sterile conditions in polythene bags and kept it laboratory at 5-10°C and the sample was air dried for isolation of bacteria. The isolation of *Azospirillum* sp. is based on utilize of N free semi-solid media containing low concentration of agar. NFb medium for *A. lipoferum*, *A. brasilense*, and afterward with a few minor alterations, was valuable to separate *A. irakense* and *A. halopraeferans, A. irakense* can be separated by the method of Khammas et al[8]. in semi-solid NFb medium containing up to 0.3 % NaCl with pH balanced to 7.0–8.5

and brooding at 33 °C. *A. doebereinerae*[9] can be separated in NFb semi-solid medium segregation, identification and biochemical characterization.

Identification and confirmation test

The well-formed separates from the Nfb semi strong medium was exchanged to Nfb agar plates. The exchange was done for each and each shaped particular pellicle. The affirmation test was done by exchanging a single colony from the agar plates to Nfb-semi strong medium containing 1.75% agar. For further confirmation, the pellicles was transferred from the semisolid medium onto BMS agar plates.

Differentiation of *Azospirillum* and biochemical test's

It is basic to distinguish the *Azospirillum* spp., from other root colonizers, so chosen colonies on BMS agar plates were streaked onto RC medium (PH 7.0)10. Distinct biochemical tests viz, Methyl Ruddy test(MR), Voges-proskauer test (VP) test, Indole test, Catalase test, Oxidase test, Citrate Utilization test, Triple sugar press test, Nitrate test, Gelatin hydrolysis test, Starch hydrolysis test and Esculin hydrolysis test were conducted to recognize the chosen confines as portrayed in Bergey's Manual Determinative Bacteriology.

Microscopic Examination of PHB

All the bacterial isolates were subjectively tested for PHB production taking after the reasonable colony method of screening utilizing Sudan Black B color11. For quick screening of PHB makers, supplement agar medium was supplemented with 1 per cent for glucose. The immunized plates were incubated at 37oC for 24 hours. The ethanolic solution of (0.02%) Sudan Black B was spread over the colonies and the plates kept undisturbed for 30 minutes. At that point the plates were washed with ethanol (96%) to remove the abundance recolor from the colonies. The dark blue colored colonies were taken as positive for PHB production.

HPLC Analysis

Ion-exclusion high pressure liquid chromatography (HPLC) was used by Karr et al.[12] to measure poly-β-hydroxybutyrate (PHB) in bacteria. The items within the acid process of PHB containing material were fractionated by HPLC on Aminex HPX-87-H particle prohibition tar for natural corrosive investigation. Crotonic acid shaped from PHB amid acid (0.014 N H2SO4 and the stream rate was 0.7 ml/min) absorption was recognized by it's seriously absorbance at 210nm. The Aminex-HPLC method gives a fast and basic chromatographic procedure for schedule analysis of organic acids. The method can degree the sum of crotonic acid produced from PHB within the tests from the regression condition determined from a standard bend gotten with crotonic acid standard arrangements. The standard bend is developed relating the top range of crotonic acid standard by concentrations.

RESULTS AND DISCUSSION

Identification and confirmation

The confirmed strains were happened white and little, dry and regularly combined colonies on the NFb agar plates. These colonies were affirmed by transferring single colony from plates to test tubes containing NFb semisolid medium which brought about within the characteristic appearance of the pellicles. So, the separation microbes was identified and confirmed as *Azospirillum*.

Figure 1: A thin, white sub-surface pellicle at 0.5 mm below from the surface

Differentiation of *Azospirillum*

In the RC medium reddish color colonies were shaped. This makes a difference in separating *Azospirillum* sp. from other root colonizers since as it were *Azospirillum* sp. has the capacity to retain Congo red and shape ruddy colonies. Thus purified separates of *Azospirillum* was obtained.

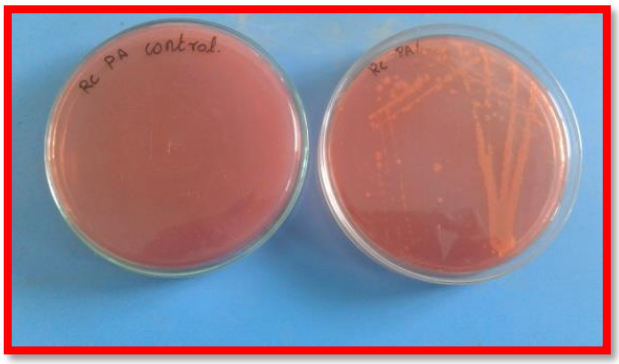

Figure 2: Plate A-Showing a red colonies of *Azospirillum* on Selective roderquez caceras medium, This helps in differentiating *Azospirillum sp.*from other root colonizers since only *Azospirillum sp.* have the ability to absorb congo red and form red colonies thus purified isolates of *Azospirillum* were obtained.

Biochemical Test:

Table 1: Biochemical Characterization

Biochemical Test	Results
Gram's Staining	-
Methyl red test	+
Indole test	-
VP test	-
Oxidase test	+
Catalase test	–
Citrate utilization test	+
TSI test	–
Gelatin test	–
Starch test	–
Esculin hydrolysis	+

VP - Voges-proskauer, TSI - Triple Sugar Iron, Negative - (-), Positive – (+)

Bacteria were isolated from soil. Table-1 shows the results of biochemical characteristics and cell morphology of the isolated bacteria and the bacteria were identified as *Azospirillum*.

Microscopic Examination of PHB

Screening of the isolates was shown dark blue particles after recoloring by using Sudan black B strain. Sudan black could be a lysochrome (fat dissolvable color). It is utilized for staining of triglycerides, lipids and lipoproteins. PHB could be a lipid like polymer of 3-hydroxybutyrate. So, Sudan black stain binds with PHB granules. In sudden black stained cells, PHB granules showed up as dark blue interior the red cells.

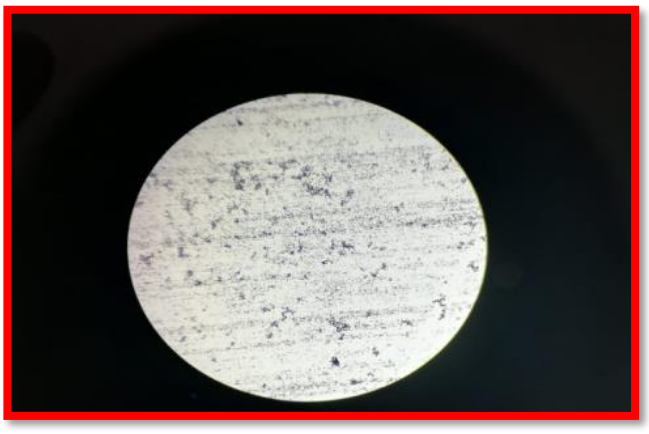

Figure 3: PHB granules – Microscopically observed as black color

HPLC Analysis:

Ion-exclusion high pressure liquid chromatography (HPLC) was utilized to degree poly-β-hydroxybutyrate (PHB) in *Azospirillum*. The items within the acid process of PHB containing material were fractionated by HPLC on Aminex HPX-87-H particle prohibition resin for organic acid analysis. Crotonic acid shaped from PHB during acid absorption was recognized by it's seriously absorbance at 210nm. The Aminex-HPLC strategy gives a fast and basic chromatographic strategy for schedule analysis of organic acids.

CONCLUSION

In the present study appeared that separation of biopolymer producing bacteria *Azospirillum* spp., which can utilized nitrogen source for PHB production has been recognized and characterized from the soil. Among the soil tests which were utilized cultivate field soil gave the number of isolated single positive and high sum of PHB collection colonies individually. Encourage bacterial biopolymer was confirmed by HPLC investigation analysis examination investigation were recognized and comes about propose that the poly-3-hydroxybutyrate compound was delivered by *Azospirillum* spp. The production of PHB was found to extend along with the increment within the biomass. Further studies are required to optimize the development media to progress the PHB abdicate and to decrease the cost of production media together with reasonable PHB induction media components.

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