



Research Article

Responses of Bacteria from Urban and Desert Soil for Bioplastic Accumulation

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Article History

Received: June 24, 2017
Revised: December 09, 2017
Accepted: December 12, 2017
Published: ---- xx, 2018

Authors' Contributions

HJ carried out the experiments and prepared the first draft. NJ supervised the project and finalized the manuscript.

Keywords

Bio-plastic production, Desert, Screening, Coconut oil, *Acinetobacter seohaensis*.

Abstract | Bacteria retain the ability to produce certain compounds to survive in unfavorable conditions; bioplastics is one of them. The aim of this study was to evaluate various environments for bioplastic producing bacteria. Bacterial strains were isolated from the desert and urban soil samples. A total of 39 strains isolated from Cholistan near Bahawalpur and 24 strains were isolated from various areas of Lahore. Bacterial strains were screened for the production and accumulation of bio-plastic. Only three strains from urban soil sample were able to produce polyhydroxyalkanoates (PHA). Strain *Acinetobacter seohaensis* produced 40.6% bio-plastic when supplemented with coconut oil. This percentage is even higher than the glucose, which was 16.6 % after 24 h of incubation. The other competent strains *i.e.* *Exiguobacterium indicum*, *Bacillus acidiceler* were not able to utilize the coconut oil but produced 5 and 22% bio-plastic when supplemented with 2% glucose after 24 h of incubation, respectively. The percentage of the bio-plastic produced when grown in nutrient broth was very low. The Fourier-transform infrared spectroscopy (FTIR) analysis of the extracted bio-plastic indicated the presence of the C=O functional group specific for the presence of polyhydroxyalkanoates, the absorbance peaks were specifically at 1600 cm⁻¹. Ribo-typing of all the six strains were carried out, strains identified as *Exiguobacterium indicum*, *Bacillus acidiceler*, *Acinetobacter seohaensis*, *Serratia proteamaculans*, *Trabulsiella guamensis* and *Serratia grimesii* and showed 98 to 100% similarity with sequences available in public domains.

To cite this article: Jabeen, H. and Jamil, N., 2018. Responses of Bacteria from Urban and Desert Soil for Bioplastic Accumulation. *Punjab Univ. J. Zool.*, 33(1): xxx-xxxx. <http://dx.doi.org/10.17582/pujz/201.....>

Introduction

The best studied and characterized class of polyhydroxyalkanoates are poly (3-hydroxybutyrate) (Lee, 1996). Polyhydroxyalkanoates are not only biodegradable rather they have a higher hydrophobicity, which makes them feasible to use as compared to other plastics (van der Walle *et al.*, 2001). Bacterially produced polyhydroxyalkanoates are commercially used in various fields *e.g.* in food industry, in drug delivery system, packaging films,

in transplantology, tissue engineering and pharmacology *etc.* (Chee *et al.*, 2010).

A number of bacterial species produce polyhydroxyalkanoates and the broadly studied bacterial species for PHA production is (*Ralstonia eutropha* or *Alcaligenes eutrophus*) (Vanechoutte *et al.*, 2004). There are many other bacterial strains that are specialized for PHA production includes *Pseudomonas* spp., *Bacillus* spp., *Rhodopseudomonas palustris* *etc.* (Verlinden *et al.*, 2007). As the environmental conditions that are favorable for the spore formation are also considered to be feasible for the synthesis of biopolymer by certain strains of *Bacillus* that are also spore formers (Chee *et al.*, 2010). There are a number of sources available for the synthesis of PHA by bacteria including

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carbohydrates, fatty acids, different plant oils, waste materials from sewage, agricultural land *etc.* (Yamane, 1993; Jau *et al.*, 2005). The soil is the major habitat for several microbial species. Another important fact is that different soils are populated with various microbes both in their number and behavior as well as to the other microorganism inhabiting the same soil (Fukui and Doi, 1998). The soils with greater amounts of organic matter are usually densely populated with the microbes as compared to the soils with lesser organic matter content (Bardgett *et al.*, 2003). Desert and urban soils differ from each other in their morphology, physical characteristics, and organic matter content as well as in the microbial load (Griffiths *et al.*, 1998). Moreover, the number and function of soil microbes differ not only in different types of soils but also differs in the different layers of the same soil (Fierer *et al.*, 2003). There are certain species of bacteria that are specialized for the antimicrobial activities, their behavior is termed as microbial antagonism in which specific species of bacteria retard or completely inhibit growth of other related or different microbial species. Mainly it is regarded as a natural phenomenon in which bacteria inhabiting a natural environment tend to compete with other microbes for space and nutrition (Hibbing *et al.*, 2010). The bio-medical importance of the bio-plastics cannot be underestimated (Ali and Jamil, 2016). Present study indicates potentials of soil bacteria collected from desert and urban area for production of polyhydroxyalkanoates.

Materials and Methods

Bacterial isolation and characterization from desert and urban soil samples

Sampling was done from Cholistan desert near Bahawalpur and three different areas of Lahore (Singh Pura, Akbari Mandi and St. Marry, Lahore). Serial dilutions of the collected soil samples were prepared up to 10^{-10} . Dilutions *i.e.* 10^{-3} , 10^{-5} , 10^{-7} and 10^{-9} were selected for spreading on Nutrient agar plates. They were incubated for 24 h at 37°C. Morphological characteristics of the selected bacterial strains were determined by gram staining, spore staining, and capsule staining and by performing motility test.

Screening of PHA production

Production of PHA by bacterial strains Nile blue and Sudan black staining was performed. Strains showing fluorescence on UV-trans illuminator and black granules against pink background after microscopy were subjected to grow on PHA agar medium supplemented with 2% glucose as well as 2 % coconut oil as a carbon source and nutrient broth with 5 and 14 h interval up to 72 h. Optical density was noted at 600nm and aliquot of 1ml was centrifuged to obtain biomass after PHA extraction from it was done for determining growth curves and time profiling of PHA production. PHA extraction and purification was done by sodium hypochlorite and chloroform method.

FTIR spectroscopy was then performed to establish spectral fingerprints of integral bacterial cell.

Identification of selected bacterial strains

Identification of selected bacterial strains was done by 16SrRNA, ribosomal gene sequence from Macrogen Korea.

Results and Discussion

In this study we aimed to isolate bacterial species for the production of bioplastics. Sixty-three various bacterial strains from desert (Cholistan) and urban (Singh Pura, Akbari Mandi and St. Marry, Lahore) soil samples were analyzed for PHA production and to check antimicrobial activity. Physical characteristics of samples were also determined, pH of the samples was nearly 7 ± 0.1 and temperature was 35-38°C. The isolated bacterial strains showing PHA production were identified as *Exiguobacterium indicum*, *Bacillus acidicer*, *Acinetobacter seohaensis*, *Trabulsiella guamensis*, *Serratia proteamaculans* and *Serratia grimesii* (Table I).

Table I: Nucleotides similarity between isolated strains and strains reported in the NCBI.

S. No	Strain	Sequence homology	% identity
1	AKL1	<i>Exiguobacterium indicum</i>	100%
2	AKL3	<i>Bacillus acidicer</i>	97%
3	SM4	<i>Acinetobacter seohaensis</i>	83%
4	DSA1	<i>Serratia proteamaculans</i>	99%
5	DSA2	<i>Trabulsiella guamensis</i>	100%
6	SM7	<i>Serratia grimesii</i>	100%

These strains when analyzed microscopically and examination showed that *Exiguobacterium indicum* and *Bacillus acidicer* were Gram's positive rods whereas rest of the strains was Gram's negative rods. All the strains were motile except *Acinetobacter seohaensis*. Of these 63 strains, only 3 bacterial strains from urban soil sample (AKL1, AKL3 and SM4) were PHA producers. None of the desert sample was positive for PHA production. The positive bacterial strains for PHA production were then allowed to grow in the PHA detection broth supplemented with two different carbon sources *i.e.* 2 % glucose and 2 % coconut oil and in the nutrient broth medium (Lemos *et al.*, 1998). Moreover, time profiling of PHA production by these three bacterial strains was determined by calculating the total biomass as well the weight of PHA produced (Chaudhry *et al.*, 2011). The isolated bacterial strains produced bio-plastic consistently when given glucose as carbon source but highest amount of biopolymer was produced by *Acinetobacter seohaensis* (40.6 %) when supplemented with coconut oil. The other selected strains were not able to utilize coconut oil to produce Bio-plastic.

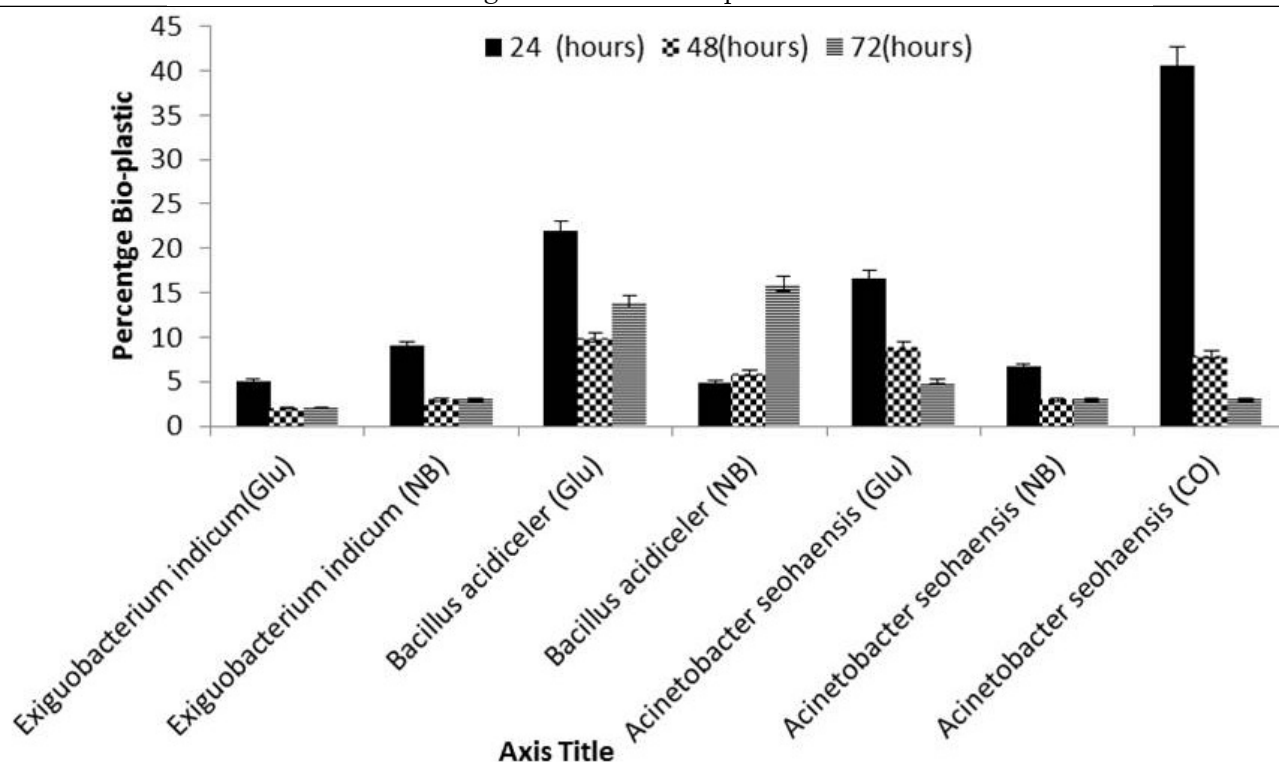


Figure 1: Comparative analysis of the bacterial strains for the production of biopolymer. The percentage production of PHA at 24, 48 and 72 h of incubation using Glucose (Glu), nutrient broth (NB) and coconut oil (CO) as nutrition sources.

Exiguobacterium indicum produced maximum PHA when supplemented with 2 % glucose *i.e.* 5 % at 24 h of incubation while in nutrient broth media it showed maximum percentage *i.e.* 9 % (Figure 1). *Bacillus acidicer* showed maximum percentage of PHA *i.e.* 22 % at 24 h incubation when 2 % glucose was added in PDA media. While in case of nutrient broth maximum yield was 4.85 % at 24 h incubation (Figure 1). *Acinetobacter seohaensis* had highest yield of 16.67 % at 24 h when the PHA medium with 2% glucose was evaluated. In case of 2 % coconut oil the percentage of PHA was 40.6% at 24 h incubation. The bio-plastic production by these bacterial species has not been reported before. The biomass from PHA producer strains were subjected to FTIR spectroscopy and for all the strains the peaks were obtained at about 1600 absorbance in both the PHA detection agar supplemented with 2% glucose and 2 % coconut oil as carbon source. The absorbance at these peaks confirms the presence of carboxylic groups, which indicates potential of these strains for bio-plastic production (de Jesus-Assis *et al.*, 2016). The bacterial strains studied in this work are not very well studied, but they have shown promising results for the production of bio-plastic and utilization of lipid carbon source, it has been observed that not a large number of bacteria are able to cleave the ester linkages in the lipids, if a bacterium can break a linkage than it can get a large amount of carbon which is not possible with monosaccharaides or simple disaccharides. The *Acinetobacter seohaensis* was not only able to utilize the coconut oil but accumulated excess carbon in the form of bio-plastic to greater extent, such microbial strains can be

manipulated in future to get useful metabolic compounds using cheap lipid carbon sources.

Conclusion

Bacteria studied in this work are able to produce bioplastics using variable carbon sources. Bioplastic accumulation response of *Acinetobacter seohaensis* was up to 40% which can be exploited for large-scale production at national and international levels.

Acknowledgments

This study was supported by Higher Education Commission research project NRPU-3443, HEC Islamabad, Pakistan.

Conflicts of interest

The authors declare no conflicts of interest.

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