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ORIGINAL ARTICLE **OPEN ACCESS**

ISOLATION AND SCREENING OF THERMOPHILES PRODUCING POLYHYDROXYALKANOATES (PHAs)

S.A. Shaikh[1]* and G.R. Pathade[2]
1. Research Scholar, Krishna Institute of Allied Sciences, KIMSDU, Karad, Maharashtra.
2.HOD and Dean, Krishna Institute of Allied Sciences, KIMSDU, Karad, Maharashtra
**For correspondence: 786swaleha.qadari@gmail.com*

ABSTRACT
Due to nonbiodegradability, conventional plastic has created environmental pollution havoc. Hence study was carried out to screen thermophiles which would produce Polyhydroxyalkanoates (PHA) - a good replacement for conventional plastic. Compost sample was collected and cultured on nutrient agar medium supplemented with glucose to isolate PHA producing thermophilic organisms. After subjecting the isolates obtained for primary and secondary screening through Sudan Black B and Nile Blue staining, respectively, one potential isolate was selected for further study. The PHA extracted from the isolate accounted to 46% of dry cell mass. The PHA produced was further characterized by Fourier Transformed Infrared Spectroscopy (FTIR) and Differential Scanning Colorimetry (DSC) to detect presence of characteristic functional groups of PHA and thermal degradation temperature of PHA. The results are encouraging.

Keywords : *Thermophiles, Polyhydroxyalkanoates, Screening.*

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INTRODUCTION
"Thermophiles" is the term used to refer group of microorganisms that love to grow at high temperature usually more than 45°C.Thermophiles can be found in various natural and man-made niches such as hot water springs, hydrothermal vents to heated compost pile [1]. Generally for large scale bioprocess mesophiles are used but this increases the risk of cross-contamination of other ubiquitously present mesophilic microbiota. Use of thermophiles resolves this difficulty as bioprocesses involving thermophiles are operated at 50-60°C [2]. The additional advantage is that it eliminates the sterilization cost and cost and energy required for cooling down the temperature elevated due to exothermic reaction.Hence one more added advantage is that the process can be operated efficiently in semi continuous and continuous modes [3].
Polyhydroxyalkanoates (PHAs)being biodegradable polymers are considered to be the greener substitute for conventional plastics and have wide range of applications [4,5]. Despite these advantages major hindrance in large scale production of PHA is exorbitant cost. Hence to cut down the cost there is need to customize both upstream and downstream processes using appropriate producer organism. Amongst different microorganisms known to produce PHA, thermophiles are less extensively studied one. Hence this study was conducted to explore more thermophilic candidates for the same.

MATERIAL AND METHODS
Sample collection
Compost samples were collected from the Karadregion of Maharashtra in sterile bags and preserved at 4°C till further processing.
Isolation of PHA producing
One gram of compost sample was serially diluted and then was spread inoculated on Nutrient agar supplemented with 1% glucose. The plates were then incubated at 50°C for 24-48 h. After incubation, well isolated representative colonies were selected and used for further study.
Screening of PHA producing organisms
Isolated strains were primarily screened by Sudan black B staining and plate assay method [6].The isolates showing positive results in primary screening were subjected to secondary screening using with

Nile blue A plate assay method for which nutrient agar medium containing 0.0005 g Nile blue was used. The isolates showing bright fluorescence on exposure with UV light were selected [7].

Production of PHA:

The 1% inoculum of selected isolate was inoculated in 100 mL production medium supplemented with 1% glucose. (Ammoniumsulfate 2.5 g/L, KH_2PO_4 1.5g/L Na_2HPO_4 3.5g/L,$MgSO_4.7H_2O$ 0.2g/L, traces of yeast extract and 1mL of trace element solution($FeSO_4.4H_2O$,$CaCl_2.H_2O$, $MnSO_4.4H_2O$,$ZnCl_2$ 1mM each)) [8] and incubatedfor 72 h at 50^{o}C.

Extraction of PHA:

After specified incubation, the production broth was centrifuged at 10,000 rpm for 15-20 minutes. The pellet obtained was completely dried and weighed. PHA was extracted using the sodium hypochlorite extraction method [9].This dry cell mass obtained was suspended in 4% sodium hypochlorite solutionand was incubatedat 30^{o}C for 30-40 min. The mixture was centrifuged at 8000 rpm for 15 min and the pellet was washed with water, ethanol and acetone successively and then air dried completely.

Quantification of PHA:

% PHA Yield :

The percent PHA yield was estimated from the weight of dry cell mass and the dry weight of dried extracted PHA using the following formula [10]:

% PHA = weight of extracted PHA x 100 / weight of dry cell mass

Characterization of Produced PHA

Fourier Transformed Infrared Spectroscopy (FT-IR) analysis

FT-IR analysis of the samples was performed on FT-IR spectrophotometer (lambda FTIR-7600) in the range 4000-400 cm^{-1}[11],availing the facility available at Yashawantrao Chavan Institute of Science, Satara, Maharashtra(India).

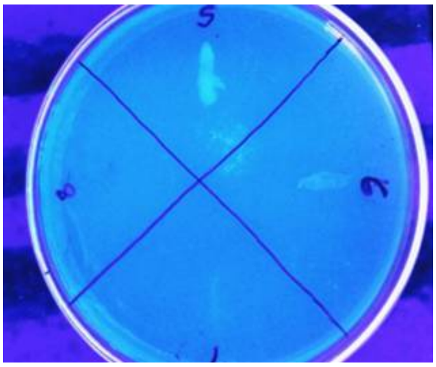
Differential Scanning colorimetry (DSC) analysis.

The thermal stability study of PHA was performed availing the service available at Shivaji University, Kolhapur. Small quantity of sample (6-8mg) was exposed to flowing nitrogen atmosphere at a heating rate of 10°C per min. in a temperature range of 20^{o}C to 500^{o}C using TA Q600 series instrument [11].

RESULTS AND DISCUSSION

Isolation and screening of PHA producers:-

On nutrient agar medium supplemented with 1% glucose, 16 isolates were obtained. Through first-line screening 6 isolates producing PHA were obtained using Sudan black Bstaining. Using secondary screening which applied Nile Blue stain, 3 isolates were found positive for PHA granules production out of them only one isolate showing strongest fluorescence was selected for production, extraction, quantification studies. (Photoplate-1). The isolate was designated as K1.5 for convenience purpose. The isolate was found to benon spore forming nonmotile gram positive cocci forming a circular, opaque, creamy and flat colony having a diameter of 2-3 mm with entire margin and moist consistency.



Photoplate 1: Fluorescence shown by isolate during screening.

Quantification of extracted PHA:-

% PHA Yield :

The percent yield of extracted PHA was found to be 46% on the basis of weight of cell pellet and dried PHA weight obtained. However, there are reports that mention accumulation of about 94 % of PHA [12].

Characterization of Produced PHA:

Fourier Transformed Infrared Spectroscopy (FTIR).

The peaks at 1631.48 and 1070.30cm^{-1} are characteristic of C=O and C-O stretching vibrations, respectively. The absorption bands at 2854.13 and 2925.48 cm-1 are corresponding to C-H stretching vibrations of methyl and methylene groups[13].These absorption bands verify the presence of Polyhydroxyalkanoates. (Fig-1)

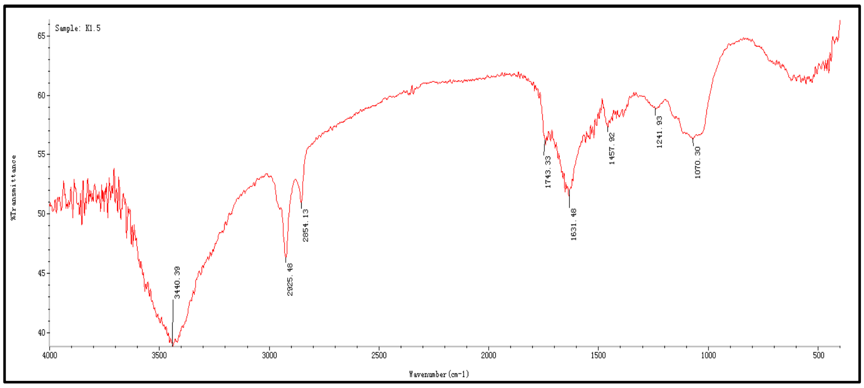


Fig.1 FTIR analysis of PHA

Differential Scanning Colorimetry (DSC) analysis of PHA polymers:

The thermal degradation study was performed using DSC and melting temperature was found to occur at 299.99°C which is quite higher. In one of the study on characterization of bioplastic, it wasdiscovered that melting temperature of starch and composite bioplastic (titanium dioxide nanoparticle with corn starch) was 297°C and 303°C respectively. Thebioplastic with higher melting temperature can serve as good packaging material [14]. (Fig-2)

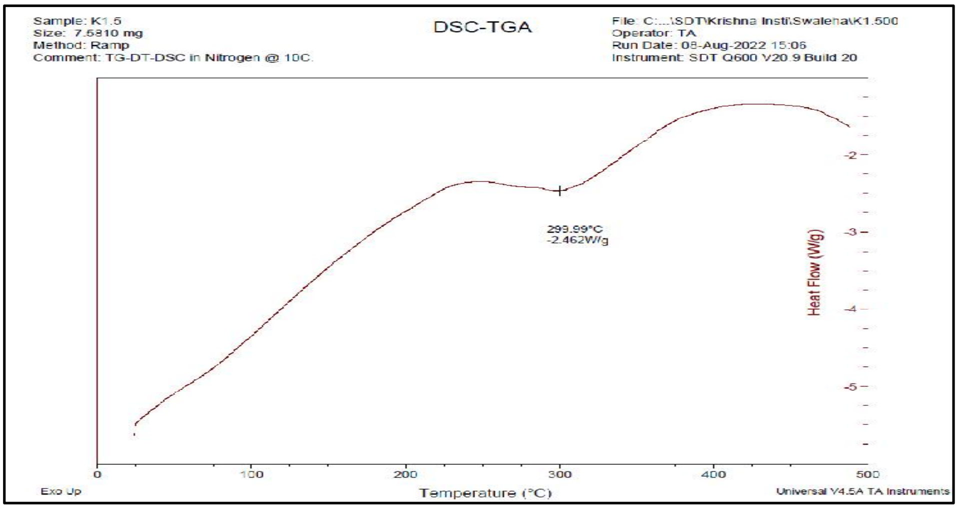


Fig.2 Thermogram of DSC analysis of PHA

CONCLUSION

The study lead to the conclusion that compost piles from Karad region can serve as a good source of thermophilic and thermotolerant isolates. When the promising isolate was explored to check its ability to produce PHA, it was found to serve the purpose of study by producing fairly good amount of PHA. This isolate can serve as good candidate for industrial application after optimization study using waste material or cheaper raw material serving as substrate for production making the process cost effective and hence would be helpful in reducing environmental pollution load created due to conventional plastic. Further study also includes characterization and identification of isolate.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

ETHICS OF HUMAN AND ANIMAL EXPERIMENTATION:
The Authors ensured that the study doesn't involve any experiment involving humans and animals.

AUTHOR'S CONTRIBUTION:
All the experimental work and preparation of manuscript was done by Swaleha Altaf Shaikh. Proof reading of manuscript wasdone by Dr Girish .P .Pathade.

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