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Research Article

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Optimization of polyhydroxyalkanoates (PHA) production from marine *Carnobacterium* sp E3

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ABSTRACT

The present study reports the production of polyhydroxyalkanoates (PHA) from marine bacteria. During the course of our microbial prospecting, bacterial strain E3 isolated from the sediment sample collected from Vellar estuarine region was found to produce PHA. Optimization of PHA production from the strain E3 was carriedout by classical one factor at a time method. In optimization studies, glucose, ammonium sulphate and sesame oil cake was found to influence the PHA production. There is no significant effect was observed on PHA production when the medium supplemented with various concentration of trace salt solutions. The potential strain E3 was characterized and identified as Carnobacterium sp (E3). This marine bacterium is a newly added source for PHA production.

Key words: Bioplastic, PHA, Carnobacterium, optimization

INTRODUCTION

Plastic material has become an integral part of contemporary life because of their many desirable properties including durability and resistance to degradation [1]. But they are undesirable to dispose because of their xenobiotic and recalcitrant nature. As the world is facing an ever increasing environmental pollution, the replacement of synthetic plastics with biodegradable plastics becomes very interesting and important [2]. Bioplastics are a new generation of biodegradable and compostable plastics, derived from renewable raw materials such as starch, cellulose, soy proteins, lactic acids and also from microbes. There are no hazards associated with the production of bioplastics and they are decomposed back into carbon dioxide, water, biomass etc., in the environment when discarded.

Polyhydroxyalkanoates are biopolyesters that are synthesized as intracellular carbon and energy reserves by a wide variety of microorganisms mainly when they are cultured under unbalanced nutrient conditions. These bioplastics have similar mechanical and thermal properties to those of plastics synthesized chemically. In contrast to classic plastics, they are fully biodegradable, biocompatible and produced from renewable materials [3]. PHA are accumulated intracellularly to levels high as 90% of the cell dry weight under conditions of nutrient stress and it act as carbon and energy reserve [4]. Bacteria accumulating PHA have been reported from various environments like soil, seawage sludge, ponds and tropical mangrove and marine environments. Use of a high PHA yielding organism, optimization of the physic-chemical parameters and the reduction of the cost of raw materials are expected to lower

the high cost of PHA production [5]. This study was under taken to produce PHA from marine bacterial strain E3 isolated from Vellar estuarine sediment, Parangipettai, Tamil Nadu.

EXPERIMENTAL SECTION

Isolation and screening of PHA producing bacteria

Sediment sample was collected from Vellar estuary, Parangipettai. Bacteria from the collected sediment sample was isolated by standard spread plate method using Nitrogen limiting minimal agar medium [6] supplemented with 2.5% NaCl. After incubation morphologically different bacterial colonies were selected and sub cultured on nutrient agar slants and stored at 4°C for further study.

Accumulation of PHA granules was detected by phase contrast microscopic observation after Sudan black staining [7]. The strains positive for Sudan black staining were inoculated in 10 ml of nitrogen limiting minimal medium (NLMM) and incubated in a rotary shaker at room temperature for the intracellular production of PHA. The cells were separated by centrifugation in a preweighed centrifuge tubes and were digested by sodium hypochlorite and the PHA was extracted with chloroform [8]. Crude PHA was concentrated by evaporating the chloroform and quantified. Bacterial strain E3 which showed maximum PHA accumulation was selected as potential strain for further studies

Production of PHA from potential strain

NLMM inoculation medium was prepared and glucose was added after autoclaving by sterilizing with syringe filter. The media was inoculated with the potential strain and inoculum in a rotary shaker for 32 hours. NLMM production media was prepared and inoculated with 10% of inoculum and incubated at a rotary shaker for 72 hours [6]. After 72 hours of incubation the cells were separated from the media by centrifugation at 7000 rpm for 5 minutes and washed in distilled water and the cells were dried in the hot air oven at 105°C for 24 hours [8]. Then the PHA containing biomass was mixed with sodium hypochlorite solution to digest the non-PHA cellular materials. The cell debris and the PHA granules were separated by centrifugation. The separated granules were extracted with chloroform and weighed again to get the post weight and thereby get the quantity of PHA produced.

Optimization of PHA production

Effect of variables such as carbon source (1%), nitrogen source (1%), trace salt solution (0.5-2.5ml) and sesame oil cake (5-25%) was studied by adopting classical one factor at a time method using NLMM broth as a basal medium. The carbon and nitrogen sources were added at 1% concentration. The basal medium was supplemented with different variables and sterilized by autoclaving. About 10 % of bacterial inoculum was added into all the reaction flask and incubated in rotary shaker for 72 hours. Then the cells were separated by centrifugation at 10000 rpm for 10 minutes and quantified. Crude PHA from the cells was extracted and quantified by adopting the method as described [1].

Characterization and identification of the potential strain

The phenotypic characteristics such as gram staining, motility, spore staining and biochemical characteristics of the potential strain was studied by adopting standard microbiological procedures and identified at genus level in comparison with the characteristics of bacteria given in Bergey's Manual of Systemic Bacteriology.

RESULTS AND DISCUSSION

Many morphologically different bacterial colonies were observed on isolation medium after 72 hours of incubation at room temperature and the colonies were selected based on the turbidity of the colonies, since the PHA accumulating strains appears turbid. Totally 18 morphologically different strains were selected from Vellar estuarine sediment (E1-E18). In Sudan black staining, six out of eighteen positive producer of PHA appeared as pink vegetative cells with black colour accumulation in the centre. This method is similar to one which was carried out by Lee and Choi [6] and Arun et al. [1]. Among the six positive isolates only the strain E3 produced thin layer of PHA in when grown on NLMM and others produced only in trace amounts.

Strain E3 showed good growth on NLMM. The amount of PHA produced was found to be 35.4mg of PHA from 180mg of dry cell weight per 100ml of medium. Effect of sugars, nitrogen sources, trace salt solution and sesame oil cake was given in table 1. Among the different variables tested, glucose, ammonium sulphate and 2.5% of sesame

oil cake was found to influence the PHA production. There is no significant effect was observed when the medium supplemented with different concentration of trace salt solution. One of the important limiting factors for the commercialization of PHA is the high cost of carbon substrates for fermentation. PHA co-polymer possess better mechanical properties compared with homopolymer of PHB but costlier co carbon substrates such as fatty acids should be supplemented in the medium for its biosynthesis. Hence the production of the polymer from unrefined and cost effective substrates rich in different carbon compounds appeared feasible for PHA production. Cultivation of bacteria on this substrate indicated variability in yields of biomass and PHA concentration in the cells [9].

Table 1. Effect of selected medium components on PHA production by the marine bacterial strain E3

Factors	Cell dry weight(mg)	PHA produced (mg)
Carbon source (1%)		
Glucose	218	45.0
Fructose	180	29.0
Lactose	190	31.0
Sucrose	205	38.0
Starch	161	25.0
Nitrogen source (1%)		
Ammonium nitrate	160	22.8
Ammonium sulphate	180	41.4
Peptone	218	Trace
Yeast extract	206	26.3
Beef extract	195	Trace
Trace salt solution (ml)		
0.5	180	41.0
1.0	180	41.5
1.5	175	41.0
2.0	183	41.3
2.5	185	42.5
Sesame oil cake (%)		
0.5	200	56.7
1.0	220	58.2
1.5	250	60.7
2.0	280	63.0
2.5	289	65.0

Table 2. Characteristics of potential bacterial strain E3

Characteristics	Strain E3
Gram staining	Gram + rods
Endospore staining	Negative
Motility	Non-motile
Growth on nutrient agar	Small irregular colourless colonies
McConkey agar	Present
MRS agar	No growth
Catalase	+
Oxidase	-
Indole	-
MR	+
VP	+
Citrate	-
NaCl Tolerance	0-2.5%
pH tolerance	5-9

Different industrial wastes like malt, soya, sesame molassess, bagasse and pharmaceutical waste were used for the PHB production. Industrial waste in different percentage was supplemented as carbon source in NLMM and it was observed that maximal yield is obtained with the sesame oil medium especially highest in 4% of sesame oil waste [1]. In the present study sesame oil cake is used as a sole carbon source in different percentage. The amount of PHA production increased with increase in percentage of sesame oil cake in aerobic condition and there was an increase of about 30 mg of PHA per 100 ml of medium.

Micromorphological, cultural and biochemical characteristics of potential bacterial strain were given in table 2. Based on the above characteristics the potential strain was tentatively identified as *Carnobacterium sp.* (E3). The genus *Carnobacterium* contains nine species, but only *C. divergens* and *C. maltaromaticum* are frequently isolated from natural environments and foods. They are tolerant to freezing/thawing and high pressure and able to grow at

low temperatures, anaerobically and with increased CO_2 concentrations. *Carnobacterium divergens* and *C. maltaromaticum* have been extensively studied as protective cultures in order to inhibit growth of *Listeria monocytogenes* in fish and meat products. *Carnobacterium maltaromaticum* can be a fish pathogen, although *Carnobacteria* are also suggested as probiotic cultures for use in aquaculture [10].

From the available literature, there is no much works on PHA accumulation by *Carnobacterium* species. The *Carnobacterium* species (E3) investigated in this study is a newly added source from Indian marine ecosystem for PHA production.

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