Indian Journal of Experimental Biology
Vol. 58, September 2020, pp. 643-650

Potential of dumpsite bacterial isolate in producing polyhydroxybutyrate under stress prone environment

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Received 16 December 2019; *revised 06 July 2020*

Different bacterial species from soil samples of waste dumpsites were isolated and screened for their ability to synthesize polyhydroxybutyrate (PHB). Out of 86 bacterial cultures isolated, 26 isolates showed the presence of lipid granules PHB. Based on high polymer production, 4 potential bacterial isolates were identified by morphological, physiological and 16S rDNA sequencing. Their nucleotide sequences were submitted in the GenBank database under accession numbers KT907042, KT907043, KT907045 and KT907047. As biomass production is analogous to PHB production, cultural parameters like carbon, nitrogen, pH and temperature were optimized for maximum production of polymer by a potential bacterium *Bacillus pumilus* K8. Growth kinetics of *B. pumilus* K8 was analyzed and found that it grows at a rate 0.112 $gh^{-1}L^{-1}$ of the production medium. It could achieve maximum production of 72.93% of PHB at the rate of 0.013 gg^{-1} of biomass h^{-1} with polymer concentration of 5.04 gL^{-1} using mannitol, yeast extract, pH 7 and temperature 35°C, respectively as favourable cultural parameters. *phbC* gene, mainly responsible for PHB production was amplified by PCR and this genotypically confirms PHB production by the bacterial isolates. *Bacillus pumilus* K8 may be exploited for further industrial production of biopolymer.

Keywords: 16S rDNA sequence, Assam, Biopolymer, Carbon source, Northeast India, *phbC*, PHB production, Plastics

Inordinate growth of human population has led to increased use of petroleum-based plastic products, and the demand is still continuing. Due to versatile properties and wide applications of these plastic products, mankind cannot ignore the virtue of this polymer. These conventional plastics accumulate in the environment because they cannot be degraded by microorganisms, thereby producing a large spectrum of pollutants[1]. Approximately, 150 million tons of fossil fuels are required for plastic production and at the same time, a huge amount of waste is produced whose depolymerization takes thousands of years[2]. Based on the present production and waste management trends, approximately 12,000 Mt of non-biodegradable polymers waste were expected to be disposed of in the landfill by 2050[3]. There is a big challenge in the methods used for disposal of plastic materials. The rates of degradation are tremendously low in landfills; and incineration process also generates toxic by-products. The other recycling option is not only time-consuming process but also causes changes in the properties of plastic materials[4]. Therefore, researchers across the world are looking for an alternative and effective polymer which is biodegradable.

Currently, there are three well-known biodegradable polyesters commercially available in the market, namely polyhydroxyalkanoates (PHAs), polylactic acid (PLA) and polybutylene adipate-co-terephthalate (PBAT)[5]. PHA is a bio-based polymer produced by many microorganisms during stress conditions when a carbon source is readily available. Polyhydroxybutyrate (PHB) is the best known polyhydroxyalkanoates (PHA) which share common physical properties to that of the petroleum-based plastics and it can be easily degraded by soil bacteria in eco-friendly manner aerobically as well as anaerobically. The diverse forms of PHAs (co-polymers and homopolymers) are found to have a variety of possible applications in packaging materials, agriculture, medicine, cosmetics and many more[6,7]. PHB has a molecular weight (Mw) of 1×10^4–3×10^6 Da, exhibiting a polydispersity of around 2. It has a glass transition temperature of 180°C, densities of crystalline as 1.26 and amorphous PHB as 1.18 $g(cm^3)^{-1}$[8]. The tensile strength and Young's modulus of PHAs are close to polypropylene (PP), but the extension to break (5%) for PHB is lower than that of PP (400%)[9]. Though PHB is a solution to these non-degradable

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plastics, its cost of production is high thereby, limiting its use as a substitute to conventional plastics[10].

The high cost of production of PHB can be overcome using cheap carbon sources, genetically engineered microbes, optimizing the bacterial cultural conditions, mutations, etc. Efforts have also been made to express the entire polyhydroxybutyrate operon of bacteria in plants with the hope to reduce its production cost[11]. Cyanobacteria have also been studied widely as it can accumulate considerable amounts of PHB as product of the wild metabolism. Genetic engineering of cynobacterial strains by heterologous transformation with genes involved in the PHB pathway of *Ralstonia eutropha* also can acquire considerable amount of PHB[12]. So, in many cyanobacteria, through poly-hydroxyalkanoate (PHA) biosynthetic pathway, PHB has been produced as a native polymer that stores carbon[13]. Therefore, it has been shown that genetic and metabolic engineering together with recombinant DNA technology have the ability to increase the accumulation of PHB to produce a considerable amount of biomass[14]. Bio-synthesis of PHB involves three important enzymatic steps of which the starting material is acetyl-coenzymeA (acetyl-CoA). The reversible condensation reaction of two acetyl-CoA molecules into acetoacetyl-CoA was catalysed by β-ketothiolase (encoded by *phbA*). It is then reduced to 3-hydroxy-butyryl-CoA by NADPH-dependant acetoacetyl-CoA reductase (encoded by *phbB*) and subsequently polymerizing 3-hydroxybutyryl-CoA monomers by P (3HB) synthase (encoded by *phbC*) produces PHB. The type of PHA produced is determined by PHB synthase enzyme when the concerned metabolic pathway and the carbon sources required for the microorganism are available. Therefore, PHB synthase (*phbC* gene) is described as the key enzyme for the biosynthesis of PHB.

Microbes are the primary and vital part of an ecosystem; they are readily influenced by the changing biotic and abiotic factors[15]. In order to adapt to this changing environment, bacteria produce PHB to derive its energy for their survival. In this context, identification of potential PHB producer from different ecological niches has also gained momentum as it may reduce the production cost of the biopolymer. Different microorganisms like *Alcaligenes latus*, *Ralstonia eutropha*, *Azotobacter beijerinckii*, *Bacillus megaterium*, *Pseudomonas oleovorans*, *etc.* have been found to accumulate polyhydroxybutyrate during unfavourable conditions. *Streptomyces* sp. also produces PHB in nitrogen deficient medium[16]. Among these microbes, *Bacillus* species have been reported and studied extensively for PHB production[17]. In industry and academia, *Bacillus* is widely studied as its replication and maintenance of plasmids is stable[18]. *Bacillus* species can grow well in cheap substrates, have high growth rate in comparison to other bacteria and most importantly the absence of lipopolysaccharide layer makes them easy to extract the polymer. *Bacillus megaterium* S29 and *Bacillus* sp. IPCB-403 have been reported to accumulate over 70% PHB content per cell dry weight in optimal conditions[19]. *Bacillus aryabhattai* T34-N4 utilizes waste starch for direct production of PHB[20]. Moreover, *Bacillus cereus* is reported to yield up to 48% of PHB content when starch was used as a source of carbon[21]. Recently, *Bacillus drentensis* strain BP17 was found to be a promising bacterium for PHB production using agricultural waste, such as pineapple peel as a low-cost carbon source for PHB production[22]. *Bacillus* spp. has shown ability to obtain high PHB yields with few fermentation factors, and hence is suitable for PHB production[23].

Soils from municipal dumpsite areas rich in carbon sources serve as a stress prone environment. This favours accumulation of lipid granules, polyhydroxy-butyrate inside the cytoplasm of bacterial cell. In the present study, a potential PHB producing *Bacillus* strain from the dumpsite soil of Southern Assam, India was isolated and characterized. Its PHB producing ability was studied using phenotypic and genotypic methods and finally, the cultural conditions (carbon sources, nitrogen sources, pH and temperatures) of the bacterium were also optimized to enhance its PHB production.

Materials and Methods

Isolation and screening of PHB producing bacteria

Soil samples were collected from different municipal dumpsite areas of Southern Assam, India. Different colonies of bacteria were isolated by serial dilution method and morphologically different colonies were separated in nutrient agar medium and sub-cultured to obtain a pure culture. These bacterial isolates were incubated in nutrient limited basally defined DM9 medium (glucose, 4 gL^{-1}; NH_4Cl, 1.0 gL^{-1};

Na_2HPO_4, 7 gL^{-1}; NaH_2PO_4, 3 gL^{-1}; 10 mL 0.01M $CaCl_2$ and 10 mL 0.1M $MgS0_4.7H_20$) for 48 h[24]. After incubation, bacterial smears were made on clean grease-free glass slides. After drying, a drop of 3% Sudan Black B was added to the slides. Then, the slides were immersed in xylene for few seconds, dried and stained with 5% safranin for 20 s. The slides were washed with distilled water, dried and observed in oil immersion microscope (Nikon E200) for detecting PHB granules[15].

Characterization of bacterial isolates

Morphological and microscopic characterization of PHB producing bacteria were investigated based on colony characteristics and Gram's reaction with microscopic examination. Biochemical characteristics were analysed according to Bergey's manual of determinative bacteriology[25]. Molecular characterization was done by extracting the genomic DNA using HiPurA™ Bacterial genomic DNA Extraction Kit and 16S rDNA gene was amplified by PCR using primers 27F and 1492R[26]. Using Genejet Gel Extraction PCR purification kit, the amplified gene product was purified and then sequenced by ABI 3500 Genetic Analyser. The 16S rDNA gene sequence analysis was carried out by Bio-Edit software and identification of bacteria was done using NCBI-BLAST (National Centre for Biotechnology Information http://www. ncbi. nml.nih.gov) program. The sequences were aligned and the phylogenetic tree was constructed by UPGMA method using MEGA 4.1. Evaluation of the tree was done with respect to bootstrap method for 1000 replica.

Optimization of PHB production

For optimum production of PHB, biomass production was optimized considering different growth parameters like pH, temperature, carbon and nitrogen sources, using slightly modified production medium. The evaluated parameters are pH (5-9), temperature (20-40°C), carbon sources (glucose, fructose, sucrose, mannitol and starch) at a fixed concentration of 4 gL^{-1} and nitrogen sources (peptone, yeast extract, ammonium chloride, tryptone and urea) at a fixed concentration of 1.0 gL^{-1}. To obtain the optimum pH for biomass production, 5 mL of production medium was taken in different test tubes and pH was adjusted from 5 to 9, keeping carbon and nitrogen at a fixed concentration. One mL of 24 h fresh culture was inoculated in each tube and incubated for 48 h at 30°C with 120 rpm. The bacterial biomass production was evaluated by measuring the OD_{600} in UV-Vis spectrophotometer (Spectrascan UV 2600) and the optimum pH was determined. Similarly, other parameters were also optimized in the same manner[15].

Extraction and estimation of PHB

Using the above optimized growth parameters extraction of PHB was carried out following Hypochlorite method with a slight modification[27]. For this, bacterial isolate was grown in 50 mL of optimized DM9 medium and incubated for 48 h at 35°C with 120 rpm. Then, the cell pellet was harvested by centrifugation at 6000×g for 10 min, washed once with 10 mL saline and re-centrifuged to get the pellet. 5 mL sodium hypochlorite (4% active chlorine) was added to the pellet and incubated at 37°C for 10 min with stirring. This was again centrifuged at 8000×g for 20 min and the PHB pellet was washed with cold diethyl ether. Finally, it was centrifuged at 8000×g to get the purified PHB and was dried to constant weight at 60°C. Amount of PHB produced was estimated by measuring its dry weight.

Determination of bacterial biomass

Bacterial biomass was determined by measuring the dry cell weight of the bacteria. For this, 50 mL of the bacterial culture broth was centrifuged and supernatant was discarded. The cell pellet was washed with distilled water and dried at 60°C until the weight does not decrease further. PHB production was quantified using the following formula[15]:

$$\text{PHB production (\%)} = \frac{\text{Weight of PHB}}{\text{Weight of biomass}} \times 100$$

Amplification of *phbC* gene

The genomic DNA of the bacterial isolates were extracted using HiPurA™ Bacterial genomic DNA Extraction Kit and the *phbC* gene of the isolates were amplified by PCR using the specific primer *phbC*-F (5'-CCTTCTCGCCTATGCTCTGG-3') and *phbC*-R (5'-GCGTGACCTTGAATGGTTGG-3'), designed on the basis of *phbC* gene sequences of other bacteria. PCR amplification was carried out with the following temperature profiles: initial denaturation (5 min at 94°C), 35 cycles of denaturation (30 s at 94°C), annealing (40 s at 55°C), extension (1.0 min at 72°C) and a final extension for 7 min at 72°C. The amplified genes of the isolates were then observed in 1% agarose gel electrophoresis[28].

Evaluation of kinetic parameters

Specific growth rate (G) was determined by the formula: G = ln W2 – ln W1 / T2 - T1 × 100, where W2 is the dry cell biomass at time T2 and W1 is the dry cell biomass at time T1. It is expressed in terms of $gh^{-1}L^{-1}$. Content of the polymer was expressed as the ratio of PHB (P) to the biomass (X) multiplied by 100. PHB content = P × 100/X. Specific PHB storage rate (μ) per unit time (t) was given as μ = P/X.t (gram of PHB produced per gram of biomass per hour). PHB yield at the end of the fermentation ($Y_{p/s}$) was calculated as the mass of PHB obtained per gram of the substrate[29].

Statistical analysis

Analysis of the results obtained for optimization of PHB production was done by using SPSS 16.0 software, where P <0.05 was considered to be statistically significant.

Results and Discussion

Screening of PHB producing bacteria

Gram-positive bacteria such as *Bacillus* spp. though it has been used extensively in industry are under exploited for PHB production. On the contrary, Gram-negative bacteria are the only commercial source for PHB production but often it has been contaminated by liposaccharides (LPS)[30]. Hence, in this study, we selected *Bacillus* sp. to screen for possible isolates that can be used in PHB production to make it economical. A total of 86 bacterial cultures were isolated and screened for their ability to accumulate PHB when grown in a stressed environment provided by DM9 medium. Screening was done using Sudan Black B staining in which the positive isolates showed blue-black coloured granules inside the bacterial cell (Fig. 1). Among these 86 isolates, 26 isolates showed accumulation of PHB in DM9 medium after 48 h of incubation at 30°C with 120 rpm and amount of PHB produced was quantified as shown in Table 1. Isolate K8 was selected for further optimizing the PHB production as it accumulates maximum amount of the polymer.

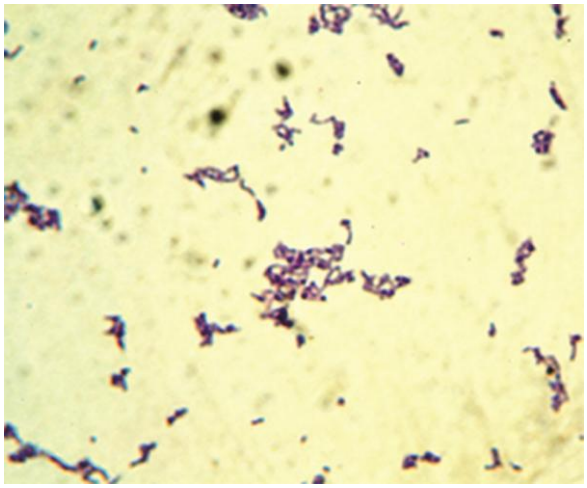

Fig. 1 — Sudan Black B staining of *Bacillus pumilus* K8 showing accumulation of polyhydroxybutyrate as blue-black coloured granules inside the bacterial cell

Characterization of bacterial isolates

Morphological and physiological characterization was investigated for the four potential bacterial isolates (Table 2) and based on its subsequent sequences of 16S rDNA gene, the bacterial isolates were identified as *Bacillus pumilus* H4, *Bacillus pumilus* H7, *Bacillus subtilis* H13 and *Bacillus pumilus* K8. The nucleotide sequences of these four bacterial cultures have been deposited in GenBank database under accession numbers KT907042, KT907043, KT907045 and KT907047. The consensus sequences of the four bacterial isolates and other related strains were aligned and the phylogenetic tree shown in Fig. 2 was constructed.

Table 1 — PHB produced by 26 positive isolates

Name of isolates	Source of isolates	OD (600 nm)	PHB (gL^{-1})
K8	dump soil from Karimganj	1.310	3.21
H4	dump soil from Hailakandi	0.764	1.75
H13	dump soil from Hailakandi	0.690	1.60
H7	dump soil from Hailakandi	0.446	1.50
H12	dump soil from Hailakandi	0.416	1.20
H15	dump soil from Hailakandi	0.355	1.17
H3	dump soil from Hailakandi	0.167	0.95
SH16	dump soil from Silchar	0.130	0.65
SH21	dump soil from Silchar	0.122	0.63
SH22	dump soil from Silchar	0.120	0.50
NCS42	dump soil from Silchar	0.113	0.43
S2	dump soil from Silchar	0.110	0.42
S8	dump soil from Silchar	0.107	0.39
S14	dump soil from Silchar	0.099	0.29
S15	dump soil from Silchar	0.091	0.25
S21	dump soil from Silchar	0.088	0.23
S24	dump soil from Silchar	0.080	0.19
S25	dump soil from Silchar	0.073	0.15
H1	dump soil from Hailakandi	0.064	0.14
H16	dump soil from Hailakandi	0.057	0.12
H18	dump soil from Hailakandi	0.050	0.10
SH1	dump soil from Silchar	0.037	0.09
SH3	dump soil from Silchar	0.029	0.07
SH4	dump soil from Silchar	0.025	0.06
SH6	dump soil from Silchar	0.014	0.04
SH13	dump soil from Silchar	0.013	0.02

[O.D- optical density taken at 600 nm]

Table 2 — Morphological and biochemical properties of bacterial isolates

Characterization				
Morphology	K8	H4	H13	H7
Colony shape	Round	Round	Round	Round
Colony colour	Milky	Off white	Creamy	Milky
Surface	Smooth	Smooth	Smooth	Smooth
Elevation	Raised	Raised	Flat	Raised
Transparency	Opaque	Translucent	Opaque	Opaque
Margin	Entire	Entire	Entire	Entire
Stickiness	Non-sticky	Sticky	Sticky	Sticky
Gram staining	+	+	+	+
Cell shape	Rod	Rod	Rod	Rod
Cultural characteristics				
Growth on NA	+	+	+	+
Growth on NB	+	+	+	+
Optimum temp.	35°C	30°C	30°C	30°C
Optimum pH	7	6	7	6
Biochemical properties				
Indole	nil	nil	nil	nil
Methyl red	nil	nil	nil	nil
Voges Proskauer	+	+	+	+
Citrate utilization	nil	nil	nil	nil
Nitrate reduction	nil	+	+	nil
Starch hydrolysis	+	nil	+	nil
Catalase	+	+	+	+
Oxidase	nil	+	nil	+
Urease	+	nil	nil	nil
Gelatin liquification	nil	nil	nil	nil
Utilization of carbohydrates				
Glucose	+	+	+	nil
Mannitol	+	+	+	nil
Rhamnose	+	+	+	+
Adonitol	nil	nil	nil	nil
Arabinose	+	+	nil	nil
Lactose	nil	nil	nil	nil
Sorbitol	nil	nil	nil	nil
Sucrose	+	+	+	nil

[NA, Nutrient agar; NB, Nutrient broth; +, positive. and nil, negative]

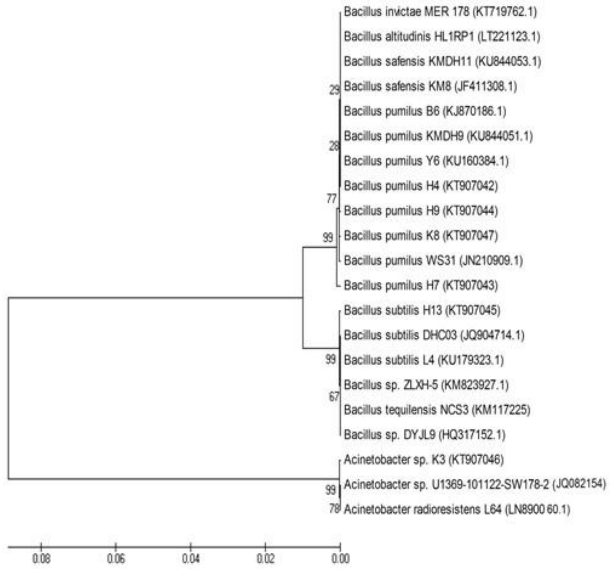

Fig. 2 — Phylogenetic relationship of the bacterial isolates with their related strains

Different *Bacillus* species isolated from different environments like waste water, sewage and sludge were found to accumulate PHB[23]. *Bacillus* is a prominent genus found in soil and water, which possess the ability to grow using cheap raw materials. Accumulation of PHB in bacteria largely depends on limiting nutritional factors like low nitrogen, phosphorus and sulphur with abundant amount of carbon. In this investigation, soils from dump sites have been collected with the view that this desired nutritional condition is naturally harboured by the dwelling bacteria and also dump soil serves as a rich source of carbon. Moreover, soil is the source for a diverse group of microorganisms where they can survive easily in unfavourable environmental conditions by accumulating PHB or by secreting extracellular metabolites to protect themselves from the noxious environment. Municipal waste sites have high BOD and COD values and also have high carbon content with less nitrogen and phosphorus[31]. This variant nutrient condition (especially carbon to nitrogen) produces a stress prone environment which triggers accumulation of PHB inside the cytosol of the bacteria.

Optimization of PHB production by isolate K8

The cultural parameters such as temperature, pH, carbon source, nitrogen source, etc. play a key role in increasing the biomass production and in turn PHB production as biomass production is analogous to PHB production[32]. Therefore, it was standardized and observed that mannitol, yeast extract, pH7 and temperature 35°C were optimum at significance (P <0.05) level. Growth of organism and PHB accumulation depends on specific carbon source taken up by the organism. Growth of *Bacillus pumilus* K8 was investigated using different sources of carbon. Fig. 3A shows that the isolate K8 could utilize mannitol efficiently among different carbon sources and growth (in terms of OD) was approximately 1.51±0.08. Fructose and glucose also supported growth (1.41±0.09 and 1.34±0.18) but they were used up moderately for accumulation of PHB. A highest cell density was obtained when yeast extract was used as the sole nitrogen source with 1.52±0.09 O.D (Fig. 3B). Peptone and ammonium chloride also promoted growth however, urea and tryptone showed very low cell densities. Bacterial growth have also greatly influenced by change in pH gradients. Fig. 3C shows that maximum bacterial growth occurred at pH 7 (1.97±0.03 O.D) and further increase in pH does not

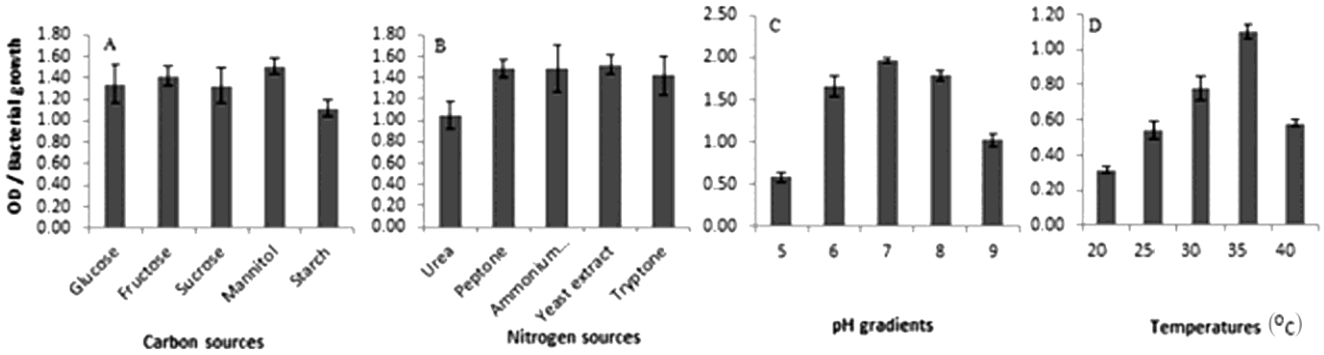

Fig. 3 — Optimization of growth parameters for PHB production by *Bacillus pumilus* K8 at different (A) carbon sources (B) nitrogen sources (C) pH gradients and (D) temperatures

favour growth of the cell and in turn PHB accumulation. Temperature also plays an important role on cell growth of the bacterium. Maximum cell growth was shown at 35°C (1.49±0.04 O.D) and minimum cell growth was observed at 20°C (0.32±0.02 O.D) (Fig. 3D). Further increase in temperature does not favour growth of *Bacillus pumilus* K8. Subsequently, under this optimized conditions, *Bacillus pumilus* K8 was found to accumulate maximum amount of 5.04 gL^{-1} PHB achieving 72.93% of PHB production.

The present result somewhat coincides with the early report that 70.5% PHA production was observed using *Bacillus* sp. in presence of 0.5 gL^{-1} of nitrogen source[20]. In another report, it has been stated that PHA production from *Bacillus* sp. varied from 13.06% by *Bacillus* sp. PHA 013 to 66.80% of dry cell weight by *B. licheniformis* strain PHA007[33]. *B. licheniformis* PHA007 produces maximum amount of 6.28 gL^{-1} PHB which is also similar to the findings of our present study. Optimum PHB production by another strain *Bacillus cereus* FA11 was 48.43% obtained at pH7, 30°C, with glucose as carbon source after 48 h of incubation. Cultural effects on PHB production was also studied in *Pseudomonas, Citrobacter* and *Bacillus* (MS2D, MS2C and SEWA) where the strains MS2D, MS2C and SEWA accumulates maximum amount of PHAs at pH7[34]. pH7 and temperature 35°C in the present optimized data are pre-eminent for the production of PHB, as *Bacillus pumilus* K8 was isolated from the environmental soil sample. Moreover, mannitol is a monosaccharide derivative which can easily assimilate by bacteria. Sucrose is a cheaper source for the production of PHB by *Bacillus mycoides* RLJ B-07. A maximum PHB content in the cell reached up to

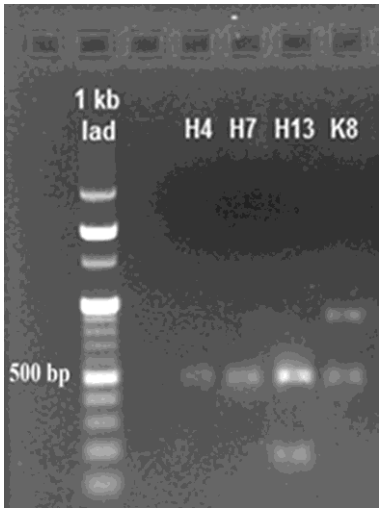

Fig. 4 — Amplified PCR product of *phbC* gene of the four bacterial isolates

39.9% in *Bacillus megaterium* after growing with glucose as the sole carbon source[35]. However in this study, mannitol was found to favour higher biomass and PHB production by *Bacillus pumilus* K8. Similarly, higher biomass and in turn higher PHB accumulation was obtained in yeast extract among other nitrogen sources used in the study. This may be due to low nitrogen content and presence of vitamin B complex and growth factor. In *B. subtilis* 25 and in *B. megaterium* 12, protease peptone was found to observe the highest level of PHB accumulation[36]. Literature also reveals that a variety of commercially available complex nitrogen sources were analyzed for PHB production and they increased the yield of PHB production in *A. vinelandii* UWD strain[37].

Amplification of *phbC* gene

A pair of specific PCR primer was designed and used for genotypic detection of *phbC* synthase gene. Approximately, 503 bp DNA fragment in the seven

bacterial strains were successfully amplified and separated on 1% agarose gel, observed with a UV trans-illuminator and documented with GelDocXR software (Biorad) (Fig. 4). Amplification of *phbC* gene genotypically confirms the production of PHB by the bacteria. Application of different genotypic methods in various PCR protocols; using degenerate primers to detect and amplify the PHA synthase gene(s) have been reported[38,39]. The polyphasic approach using combination of phenotypic and genotypic screening method was successfully applied[40]. This polyphasic approach is more preferred than the individual detection method. Also, different molecular approaches were successfully applied for detection of PHAs accumulation by a variety of bacteria[41,42]. Thus, amplification of *phbC* gene may help to acquire the knowledge in molecular genetics of PHB production which may further facilitate in analyzing the metabolic process of PHB biosynthesis in *Bacillus pumilus* K8.

Evaluation of kinetic parameters

The kinetic parameters for the batch experiment were evaluated with respect to PHB and biomass production on the optimized culture medium. After optimization, *Bacillus pumilus* K8 grow at the rate 0.112 $gh^{-1}L^{-1}$ of the production medium and produces 72.93% of PHB at the rate of 0.013 g g^{-1} of the biomass produced h^{-1}. But in another study[29], it has been reported that *B. subtilis* NG05 grows at the rate 0.14 $gh^{-1}L^{-1}$ of the production medium and accumulate 50% of PHB at the rate of 0.007 g g^{-1} of the biomass produced h^{-1}.

Conclusion

The results suggest that *Bacillus pumilus* K8, a Gram-positive bacterium could be a potential bacterium for PHAs production. It grows at a rate of 0.112 $gh^{-1}L^{-1}$ of the production medium and could achieve maximum production of 72.93% of PHB at the rate of 0.013 gg^{-1} of biomass h^{-1} with polymer concentration of 5.04 gL^{-1} in an optimized condition. This study revealed that dumpsite soil could be a good resource for PHB producing bacterial species. *Bacillus pumilus* was found to be a new *Bacillus* sp. capable to accumulate PHB efficiently. Moreover, *Bacillus* sp. has the capability to produce different ranges of monomeric composition as it can incorporate both short chain length (scl) and medium chain length (mcl) PHAs. Therefore, PHAs production by Gram-positive *Bacillus* sp. has a distinct characteristic which needs further intensive studies. *Bacillus pumilus* could be utilized for industrial production of PHB as it can produce the polymer very efficiently and hence may help in reducing the production cost of PHB. Also, the result of this study may serve as a reference for further investigation of new species of *Bacillus* from some other unexplored niches.

Acknowledgement

Authors acknowledge the Dept. of Biotechnology, Govt. of India, New Delhi for instrument facility at Microbial Molecular Biology Laboratory, Department of Biotechnology, Assam University, Silchar, Assam, India which were used in this work.

Conflict of Interests

Authors declare no conflict of interests.

References

1 Laxuman S, Akhilesh KS, Bhabatarini P & Nirupama M, Process optimization for poly-beta-hydroxybutyrate production in a nitrogen fixing cyanobacterium, *Nostoc muscorum* using response surface methodology. *Bioresour Technol*, 98 (2007) 987.

2 Hempel F, Bozarth AS, Lindenkamp N, Klingl A, Zauner S, Linne U, Steinbüchel A & Maier UG, Microalgae as bioreactors for bioplastic production. *Microb Cell Fact*, 10 (2011) 81.

3 Yeo JCC, Joseph KM, Warintorn T, Zibiao L & Chaobin H, Recent advances in the development of biodegradable PHB-based toughening materials: Approaches, advantages and applications. *Mater Sci Eng C*, 92 (2018) 1092.

4 Costa SS, Miranda AL, de Morais MG, Costa JAV & Druzian JI, Microalgae as source of polyhydroxyalkanoates (PHAs)—A review. *Int J Biol Macromol*, 131 (2019) 536.

5 Kim MY, Kim C, Moon J, Heo J, Jung SP & Kim JR, Polymer film-based screening and isolation of polylactic acid (PLA)-degrading microorganisms. *J Microbiol Biotechnol*, 27 (2017) 342.

6 Akaraonye E, Keshavarz T & Roy I, Production of polyhydroxyalkanoates: the future green materials of choice. *J Chem Technol Biotechnol*, 85 (2010) 732.

7 Gumel AM, Annuar MSM & Heidelberg T, Current application of controlled degradation processes in polymer modification and functionalization. *J Appl Polym Sci*, 129 (2013) 3079.

8 Dipa R, Current barriers and future needs towards industrial development in *Biocomposites for High-Performance Applications* (Woodhead Publishing, Elsevier Ltd., ON, Canada), 2017, 336.

9 Shilpi K & Ashok KS, A simple structured mathematical model for biopolymer (PHB) production. *Biotechnol Prog*, 21 (2005) 830.

10 Ojumu TV, Yu J & Solomon BO, Production of polyhydroxy-alkanoates, a bacterial biodegradable polymer. *Afr J Biotechnol*, 3 (2004) 18.

11 Mozes-Koch R, Tanne E, Brodezki A, Yehuda R, Gover O, Rabinowitch HD & Sela I, Expression of the entire polyhydroxybutyrate operon of *Ralstonia eutropha* in plants. *J Biol Eng*, 11 (2017) 44.

12 Miyake M, Takase K, Narato M, Khatipov E, Schnackenberg J, Shirai M, Kurane R & Asada Y, Polyhydroxybutyrate production from carbon dioxide by cyanobacteria. *Appl Biochem Biotechnol*, 84 (2000) 991.

13 Carpine R, Du W, Olivieri G, Pollio A, Hellingwerf KJ, Marzocchella A & Branco dos Santos F, Genetic engineering of *Synechocystis* sp. PCC 6803 for polyhydroxy-butyrate overproduction. *Algal Res*, 25 (2017) 117.

14 Akhilesh KS, Laxuman S, Nirupama M & Jyoti M, Progress and challenges in producing polyhydroxyalkanoate biopolymers from cyanobacteria. *J Appl Phycol*, 29 (2017) 1213.

15 Swati M, Pravanshu M, Biplab S, Akshay D, Chitranjan K & Devi PS, Production of polyhydroxyalkanoates (PHAs) by *Bacillus* strain isolated from waste water and its biochemical characterization. *Proc Natl Acad Sci India Sect B Biol Sci*, 4 (2015) 1. DOI: 10.1007/s40011-015-0626-6.

16 Wani SJ & Sayyed RZ, Production, efficient recovery and partial characterization of biodegradable polymer produced by soil *Streptomyces* sp. *Ind J Biotechnol*, 15 (2016) 127.

17 Vishnuvardhan RS, Thirumala M & Mahmood SK, Production of PHB and P (3HB-co-3HV) biopolymers by *Bacillus megaterium* strain OU303A isolated from municipal sewage sludge. *World J Microbiol Biotechnol*, 25 (2009) 391.

18 Biedendieck R, Gamer M, Jaensch L, Meyer S, Rohde M, Deckwer WD & Jahn D, A sucrose inducible promoter system for the intra- and extracellular protein production in *Bacillus megaterium. J Biotechnol*, 132 (2007) 426.

19 Rodriguez-Contreras A, Koller M, de Sousa Dias MM, Calafell M, Braunegg G & Marque´s-Calvo MS, Novel poly [(β)-3-hydroxybutyrate]-producing bacterium isolated from a Bolivian hypersaline lake. *Food Technol Biotechnol*, 51 (2013) 123.

20 Bomrungnok W, Arai T, Yoshihashi T, Sudesh K, Hatta T & Kosugi A, Direct production of polyhydroxybutyrate from waste starch by newly-isolated *Bacillus aryabhattai* T34-N4. *Environ Technol*, (2019) DOI: 10.1080/09593330. 2019.1608314.

21 Tian PY, Shang L, Ren H, Mi Y, Fan DD & Jiang M, Biosynthesis of polyhydroxyalkanoates: Current research and development. *Afr J Biotechnol*, 8 (2009) 709.

22 Penkhrue W, Jendrossek D, Khanongnuch C, Pathomaree W, Aizawa T, Behrens RL & Lumyong S, Response surface method for polyhydroxybutyrate (PHB) bioplastic accumulation in *Bacillus drentensis* BP17 using pineapple peel. *PLoS One*, 15 (2020) e0230443.

23 Thirumala M, Vishnuvardhan RS & Mahmood SK, Production and characterization of PHB from two novel strains of *Bacillus* spp. isolated from soil and activated sludge. *J Ind Microbiol Biotechnol*, 37 (2010) 271.

24 Wei YH, Chen WC, Huang CK, Wu HS, Sun YM, Lo CW & Janarthanan OM, Screening and evaluation of poly-hydroxybutyrate producing strains from indigenous isolate *Cupriavidus taiwanensis* strains. *Int J Mol Sci*, 12 (2011) 252.

25 Holt JG, Krieg NR, Sneath PHA, Staley JT & Williams ST, In: *Bergey's Manual of Determinative Bacteriology*, (9th ed Williamsons and Wilkins, Balitomore, USA), 1994.

26 Weisburg WG, Barns SM, Pelletier DA & Lane DJ, 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*, 173 (1991) 697.

27 Rawte T & Mavinkurve S, A rapid hypochlorite method for the extraction of polyhydroxyalkanoates from bacterial cells. *Indian J Exp Biol*, 40 (2002) 924.

28 Hamid G, Sara A & Maisam M, Cloning of poly (3-hydroxy-butyrate) synthesis genes from *Azotobacter vinelandii* into *Escherichia coli*. *Jundishapur J Microbiol*, 2 (2009) 31.

29 Gulab S, Anish K, Arpana M, Varsha G, Anita Y & Neeraj KA, Cost effective production of polyhydroxybutyrate by *Bacillus subtilis* NG05 using sugar industry waste water. *J Polym Environ*, 21 (2013) 441.

30 Ram KPS, Deepak V, Kalishwaralal K, Ramesh KN, Jeyaraj M & Gurunathan S, Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by *Bacillus megaterium* SRKP-3. *Bioresour Technol*, 101 (2010) 705.

31 Dircks K, Beun JJ, Van LM, Heijnen JJ & Henze M, Glycogen metabolism in aerobic mixed cultures. *Biotechnol Bioeng*, 73 (2001) 85.

32 Koller M, Atlic A, Dias M, Reiterer A & Braunegg G, Microbial PHA production from waste raw materials. In: *Plastics from Bacteria: Natural Functions and Applications*, *Microbiology Monographs*, (Ed. Chen G; Springer Verlag Berlin, Heidelberg, Germany), 2010, 85.

33 Kanokphorn S & Poonsuk P, Screening and identification of polyhydroxyalkanoates producing bacteria and biochemical characterization of their possible application. *J Gen Appl Microbiol*, 58 (2012) 173.

34 Arshad MU, Jamil N, Naheed N & Husnain S, Analysis of bacterial strains from contaminated and non-contaminated sites for the production of biopolymers. *Afr J Biotechnol*, 6 (2007) 1115.

35 Borah B, Thakur PS & Nigam JN, The influence of nutritional and environmental conditions on the accumulation of poly-hydroxybutyrate in *Bacillus mycoides* RLJ B-017. *J Appl Microbiol*, 92 (2002) 776.

36 Yuksekdag NZ, Belma A, Yavuz B & Nazim M, Effect of carbon and nitrogen sources and incubation times on PHB synthesis by *Bacillus subtilis* and *Bacillus megaterium*. *Afr J Biotechnol*, 3 (2004) 63.

37 Page WJ, Production of poly-β-hydroxybutyrate by *Azotobacter vinelandii* UWD in media containing sugars and complex nitrogen sources. *Appl Microbiol Biotechnol*, 38 (1992) 117.

38 Shamala RT, Chandrashekar A, Vijayendra SVN & Kshama L, Identification of polyhydroxyalkanoate (PHA) producing *Bacillus* sp. using the polymerase chain reaction (PCR). *J Appl Microbiol*, 94 (2003) 369.

39 Solaiman DKY & Ashby RD, Genetic characterization of the poly (hydroxyalkanoate) synthases of various *Pseudomonas oleovorans* strains. *Curr Microbiol*, 50 (2005) 329.

40 Chien CC, Kung SS & Chuang CH, Isolation of polyhydroxy-alkanoates-producing bacteria using a combination of phenotypic and genotypic approach. *Lett Appl Microbiol*, 44 (2007) 364.

41 Sheu DS, Wang YT & Lee CY, Rapid detection of polyhydroxy-alkanoate accumulating bacteria isolated from the environment by colony PCR. *Microbiology*, 146 (2000) 2019.

42 Castano DM, Romo DMR, Grosse MV & Solano NCM, A most effective method for selecting a broad range of short and medium chain-length polyhydroxyalkanoate producing microorganisms. *Electronic J Biotechnol*, 10 (2007) 348.