Kuwait J. Sci. **42 (2)** pp. 236-249, 2015

Polyhydroxyalkanoates production using canola oil by bacteria isolated from paper pulp industry

TAYYABA YOUNAS, IFTIKHAR ALI* AND NAZIA JAMIL

Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore-54590, Pakistan.
**Corresponding author: iftikhar_ali_iftikhar@yahoo.com*

ABSTRACT

Bacterial polyhydroxyalkanoates (PHAs) are the biopolyesters that are produced under unfavorable conditions. Environmental samples were taken from paper pulp and mixed organic wastes of an industry for isolation of PHA producing bacterial strains. High PHA production ability was found in six strains belonging to *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Escherichia* genera. The PHA production optimization of the stains was done at various concentrations of $(NH_4)_2SO_4$ (0.2%, 0.4%, and 0.6%), pH (5, 6, and 7) and temperatures (4°C, 37°C, 45°C). Strain WC9 produced 19% PHA content from canola oil and 21% with glucose. Strain WC20 was found to produce 28% and 16% PHA content with glucose and canola oil respectively at 37°C, under 0.2% of (NH4)2SO4. Strain WC20 was found to belong to *Pseudomonas* spp. according to 16S rRNA gene sequencing. PCR amplification of the phaC gene was also successfully performed for the *Pseudomonas* sp. WC20.

Keywords: Canola oil; industrial waste; *pha*Cgene; polyhydroxyalkanoates.

INTRODUCTION

Widespread use of synthetic plastic makes it highly produced polymer, but this polymer is non-renewable and non-biodegradable (Demarco, 2006). The solution to this problem is the use of bioplastics, which are a potential replacement to the synthetic plastics. Due to their biodegradability and origin from renewable resources, PHAs have attracted much interest by industrialists and many technical applications have been developed for them in the last few decades (Anderson & Dawes, 1990; Hocking & Marchessault, 1994).

Polyhydroxyalkanoates (PHAs) are polyesters produced by a wide variety of bacteria as an intracellular storage material of carbon and energy (Tsuge, 2002) and are usually produced when an essential nutrient element such as nitrogen, phosphorus, oxygen, sulfur, or potassium is limited and carbon source is in excess (Lee, 1996).

The use of PHAs in a wide range of applications has been hampered mainly by their high production cost compared with oil-derived plastics (Lee & Choi, 1999). With the aim of commercializing PHA, great efforts have been employed to reduce

the production cost by the development of bacterial strains and more efficient fermentation/recovery process (Lee *et al*., 2000) The cost of raw material only accounts for 40-50 % of the total production cost (Purushothaman *et al*., 2001). Sugars, plant oils and agricultural byproducts are the various inexpensive carbon sources that can be converted to PHA (Loo & Sudesh, 2007). Because of the compatibility of plant oils for PHA production, we decided to use canola oil for PHA production by indigenous bacteria found in wastewater of paper industry. The paper industry waste was used to search for some better PHA producer, able to utilize complex carbon source such as, canola oil, as they are resident of a nutritively complex environment.

Plant oils are the renewable inexpensive carbon sources and hence these are being used for the production of polyhydroxyalkanoates such as soybean, sunflower, palm, canola, cartamo, soja, maize, and olive oil (Kobayashi*et al*., 1994; Fukui & Doi, 1998; Kahar *et al*., 2004; Loo *et al*., 2005; Ma del Rocio *et al*., 2007; Din *et al*., 2012). Recently the bacterial strains have also been engineered to utilize the plant oils efficiently for the PHA production (Eskin & McDonald, 1991; Mifune *et al*., 2008).

The canola oil composition with regard to fatty acids is similar to that of olive oils with respect to the high level of oleic acid (Eskin & McDonald, 1991) and novel polyhydroxyalkanoates production from long chain fatty acids has already been reported (Eggink *et al*., 1995). This study deals with the screening of PHA producing strains when canola oil is provided to them and the optimization of PHA production from these strains.

MATERIALS AND METHODS

Bacterial isolation

Two types of samples i.e. paper pulp and mixed organic wastes were collected from the effluent of Packages Industries Pvt. Ltd (paper industry located in Lahore) for the isolation of PHA producing strains. Dilutions were made and inoculated into mineral salt medium broth (Ali & Jamil, 2014) containing Canola oil as carbon source, followed by incubation at 37°C for two days for enrichment of oil utilizing bacterial strains. Colony count, morphological and biochemical analysis (Cappuccino & Sherman, 2007) of the twenty five purified strains was done. Total bacterial load for the both samples was determined.

Screening of PHA producers

Screening of PHA producers was done on PHA detection agar (PDA) supplemented with 0.5 µg/ml Nile blue A (Spiekermann *et al*., 1999) having canola oil as the only carbon source, for viable colony staining. The cultures were incubated at 37°C for 24

hours. PHA producers give fluorescence, when the plates are illuminated with UV light. Sudan black B staining (Lee & Choi, 1999) was also used as a confirmation for the presence of intracellular PHA granules.

Bacterial growth and extraction of PHA

Bacterial strains (WC9, WC12, WC13, WC20, WC27 and WC28) were grown in 500 ml Erlenmeyer flasks containing 50 ml of mineral salt medium (Chaudhry *et al*., 2011) containing glucose or canola oil at 200 rpm. Bacterial cultures were centrifuged at 8000 *g* for 10 min, resuspended in 0.85% NaCl solution and centrifuged and freeze dried. PHA extraction was performed by direct addition of sodium dodecyl sulphate (SDS) (Kim *et al*., 2003). Lyophilized bacterial cells called as (DCW) were agitated with SDS solution at 30°C for 2 hours at 200 rpm. The soltion was then treated with chloroform so that PHA contents from solution are dissolved in it. This organic phase was separted by centrifugation, dried and weighed to measure PHA.

The PHA % was calculated by following formula,

$$\text{PHA \%} = \frac{\text{DCW (g)}}{\text{PHA (g)}} \times 100$$

For each strain the experiment was carried out in triplicate flasks under following conditions.

i) Bacterial growth on glucose and canola as sole sources of carbon

Two carbon sources, glucose and canola oil, were used to check the effect of carbon source variety on PHA production efficiency of bacteria. Flasks of MSM broth containing either 2% glucose or canola oil as carbon source, were prepared. The flasks were inoculated and incubated for 72 hours at 37°C with 0.2% nitrogen source and at pH 7.

ii) At various concentrations of nitrogen

Flasks containing 50 ml MSM broth were prepared with varying concentrations of nitrogen i.e., (NH4)2SO4 at 0.2%, 0.4%, and 0.6%, using glucose as carbon source. The flasks were inoculated and incubated for 72 hours at 37°C.

iii) At different values of pH

Flasks of MSM broth with three different pH values were prepared i.e., pH 5, 6 and 7 using glucose as carbon source. The flasks were inoculated and incubated for 72 hours at 37°C and 0.2% nitrogen source.

iv) At different temperatures

Flasks containing 50 ml MSM broth were prepared, using glucose as carbon source, the flasks were inoculated and incubated for 72 hours at three different temperatures i.e. 4°C, 37°C, and 45°C, while providing 0.2% nitrogen source and pH 7.

PCR amplification of16S rRNA and phaC gene fragments

The genomic DNA of the bacterial strains was isolated by CTAB/ NaCl method (Ausubel, 2002). The DNA was observed on agarose gel electrophoresis by the method used by Sambrook & Russell (2001). The 16S rRNA gene was amplified and sequenced by Macrogen, Inc. Korea by using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The sequence was submitted to NCBI GENBANK for the Accession number. DNA amplification of the phaC gene fragment was carried out through PCR using the primers and conditions as described by Chaudhry *et al*.(2011). Forward primer 179–L (5′-ACAGATCAAGTTCTACATCTTCGAC-3′) and the reverse primer 179–R (5′-GGTGTTGTCGTTCCAGTAGAGGATGTC-3′) were used for amplifications carried out with initial denaturation at 110°C for 10 min followed by 30 cycles of amplification (95°C for 2 min, 56°C for 1 min and 72°C for 2 min).

RESULT AND DISCUSSION

Plant oils can be used as carbon sources for PHA production by bacteria, as they have proved to be much cheaper as compared to processed sugars and fatty acids. So this study was aimed to isolate and identify the PHA producing strains using a cheaper carbon source. We used canola oil for countering the cost issues related to production and application of PHA. PHA production was analyzed for various bacterial strains and optimization was carried out for different conditions of PHA production for best producing strains. Samples were collected from Packages Paper Industry Pvt. Ltd., that contained complex high carbon number nutrient sources.

Bacterial load was found to be 2.5 x103 CFU/ml and 2.9x104 CFU/ml on MSM agar, while it increased to3x104 CFU/ml and 3.2x104 CFU/ml on nutrient agar for paper pulp waste sample and mixed organic waste sample respectively. A total of 25 strains were selected, out of which thirteen strains (WC1 to WC13) were from paper pulp sample and twelve strains (WC14 to WC23, WC27 and WC28) were from mixed organic waste sample. Screening of isolated strains for PHA production revealed that 18 out of 25 strains were found to be PHA producers based on their positivity for Nile blue A staining (Figure 1) and Sudan black B staining.

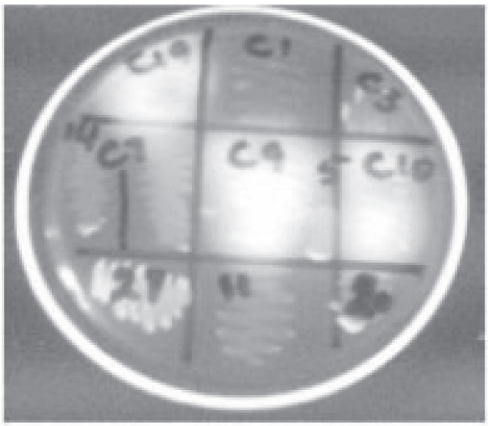

Fig 1. PHA producing strains fluoresce under UV light when grown on a nitrogen limited minimal salt medium supplemented with 0.5µg/ml Nile blue A dye.

The microscopic analysis of bacterial strains for gram reaction, spore staining and the shape and arrangement of cells was performed. Most of the PHA producers were gram positive, non-spore forming rods. The gram staining of strains revealed that out of 25, 7 strains (WC14, WC15, WC16, WC18, WC20, WC27, WC28) were gram negative rods, 3 strains (WC4, WC11, WC23) were gram negative cocci, 11 strains (WC1,WC2,WC3, WC5, WC7, WC8, WC9, WC10, WC12,WC19, WC21) were gram positive rods, 4 strains (WC6, WC13, WC17, WC22) were gram positive cocci. Spore staining of the isolates revealed that 5 strains (WC1, WC2, WC9, WC10 and WC12) were spore former. The isolated bacterial strains were morphologically and biochemically characterized and found to belong to genera *Bacillus, Staphylococcus, Micrococcus, Corynebacterium, Pseudomonas, Klebsiella and Escherichia*, indicating the ability of these bacteria to utilize the paper pulp wastes. The isolated WC20 was found to belong *Pseudomonas* genus and its 16S rRNA sequence showed more than 99% identity to Pseudomonas aeruginosa. The 16S rRNA gene sequence was submitted to NCBI GENBANK under accession number **KJ784469** as *Pseudomonas* sp. WC20.

A variety of biochemical tests were performed for bacterial identification. Among gram positive rods two genera were found to be present, i.e., *Bacillus* (WC1, WC2, WC9, WC10 and WC12) and Corynebacterium (WC3, WC5, WC7, WC8, WC19 and WC21).The biochemical characterization results for gram positive cocci revealed that the strains belonged to genera Staphylococcus (WC6, WC13, and WC22) and *Micrococcus* (WC17). The biochemical characterization results (API 20E kit) for gram negative rods revealed that the strains were found to belong to genera Klebsiella (WC14), *Pseudomonas* (WC15, WC16, WC20 and WC28) and *Escherichia* (WC18, WC27).

PHA extraction results showed that high PHA production ability was present in 6 strains (WC9, WC12, WC13, WC20, WC27 and WC28), whereas the rest produced PHA at very low concentrations. All the strains produced higher PHA yield, when provided with 0.2% (NH4)2SO4concentration. The lowest yield resulted on 0.6% nitrogen concentration. The reason could be that limiting amount of nitrogen is required for PHA production (Figure 2). Two carbon sources were used and the maximum PHA production for all the six strains was found to be on glucose. PHA yields were good on canola oil, but they were less as compared to glucose (Figure 3).

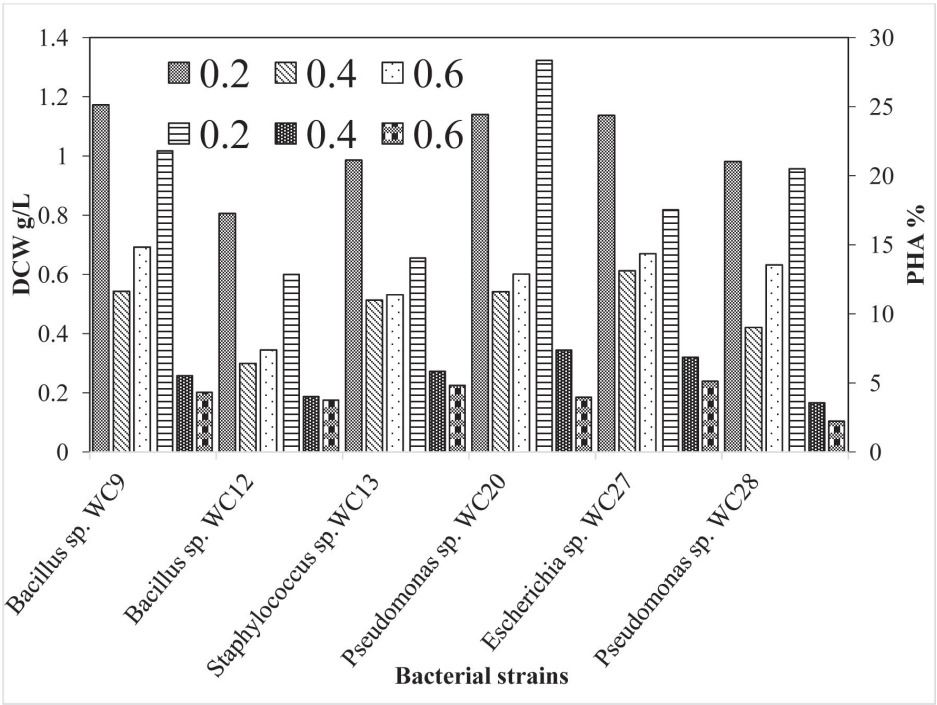

Fig 2. PHA production at different nitrogen concentrations. (NH4)2SO4 was provided at 0.2 %, 0.4% and 0.6% in the mineral medium supplemented with glucose. B represents biomass in DCW and P represents PHA contents % with respect to different nitrogen concentrations (at 37°C and pH7).

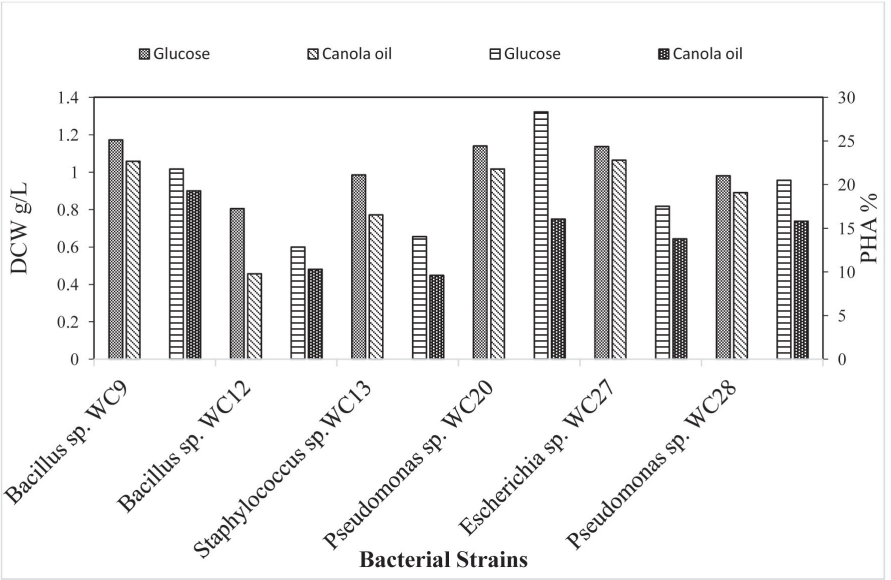

Fig 3. PHA production from glucose and canola oil (at 37°C, 0.2% (NH4)2SO4 and pH7). In case of each strain first two bars represent biomasses for glucose and canola as carbon source and next two bars represent PHA %.

It might be due to the un-revealed enzymatic mechanisms not been able to utilize canola oil as efficiently as glucose. Canola oil has shown very high biomass production (92g/L) for *Cupriavidusnecator* if it is given under controlled fed-batch fermentations (Rathinasabapathy *et al*., 2013). With fructose as carbon source C. *necator* can produce 20% PHB content at cell density of 44 g/L. The use of fed-batch fermentations for PHA production from *Pseudomonas* sp.has not been reported for canola oil but with C. *necator* it may reach up to 90% of DCW (López-Cuellar *et al*., 2011). Three temperatures (4°C, 37°C, 45°C) were used and the maximum PHA production for all the six strains was found to be at 37°C, the lowest growth and PHA production was seen at 4°C for all strains (Figure 4). Three pH values (5, 6 and 7) were used and the maximum PHA production for all the six strains was found to be at pH 7, the lowest growth and PHA production was seen at pH 5 (Figure 5). As compared to chemically synthesized plastics, PHAs production is a more descriptive process that depends upon various factors like C/N ratio, the availability of major elements e.g., magnesium, potassium, oxygen, phosphate and iron (Loo & Sudesh, 2007).

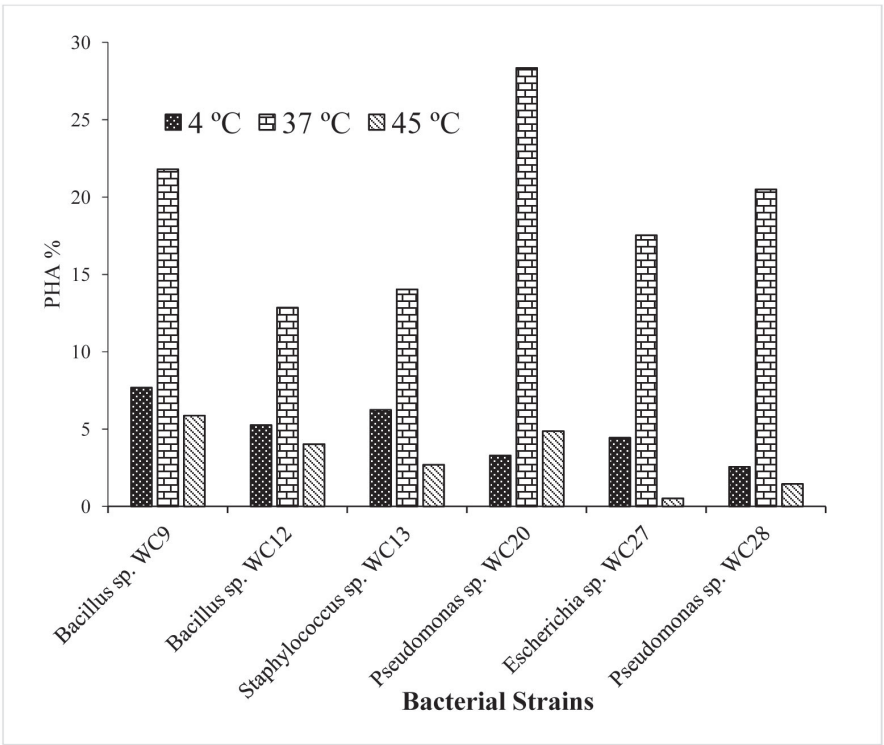

Fig 4. PHA production at different temperature values when glucose was used with 0.2% (NH4)2SO4 and at pH7.

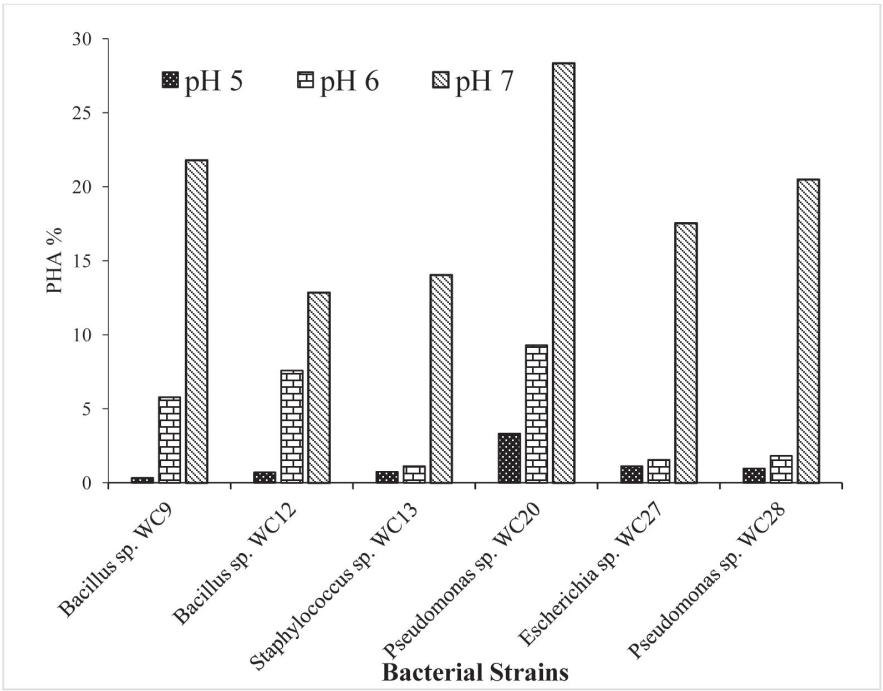

Fig 5. PHA production at different pH values under MSM medium supplemented with glucose and 0.2% (NH4)2SO4 incubated at 37°C.

Strains WC9, WC12, WC13, WC20, WC27 and WC28 were selected on the basis of their high PHA production ability under different conditions of temperature, pH, carbon source and nitrogen concentrations. PHA production from two different carbon sources that are glucose and canola oil was compared. Escherichia sp. WC27 was able to grow very well on glucose and produced DCW of about 1.13 g/L and PHA content of 17.53%, when compared to canola oil it produced 13.79% PHA and 1.06 g/L DCW. PHA production was increased from 15.83% to 20.50% when, canola oil was replaced by glucose in case of *Pseudomonas* sp. WC28. *Staphylococcus* sp. WC13 was found to be least useful in case of canola oil producing only 9.62% PHA. *Bacillus* sp. WC9 was able to produce almost double quantities of PHA, when compared to *Bacillus* sp. WC12. *Pseudomonas* sp. WC20 was found to produce maximum amount of PHA with 28.35%. Glucose gave greater yield as compared to canola oil because the glucose is simple sugar, easily metabolized and compatible with bacterial cells. Oil is a complex carbon source and bacteria have to digest it extracellularly before uptake that is why the lower yield of PHA was observed by canola oil. The PHAs yield from glucose can vary depending upon the strains and growth conditions (Jau *et al*., 2005). Since oils from plants produce more PHA contents by bacteria than simple sugars which result

in 2 fold yield of PHA polymer from oils (Akiyama *et al*., 2003), hence these oils hold a good scope for use as an efficient, cheap carbon source for PHA production in future. PHA accumulation is affected greatly by the concentration of nitrogen present in the medium. The effect of varying nitrogen concentrations on PHA production was checked at three concentration of nitrogen that are 0.2%, 0.4% and 0.6% were used. PHA content of the cell was maximum, when grown at 0.2% (Figure 2). The doubling of salt concentration decreased the PHA contents up to one-fourth in case of WC20 bacterium. While it dropped from 20.5% to only 2.23%, when salt concentration was increased from 0.2% to 0.6%. As limiting concentration is necessary for PHA production, but too high concentration as 0.6% did not give higher yield, due to low C/N ratio in the medium. Casini *et al*. (1997) reported the utilization of linseed oil hydrolysate by *Pseudomonas putida* KT2442in shake flask studies that resulted 20% PHA contents in terms of dry cell weight. Fed-batch studies have been very successful to get very high cell density from bacteria (~141 g/L) using various plant oils as well (Shang *et al*., 2008; Tan *et al*., 1997 and Lee *et al*., 2000).

As pH and temperature are crucial factors for PHA production the effect of varying pH (5, 6, and 7) and temperature (30°C, 37°C and 45°C) was checked. The reason for only checking the effect of pH in acidic range is that minimal salt broth media components get precipitated as the pH is shifted towards basic values. PHA contents decreased from 28.35% to 3.3%, when pH was changed from 7 to 5 in case of WC20 strain. Other strains had severe effect on PHA production as the pH decreased in initial medium. The PHA production was maximum at neutral pH (Figure 4). The PHA production at different temperatures was studied, bacterial strains failed to grow properly at 4°C and 45°C, but accumulated maximum PHA at 37°C. (Figure 5). These strains did not show promising PHA contents, when temperature was either increased to 45°C or decreased to 4°C. Only strain WC9 was able to give highest PHA content at 4°C (7.69%) and 45°C (5.88%). WC28 produced 2.56% PHA at 4°C and 1.45% PHA at 45°C.For strain WC20 PHA content were higher at 45°C (4.87%) and lower at 4°C (3.3%).The suitable conditions for the strains WC9, WC12 and WC20 were found to be 72 hours incubation at 37°C with mineral medium containing 0.2% nitrogen set at pH 7. However, the PHA % content were variable for respected strains.

Conserved PHA synthase gene (*phaC*) fragment of 540bp was amplified in strain WC20 strain using *Pseudomonas phaC* gene specific primers (Fig 6). The phaC polymerase enzyme is the key enzyme in synthesizing the PHA granules from their repeating subunits. Moreover the *phaC* synthase of Pseudomonas sp. is found to be in two classes i.e., phaC1 and phaC2 that consist of about 1700 bp sequences, but they do share almost 75% similarity in their nucleotide sequences. The amplified product of phaC gene was sequenced and submitted to NCBI GENBANK under the accession number KM234124. This sequence showed 98% homology with the PHA synthase of *Pseudomonas aeruginosa* MTB-1.

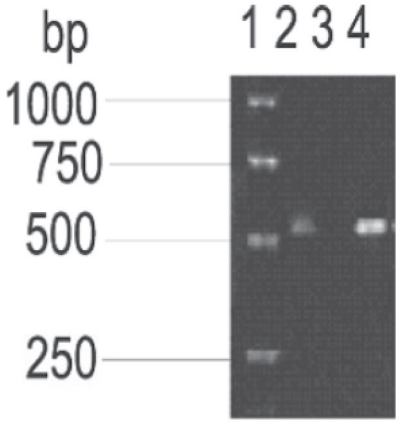

Fig 6. PCR Amplification of the phaC gene of 540 bp. Lane 1 has marker DNA, lane 4 has amplified phaC gene of strain WC20.

The PHA extraction results from the selected strains showed that canola oil can be used as a cost effective, cheap carbon source and can be used on industrial scale for biodegradable plastic production if the engineered strains are used, as this study deals only with local isolates. For using canola oil as carbon source, there is still need for further strain modification and optimization.

ACKNOWLEDGMENT

This research work was supported financially by the International Foundation for Science (IFS) Sweden.

REFERENCES

Akiyama, M., Tsuge, T. & Doi, Y. 2003. Environmental life cycle comparison of polyhydroxyalkanoates produced from renewable carbon resources by bacterial fermentation. Polymer Degradation and Stability **80**:183-194.

Ali, I. & Jamil, N. 2014. Enhanced biosynthesis of poly (3-hydroxybutyrate) from potato starch by Bacillus cereus strain 64-INS in a laboratory scale fermenter. Preparative Biochemistry and Biotechnology **44**: 822-833.

Anderson, A. J. & Dawes, E. A.1990. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiological reviews **54**:450-472.

Ausubel, F. M. 2002. Short protocols in molecular biology. Wiley, New York

Cappuccino, J.G. & Sherman, N.2007. Microbiology: A Laboratory Manual. 7th ed. Pearson Education.

Casini, E., de Rijk, T. C. & de Ward, P.1997. Synthesis of poly(hydroxyalkanoate) from hydrolyzed linseed oil. Journal of Polymer and Environment **5**:153–158.

Chaudhry, W. N., Jamil, N., Ali, I., Ayaz, M. H. & Hasnain, S.2011. Screening for polyhydroxyalkanoate (PHA)-producing bacterial strains and comparison of PHA production from various inexpensive carbon sources. Annals of Microbiology **61**:623-629.

Demarco, S. M.2006. Advances in Polyhydroxyalkanoate Production in Bacteria for Biodegradable Plastic. MMG 445 BasicBiotechnology eJournal **1**:1-4.

Din, M., Fadil, M., Ujang, Z., Van Loosdrecht, M. & Ahmad, M. A.2012. Polyhydroalkanoates (PHAs) Production from Saponified Sunflower Oil in Mixed Cultures under Aerobic Condition. Jurnal Teknologi **48**:1–19.

Eggink, G., Waard, P. D. & Huijberts, G. N.1995. Formation of novel poly (hydroxyalkanoates) from long-chain fatty acids. Canadian Journal of Microbiology **41**:14-21.

Eskin, N. M. & McDonald, B.1991. Canola oil. Nutrition Bulletin **16**:138-146.

Fukui, T. & Doi, Y.1998. Efficient production of polyhydroxyalkanoates from plant oils by Alcaligenes eutrophus and its recombinant strain. Applied Microbiology and Biotechnology **49**:333-336.

Hocking, P. & Marchessault, R.1994. Biopolyesters. In: Griffin GJL (ed) Chemistry and technology of biodegradable polymers. London, UK: Blackie Academic & Professional., pp 48-96.

Jau, M. H., Yew, S. P., Toh, P. S., Chong, A. S., Chu, W. L., Phang, S. M., Najimudin, N. & Sudesh, K. 2005. Biosynthesis and mobilization of poly(3-hydroxybutyrate) [P(3HB)] by Spirulina platensis. International Journal of Biological Macromolecules **36**:144-151.

Kahar, P., Tsuge, T., Taguchi, K. & Doi, Y.2004. High yield production of polyhydroxyalkanoates from soybean oil by Ralstonia eutropha and its recombinant strain. Polymer Degradation and Stability **83**:79-86.

Kim, M., Cho, K-S., Ryu, H. W., Lee, E. G. & Chang, Y. K.2003. Recovery of poly (3-hydroxybutyrate) from high cell density culture of Ralstonia eutropha by direct addition of sodium dodecyl sulfate. Biotechnology Letters **25**:55-59.

Kobayashi, G., Shiotani, T., Shima, Y. & Doi, Y.1994. Biosynthesis and Characterization of Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) from Oils and Fats by Aeromonas sp. OL-338 and Aeromonas sp. FA-440. In: Doi Y and Fukuda K. (eds) Biodegradable Plastics and Polymers. Elsevier, Amsterdam, pp 410-416.

Lee, S. & Choi, J.1999. Polyhydroxyalkanoates: biodegradable polymer. In: Demain A L, Davies J E and Atlas R M. (eds) Manual of Industrial Microbiology and Biotechnology, vol 2. 2 edn., Washington, D.C. American Society of Microbiology, pp 616-627.

Lee, S. Y., Wong, H. H. & Choi, J. I.2000. Production of medium-chain-length polyhydroxyalkanoates by high cell density cultivation of Pseudomonas putida under phosphorus limitation. Biotechnology and Bioengineering **68**:466–470.

Lee, S. H., Oh, D. H., Ahn, W. S., Lee, Y., Choi, Ji. & Lee, S. Y.2000. Production of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) by high-cell-density cultivation of Aeromonas hydrophila. Biotechnology and Bioengineering **67**:240-244.

Lee, S. Y.1996. Bacterial polyhydroxyalkanoates. Biotechnology and Bioengineering **49**:1-14.

Loo, C-Y., Lee, W-H., Tsuge, T., Doi, Y. & Sudesh, K.2005. Biosynthesis and characterization of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) from palm oil products in a Wautersia eutropha mutant. Biotechnology Letters **27**:1405-1410.

Loo, C-Y. & Sudesh, K.2007. Polyhydroxyalkanoates: bio-based microbial plastics and their properties. Malaysian Polymer Journal **2**:31-57.

López-Cuellar, M. R., Alba-Flores, J., Rodríguez, J. N. & Pérez-Guevara, F.2011. Production of polyhydroxyalkanoates (PHAs) with canola oil as carbon source. International Journal of Biological Macromolecules **48**: 74-80.

Ma del Rocio, L-C., Noel, G-R. J. & Fermín, P-G.2007. Production of polyhydroxyalcanoates by Wautersia eutropha using vegetable oils as carbon source. Journal of Biotechnology **131**:S156.

Mifune, J., Nakamura, S. & Fukui, T.2008. Targeted engineering of Cupriavidus necator chromosome for biosynthesis of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) from vegetable oil. Canadian Journal of Chemistry **86**:621-627.

Purushothaman, M., Anderson, R., Narayana, S. & Jayaraman, V.2001. Industrial byproducts as cheaper medium components influencing the production of polyhydroxyalkanoates (PHA)–biodegradable plastics. Bioprocess and Biosystems Engineering **24**:131-136.

Rathinasabapathy, A., Ramsay, B. A., Ramsay, J. A. & Pérez-Guevara, F.2013. A feeding strategy for incorporation of canola derived medium-chain-length monomers into the PHA produced by wild-type Cupriavidusnecator. World Journal of Microbiology and Biotechnology 1-8.

Sambrook, J. & Russell, D. W.2001. Molecular cloning: a laboratory manual. Volume 1–3. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press,

Shang, L., Jiang, M., Yun, Z., Yan, H. Q. & Chang, H. N.2008. Mass production of medium-chain-length poly (3-hydroxyalkanoates) from hydrolyzed corn oil by fed-batch culture of Pseudomonas putida. World Journal of Microbiology and Biotechnology **24**: 2783-2787.

Spiekermann, P., Rehm, B., Kalscheuer, R. & Baumeister, D.1999. A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. Archives for Microbiology **171**:73-80.

Tan, I. K. P., Sudesh, K. & Theanmalar, M.1997 . Saponified palm kernel oil and its major free fatty acids as carbon substrates for the production of polyhydroxyalkanoates in Pseudomonas putida PGA1. Applied Microbiology and Biotechnology **47**:207–211.

Tsuge, T.2002. Metabolic improvements and use of inexpensive carbon sources in microbial production of polyhydroxyalkanoates. Journal of Bioscience and Bioengineering **94**:579-584.

Submitted **:** 04/06/2014.

Revised **:** 28/10/2014

Accepted **:** 29/10/2014

إنتاج البولي هيدوكسي الكانويت من زيت الكانولا باستخدام بكتريا معزولة من صناعات عجينة الورق

طياب يوناس ، *افتخار علي ، نازيا جميل

قسم الأحياء الدقيقة والوراثة الجزيئية، جامعة البنجاب في لاهور – 54590، باكستان

خلاصة

البولي هيدوكسي الكانويت (PAHs) التي تنتجها البكتيريا هي متعدد البوليستر العضوي (biopolyesters) التي يتم إنتاجها في ظل ظروف الغير مواتية. تم أخذ عينات بيئية من عجينة والنفايات العضوية لصناعة الورق لعزل سلالات بكتيرية قادرة على إنتاج PHA. تم العثور على ست سلالات تنتمي إلى Pseudomonas, Bacillus, Staphylococcus and Escherichia قادرة على إنتاج PHA بوفرة. وقد تم تحسين إنتاج PHA من السلالات المختلفة استخدام $(NH_4)_2SO_4$ بتركيزات مختلفة (0.2 ٪، 0.4 ٪ و 0.6 ٪) ودرجات حموضة (5 و 6 و 7) ودرجات حرارة (4°C، 37°C، 45°C). أنتجت سلالة WC9 محتوى PHA 19 ٪ من زيت الكانولا و 21 ٪ مع الجلوكوز بينما أنتجت سلالة WC20 28 ٪ و 16 ٪ محتوى PHA من الجلوكوز وزيت الكانولا على التوالي عند 37°C درجة مئوية تحت تركيز 0.2 ٪ من $(NH_4)_2SO_4$. تم تحديد انتماء WC20 إلى Pseudomonas spp. وفقاً للتسلسل الجيني للرنا الريبوسومي لـ S16. تم أيضاً إجراء التفاعل المسلسل لتضخيم الدنا من جين phaC في سلالة WC20.