Biosynthesis Of PHA By *Fusarium moniliforme*

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Abstract:
Fusarium moniliforme isolated from infected roots of rice can accumulate polyhydroxyalkanoates (PHA) upto 45% of its cell biomass.This microorganism can cause bakane disease in rice which results in premature growth of plant ultimately yield of rice become decreases. Production of PHA commercially and at comparable cost has been the main focus in this area.

Introduction:

Demand of plastic goes on increasing day by day. But the dependence on conventional plastic resulted in waste accumulation and green house gas emissions. Due to this reason recent technologies are directed towards the development of biogreen material that has negligible effect on environment. Now days, major interest is in the development of polyhydroxyalkanoates (PHA) by using some bioproduct. As it possess similar properties to conventional plastic and its biodegradability. PHA is produced by a number of microorganisms in the form of PHB (Polyhydroxybutyrate) .But it is not very useful because of its brittle nature. This limitation can be overcome by converting PHB into its copolymer with other PHA by using a varying combination of carbon substrates for growth.
Fusarium moniliforme accumulate upto45% PHA of the cell biomass and the copolymers are synthesized by using propionate or valerate along with sucrose as carbon sources. In the present work we describe the synthesis of PHA by *Fusarium moniliforme* in sucrose medium.

"Fig.1-*Fusarium moniliforme*"

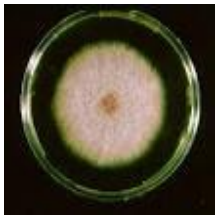
Materials and methods:

Extraction of microorganism and its growth:

Fusarium moniliforme a fungus found in infected roots of rice plant. This causes bakane disease in plants resulting in reduced yield.

"Fig:2- The disease: BAKANAE and FOOT ROT"

For its culture infected portion of the root is cut and inoculate in the petridish in the form of strands with the help of loop having potato dextrose media (PDA). Labelled the petri dish and placed in BOD at 25^0C temperature for 8 days. After 8 days colonies come.



"Fig.3- Cultured *Fusarium moniliforme*"

Preparation of potato dextrose media (PDA):

Boiled 300g of potatoes in 1L of water for extraction of starch and after that add 2g glucose as carbon source and 0.2g agar-agar for solidification of media.

PHA production in Fusiform moniliforme:

PHA production was carried out in 500ml Erlenmeyer flask having 100ml liquid media contained different C:N ratios such as $(NH_4)_2SO_4FeSO_4$1g/l with different amount of sucrose like 10g, 20g, 30g or 20g/l sucrose with 2g, 4g, & 6g of $(NH_4)_2SO_4FeSO_4$. Incubations were carried out for 72 hours at 250rpm and 30^0C temperature.

Biomass estimation:

After centrifugation of culture media cell biomass was collected and washing of pellet was carried out with distilled water. It was dried in airflow drier at temperature 70^0C.

Polysaccharides estimation:

To precipitate out polysaccharides isopropanol was used. Polysaccharides were dried at 70^0C upto a constant weight.

PHA estimation:

For the extraction of PHA from biomass solvent extraction was used. The solvent employed for this was chloroform. As chloroform extraction is a very simple and effective method to separate PHA granules from biomass. PHA obtained by this method was highly purified and without any degradation of PHA molecule.

PHA production with varying concentration of carbon and nitrogen:

PHAs are mainly synthesized in the presence of excess of carbon with limiting amount of other nutrients like N, O, S, P etc. Production of PHA increases with increasing concentration of substrate that provide carbon and decreases with increasing concentration of substrate that provide nitrogen. Results are shown in the following table:

Table1- Production of PHA with varying concentration of carbon and nitrogen

Sucrose (g/l)	Ammonium ferrous sulphate (g/l)	Biomass (g dry wt/l)	PHA (g/l)
10	1	3	0.85
20	1	4	1
30	1	4.5	1.5
20	2	4	1
20	4	2.5	0.8
20	6	1	0.5

Result and discussion:

PHA synthesized by Fusarium moniliforme is mainly polyhydroxybutyrate(PHB). As its NMR spectrum has given the following signals:
δ 1.1ppm (doublet for methyl group)
δ 2.5ppm (doublet of quadruplet for methylene group)
δ 5.28ppm (multiplet for methyne group)

PHB synthesized by *Fusarium moniliforme* in its homopolymer form is relatively stiff and brittle. PHB is 100% stereospecific with asymmetric carbon atoms having D(-) configuration, that's why highly crystalline. Its melting point is 175^0C which is slightly lower than its degrading temperaturei.e.185^0C.Yet PHB has several useful properties such as moisture resistance, water insolubility and optical purity. PHB also shows good oxygen impermeability. The various properties of PHB thus formed is shown in following table:

Table 2 shows the physical properties of PHB

Property	PHB
Melting point (^{0}C)	175
Glass-transition temperature (^{0}C)	15
Crytalline (%)	80
Young's modulus	3.5

Tensile strength (MPa)	40
Elongation to break(%)	6
Impact strength (v/m)	50

Biodegradability of PHA synthesized:

PHB synthesized by *F.moniliforme* degrades in microbially active environment.Microorganisms colonize on the polymer surface and secrete enzyme that convert PHB into hydroxybutyrate (HB). These monomer units are then utilized by cell as carbon source.

Degradability rate dependa on various factors like surface area, pH, temperature, pressure of other nutrients and moisture etc. In aerobic environment carbon dioxide and water are the end products of PHB degradation.

Future outlook:

PHAs production has drawn major attention due to its biocompatibility and biodegradability with conventional plastic. But the production of PHAs through this method is very costly which a major drawback is. Consequently, researches are going on to produce PHA at lower cost by developing some mutant strains and using some alternative carbon sources.

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References:

[1] . Forsyth WGC, Hayward AC, Roberts JB. "Occurrence of poly-β-hydroxybutyric acid in aerobic Gram-negative bacteria." *Nature*, **1958**, 800-801.

[2] Findlay RH, White DC. "Polymeric beta-hydroxyalkanoates from environmental samples and *Bacillus megaterium". Applied and Environmental Microbiology*, **1983**, 71-78.

[3] Williamson DH, Wilkinson JF. "The isolation and estimation of the poly-β-hydroxy-butyrate inclusions of *Bacillus* species".*Journal of General Microbiology*, **1958**, 198-209.

[4] Jau MH, Yew SP, Toh PSY, Chong ASC, Chu WL, Phang SM, Najimudin N, Sudesh K. "Biosynthesis and mobilization of poly(3-hydroxybutyrate) [P(3HB)] by *Spirulina platensis". International Journal of Biological Macromolecules*, **2005**, 144-151.

[5] Doi Y. "*Microbial polyesters"*. New York, VCH. **1990**

[6] Hassan MA, Shirai Y, Kusubayashi N, Karim MIA, Nakanishi K, Hashimoto K. "Effect of organic acid profiles during anaerobic treatment of palm oil mill effluent on the production of polyhydroxyalkanoates by *Rhodobacter sphaeroides". Journal of Fermentation and Bioengineering*, **1996**, 151-156.

[7] Hassan MA, Shirai Y, Kusubayashi N, Karim MIA, Nakanishi K, Hashimoto K. "The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by *Rhodobacter sphaeroides". Journal of Fermentation and Bioengineering*, **1997**, 485-488.

[8] Hashimoto K, Tsuboi H, Iwasaki S, Shirai Y. "Effect of pH on the production of poly-β-hydroxybutyrate by photosynthetic bacterium, *Rhodospirillum rubrum". Journal of Chemical Engineering of Japan*, **1993**, 56-58.

[9] .Hassan MA, Shirai Y, Kubota A, Abdul Karim MI, Nakanishi K, Hashimoto K. "Effect of oligosaccharides on glucose consumption by *Rhodobacter sphaeroides* in polyhydroxyalkanoate production from enzymatically treated crude sago starch".
Journal of Fermentation and Bioengineering, **1998**, 57-61

[10] Jensen TE, Sicko LM. "Fine structure of poly-β-hydroxybutyric acid granules in a blue-green alga, *Chlorogloea fritschii". Journal of Bacteriology*, **1971**, 683-686.

[11] Dawes EA, Senior PJ. "The role and regulation of energy reserve polymers in microorganisms". *Advance in Microbial Physiology*, **1973**, 135-266.

[12] Macrae RM, Wilkinson JF. "The influence of culture conditions on poly-β-hydroxybutyrate synthesis in *Bacillus megaterium".Proceedings of the Royal Physical Society of Edinburgh*, **1958**, 73-78.

[13] Macrae RM, Wilkinson JF. "Poly-beta-hyroxybutyrate metabolism in washed suspensions of *Bacillus cereus* and *Bacillus megaterium". Journal of General Microbiology*, **1958**, 210-222.

[14] Steinbüchel A, Valentin HE. "Diversity of bacterial polyhydroxyalkanoic acids". FEMS *Microbiology Letters*, **1995**, 219-228.

[15] Sudesh K, Abe H, Doi Y. "Synthesis, structure and properties of polyhydroxyalkanoates: Biological polyesters". *Progress in Polymer Science*, **2000**, 1503-1555.

[16] Wallen LL, Rohwedder WK. "Poly-β-hydroxyalkanoate from activated sludge". *Environmental Science and Technology*, **1974**, 576-579.

[17] De Smet MJ, Eggink G, Witholt B, Kingma J, Wynberg H. "Characterization of intracellular inclusions formed by *Pseudomonas oleovorans* during growth on octane". *Journal of Bacteriology*, **1983**, 870-878.

[18] Witholt B, Kessler B. "Perspectives of medium chain length poly (hydroxyalkanoates), a versatile set of bacterial bioplastics". *Current Opinion in Biotechnology*, **1999**, 279-285.

[19] Kunioka M, Kawaguchi Y, Doi Y. "Production of biodegradable copolyesters of 3-hydroxybutyrate and 4-hydroxybutyrate by *Alcaligenes eutrophus". Applied Microbiology and Biotechnology*, **1989**, 569-573.

[20] Saito Y, Nakamura S, Hiramitsu M, Doi Y. "Microbial synthesis and properties of poly(3-hydroxybutyrate-co-4-hydroxybutyrate)". *Polymer International*, 1996, 169-174.
[21] Kellerhals MB, Kessler B, Witholt B, Tchouboukov A, Brandl H. "Renewable long-chain fatty acids for production of biodegradable medium-chain-length polyhydroxyalkanoates (mcl-PHAs) at laboratory and pilot plant scales". *Macromolecules*, **2000**, 4690-4698.
[22] Lee SY, Wong HH, Choi JI, Lee SH, Lee SC, Han CS. "Production of medium-chain-length polyhydroxyalkanoates by high cell-density cultivation *Pseudomonas putida* under phosphorus limitation". *Biotechnology and Bioengineering*, **2000**, 466-470.
[23] Yu P, Chua H, Huang AL, Ho KP. "Conversion of industrial food wastes by *Alcaligenes latus* into polyhydroxyalkanoates". *Applied Biochemistry and Biotechnology*, **1999**, 445-454.
[24] Kshama Lakshman & Tumkur Ramachandriah Shamala : Department of food and microbiology, Central food technological research institute , Mysore 570013, India, "Enhanced biosynthesis of PHA in a mutant strain of Rhizobium meliloti", **2002.**
[25] Loo CY, Lee WH, Tsuge T, Doi Y, Sudesh K. "Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) from palm oil products in a *Wautersia eutropha* mutant". *Biotechnology Letters*, **2005**, 1405-]. 1410
[26] Thakor N, Trivedi U, Patel KC. "Biosynthesis of medium chain length poly (3-hydroxyalkanoates) (mcl-PHAs) by *Comamonas testosteroni* during cultivation on vegetable oils". *Bioresource Technology*, **2005**, 1843-1850.
[27] Yu J, Stahl H. "Microbial utilization and biopolyester synthesis of bagasse hydrolysates". *Bioresource Technology*, **2008**, 8042-8048.
[28] Lee WH, Loo CY, Nomura CT, Sudesh K. "Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3-hydroxyvalerate precursors". *Bioresource Technology*, **2008**, 6844-6851.
[29] Cavalheiro JMBT, de Almeida MCMD, Grandfils C, da Fonseca MMR. "Poly (3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol". *Process Biochemistry*, **2009**, 509-515.
[30]Chee JY, Tan Y, Samian MR, Sudesh K. "Isolation and characterization of a Burkholderia sp. USM (JCM15050) capable of producing polyhydroxyalkanoate (PHA) from triglycerides, fatty acids and glycerols". *Journal of Polymer and the Environment*, **2010**, in press, doi: 10.1007/s10924-010-0204-1.
[31]Fonseca GG, Antonio RV. "Polyhydroxyalkanoates production by recombinant *Escherichia coli* harboring the structural genes of the polyhydroxyalkanoate synthases of *Ralstonia eutropha* and *Pseudomonas aeruginosa* using low cost substrate". *Journal of Applied Sciences*, **2006**, 1745-1750.
[32] Marsudi S, Unno H, Hori K. "Palm oil utilization for the simultaneous production of polyhydroxyalkanoates and rhamnolipids by *Pseudomonas aeruginosa"*. *Applied Microbiology and Biotechnology*, **2008**, 955-961.
[33] Fernadez D, Rodrauez E, Bassas M, Viñas M, Solonas AM, Llorens J, Marques AM, Manresa A. "Agro-industrial oily wastes as substrates for PHA production by the new strain *Pseudomonas aeruginosa* NCIB 40045: Effect of culture conditions". *Biochemical Engineering*, **2005**, 159-167.
[34]Simon-Colin C, Raguenes G, Crassous P, Moppert X, Guezennec J. A novel "mcl-PHA produced on coprah oil by *Pseudomonas guezennei biovar. tikehau*, isolated from a "kopara" mat of French Polynesia". *International Journal of Biological Macromolecules*, **2008**, 176-181.
[35] Pantazaki AA, Papaneophytou CP, Pritsa AG, Liakopoulou-Kyriakides M, Kyriakidis DA. "Production of polyhydroxyalkanoates from whey by *Thermus thermophilus* HB8". *Process Biochemistry*, **2009**, 847-853.
[36] Omar S, Rayes A, Eqaab A, Vo β I, Steinb · hel A. "Optimization of cell growth and poly (3-hydroxybutyrate) accumulation on date syrup by a *Bacillus megaterium* strain". *Biotechnology Letters*, **2001,** 1119-1123.
[37]. "Shake flask studies and Fed batch fermented studies in *Alcaligenes latus* & *Alcaligenes eutrophus"*. by middle east technical university, Department of biological sciences, Biotechnology research unit 06531 Ankara-Turkey
[38]. Ojumu, T.V., Yu, J. and Soloman, B.O.: "Biodegradation of polymer engineering material" department institute, P.M.B 611, Akure, Nigeria Hawaii Natural energy institute, university of Hawaii, Honolulu HI 96822, USA Department of chemical engineering, Obafemi Awolowo university, Nigeria, **2003.**
[39]. Robert G Kranz, Karen K. Gabbert, Terry A. locke and Michael T. madigan (1997): "PHA production in *Rhodobacter capsulatus"*. Department of biology, Washington University, St. louis Missouri 63130& Department of microbiology, Southern Illinois university Carbondale Illnois 62901-6508
[40] Noda I. "Process for recovering polyhydroxyalkanoates using air classification". United States Patent, **1998**, 849,854.