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# Utilization of agro-industrial waste for polyhydroxyalkonates and exopolysaccharide production from *Bacillus* sp. 2-11

S. Jenny angel and R. Dhandapani

Department of Microbiology, Periyar University, Salem-636011.

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#### ABSTRACT

Currently many bacteria have been exploited for production of biopolymers for various industrial applications. The need for biopolymers has been increasing constantly along with increase in human needs. In the present study two important biopolymers- edo polymer (PHA) and exopolymer (EPS) was extracted and characterized from the *Bacillus* sp. 2-11. The ability of *Bacillus* sp.2-11 to produce PHA was identified by simple and rapid Sudan black B staining method. Similarly the presence of exopolysaccharides was identified by Alcian Blue staining method. The ability of the organism to utilize cheap substrates as carbon source for polyhydroxyalkonates (PHA) and exopolysaccharide (EPS) production was determined. Rice bran, wheat bran, molasses and sago liquid wastes were supplemented as carbon substrate in nitrogen free production medium. The most suitable carbon source was sago liquid waste as it showed 42.15% and 50.80% PHA and EPS yield respectively. It was also observed that PHA yield was growth associated whereas EPS production was observed at the late exponential phase. Both FT-IR and GC-MS analysis confirmed the presence of PHA and EPS in *Bacillus* sp.2-11.

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#### Introduction

In recent years there is a continuous search for different biopolymers as they have wide range of applications in different sectors like packaging, building materials, consumer products and many more. The currently available biopolymers in market are polyglycolic acid (PGA), polyhydroxyalkonate (PHA) and polylactic acid (PLA). Others polymers such as exopolysaccharide, inorganic polyanhydrides, polyesters, and polyamides are also coming in the forefront [1, 2, 3]. The first biodegradable polymer identified was polyhydroxyalkonates, which was isolated from bacteria such as Alcaligenes latus, Ralstonia eutropha, Azotobacter beijerinckii etc.,. The main role of this intracellular polymer is that it can act as carbon and energy reserve material which was produced under nitrogen, phosphate, or oxygen limited conditions pha-6 [4, 5]. Under stress conditions energy from this reserve material is utilized for its metabolic activities. Depending upon certain growth conditions PHA is made of either short-chain length or medium-chain length or both forms.

Another important polymer other than intracellular polymers is extracellular polymers. Certain bacteria produce extracellular polymers known as exopolysaccharides (EPS) which are released outside the cell under stressed condition. These are mainly produced as a defense material against high salinity and toxic dessication, environment. Exopolysaccharides are high-molecular-weight polymers which are mainly composed of sugar residues. Both these polymers are identified as the potential biopolymers that could replace synthetic polymers due to its biodegradability and biocompatibility property. Numerous microorganisms such as Azotobacter chroococcum strain 6B, Azotobacter beijerinckii WDN-01, Ralstonia eutropha and Bacillus sp. can produce both PHAs and EPS [6, 7, 8].

The production of biodegradable polymers on a large scale is limited because of expensive raw materials, substrate composition, fermentation conditions and high recovery cost. According to Halami 2008 [9], about 50% of production cost is utilized for the cost of carbon source. Hence the ideal way to reduce the polymer production cost is to replace expensive carbon source with cheap raw materials. Currently a lot of research is going on using agro- industrial wastes such as palm oil, whey, corn and cassava residues, olive oil mill effluents, sugarcane molasses and paper mill waste as the carbon source rather than refined organic substrates [10]. An added advantage in utilizing waste raw materials as carbon source is recycling of agro-industrial wastes. Similarly the recycling of waste generated from biopolymer production is also economical due to its biodegradablility [11, 12]. In the current study wheat bran, rice bran, molasses and sago liquid waste are used for production of both PHA and EPS from the same organism.

# Materials and method

# Source of bacterial strains and maintenance

The wild type bacterium *Bacillus* sp. 2-11 was procured from Fermentation laboratory of Periyar University, Salem in Tamil Nadu. The isolate was stored on Nutrient Agar medium (Himedia) slopes at 4° C and stock cultures were maintained at -20 °C in 0.5% (v/v) glycerol broth.

# Preliminary screening of PHA and EPS production

A rapid PHA detection method using Sudan Black B was followed as described by Yu-Hong Wei *et al.*, 2011 [13]. A loopful of Bacillus sp. 2-11 was streaked on Nutrient agar supplemented with 1 % glucose and incubated at 37C for 24h. After incubation the plate was flooded with Sudan Black B and kept undistributed for 30 minutes.

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The excess stain was removed and then rinsed with 96% ethanol [14]. Presence of acidic exopolysaccharide in Bacillus sp. 2-11 was determined by alcian blue staining. The 5-dayold plate was stained with alcian blue (1.0% (w/v) solution in 95% (v/v) ethanol) followed by carbol-fuchsin as a counterstain and observed under a phase-contrast microscope. **Pre-preparation of solid substrate as carbon source** 

All the waste materials used in this study like rice bran, wheat bran, molasses and Sago liquid waste was initially processed before using as carbon substrate for production. Wheat bran and rice bran were purchased from the local market, Salem district of Tamil Nadu, India. It was then sun dried for 6-8 days. Sugar molasses was procured from Sugar refinery factory, Perambalur district of Tamil Nadu, India. The raw molasses was clarified in order to eliminate interference from solid residues as described by Roberta Fusconi-a et al.2010 [15]. The raw molasses was diluted with distilled water (1:1) containing a NaH<sub>2</sub>PO<sub>4</sub> (final concentration of 1.5 g/L), autoclaved at 120°C for 30 min and allowed to settle for 24 h. The liquid phase (clarified molasses) was then separated and diluted in distilled water. The pH was adjusted to 7.0  $\pm$ 0.2 and the media were autoclaved at 121°C for 15 min. The sago liquid waste was obtained from Sago manufacturing unit, Salem district of Tamil Nadu, India. The sago liquid waste was filtered with cheese cloth and autoclaved for 121°C for 20 min [16].

## Utilization of waste as the sole carbon source

All the four substrates were added as the carbon source at the concentration of 2% to nitrogen free production medium comprising of KH<sub>2</sub>PO<sub>4</sub> 13.3(g/L); MgSO<sub>4</sub> 1.3(g/L); Citric acid 1.7(g/L); trace element solution 10mL/L (FeSO<sub>4</sub>.7H<sub>2</sub>O 10(g/L); ZnSO<sub>4</sub>.7H<sub>2</sub>O 2.25(g/L); CuSO<sub>4</sub>.5H<sub>2</sub>O 1(g/L); MnSO<sub>4</sub>.5H<sub>2</sub>O 0.5 (g/L); CaCl<sub>2</sub>.2H<sub>2</sub>O 2 (g/L); Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.10H<sub>2</sub>O 0.23(g/L); (NH<sub>4</sub>)<sub>6</sub>MO<sub>7</sub>O<sub>2</sub>4 0.1(g/L); HCl (10mL); pH of the medium was adjusted to 7.0 [17]. To the production medium 1% of 24h grown culture of Bacillus sp.2-11 was added and incubated at 37°C for 48-72 hours at 150 rpm in an orbital shaker.

#### **Determination of bio mass:**

To determine the bio mass content (DCW: dry cell weight) the cells were harvested by centrifugation 13,000 rpm for 15 minutes at  $4^{\circ}$ C. The pellets were re-suspended in distilled water, washed and centrifuged at 13,000 rpm. Pellets were collected aseptically and the dry weight of the pellet was measured.

## **Extraction and characterization of PHA**

From the production medium after 72 h of incubation culture broth was withdrawn and centrifuged at 8000 rpm for 15 min. To the pellet 10 mL of sodium hypochlorite was added and incubated at  $50^{\circ}$ C for 1 h for lyses of cells. It was then centrifuged at 12000 rpm for 30 min and the pellet was washed with distilled water [18,19]. Cell lipids and other molecules were extracted from the lysed cells by addition of 5 mL of 96% ethanol and acetone. Later the pellet was dissolved in 10 mL chloroform and incubated overnight at  $50^{\circ}$ C and allowed to dry at room temperature.

The cellular PHA content was determined by gas chromatography-mass spectrometry which was carried out at The South India Textile Research Association, Coimbatore. The analysis was performed using a Trace GC-MS equipped with a Thermo GC – Trace ultra Ver: 5.0, Thermo MS DSQ II) column (DB 5 - MS Capillary standard non - polar column).The polymer was dissolved in 2 mL of chloroform and 2 mL of methanol and acidified with 3% (v/v)  $H_2So_4and$ heated at 100°C for 3.5 h for depolymerization and methanolysis of polyesters. The sample (1µl) in methanol was injected with helium (1mL min–1) as the carrier gas. The injector temperature was maintained at 70°C and the column temperature was increased from 260°C and held at the final temperature for 6 minutes. For quantitative analysis of PHA, cell culture was grown as described earlier and cell pellet was dried to estimate the dry cell weight (DCW) in units of g/L [20][21]. This was calculated to determine the cellular weight and accumulation other than PHAs. The percentage of intracellular PHA accumulation is estimated as the percentage composition of PHA present in the dry cell weight:

% PHA content recovered= Dry weight of extracted PHA (g/L)/Dry cell weight  $(DCW)(g/L) \ge 100$  The PHA content was defined as the ratio of PHA to cell dry weight and expressed in percentage.

# Extraction and characterization of exopolysaccharide

For EPS quantification liquid media was diluted to reduce the viscosity, the cells were harvested by centrifugation at 7000 rpm for 20 min. To the crude supernatant 3 volumes of ice cold ethanol was added and incubated overnight at 4°C [22]. After incubation, it was again centrifuged at 7000 rpm for 20 min. The pellet was again re-extracted with ethanol for effective extraction. The obtained pellet was dialyzed (cellulose dialysis tube, molecular weight cutoff 12,000-16,000) against distilled water for 48 h (water was changed three times each day) to eliminate residual sugars from the culture medium. Total carbohydrates were measured by the phenol-sulfuric acid method [23] in order to estimate the amounts of total, free and capsular EPS produced. The amount of EPS was expressed as g L-1 using glucose as standard. The bacterial exopolysaccharides was characterized using a Fourier transform infrared spectrophotometer [24, 25]. One part of extract was mixed with ninety nine parts of dried potassium bromide (KBr) separately and then compressed to prepare a salt disc of 3mm diameter. These discs were subjected to IR- spectra measurement in the frequency range of 400 and 4000 cm-1

#### **Result and discussion**

Numerous bacteria have been identified as potent PHA and EPS producers, but a combined study on the production of endo and exopolymers are limited. In the present work, Bacillus sp.2-11 was identified to produce both PHA – endopolymer and EPS – exopolymer under nitrogen limitation. Rapid screening of PHA was carried out by Sudan Black B staining as it a cheap and simple method. On addition of Sudan Black stain the colony of Bacillus sp. 2-11 was stained blue black indicating the presence of PHA. Sudan Black B staining is a fat-soluble dye which is mainly used for staining of neutral triglycerides, lipids and lipoproteins [26]. Alcian Blue stain as it is specific to acidic polysaccharides [27] was used for the detection of polysaccharides. The presence of polysaccharides was observed as the bacillus was stainable with Alcian Blue.

One of the major limitations to the commercial production of biodegradable polymers is the lack of inexpensive feedstock that can compete with conventional thermal plastics. Henceforth the selection of suitable carbon substrate is a critical factor that determines the overall performance of the bacterial fermentation and the cost of the final product [36]. Many reports are available regarding the application of cheap substrates such as edible oil, cassava waste, oil waste and cardboard pulp waste [28] as carbon source. In the present study, the ability of the organism to utilize cheap substrates such as Rice bran, wheat bran, molasses and sago liquid waste as carbon source were assessed. These raw materials were selected based on its chemical composition and availability. Wheat and rice bran are almost equal in their chemical composition containing starch content of 34.0%, fibre 9.2%, crude protein 1.4%, free reducing sugar 1.9 % and starch content 33.5%, fibre 8.6%, Protein 1.7%, free reducing sugar 1.3 % respectively [25,26]. Sugarcane molasses contains high amount of carbon 54% and trace amount of vitamins such as thiamine, riboflavin, pyridoxine and niacinamide [27, 28]. Sago mills generate both solid and liquid waste during sago preparation from tapioca roots. This sago liquid waste also contains high starch content of 58.0% and lower amount of protein 23% and 11% of lipid [29, 30]. The pre-processed waste materials were added to the production medium as a sole source of carbon and nitrogen source was excluded to create nitrogen limited condition. The bacterial growth was assessed by estimating the dry cell weight (DCW) and PHA and EPS yield in percentage was also determined simultaneously (Fig 1).



As Bacillus is known for its metabolic versatility, it was able utilize all the four waste material as carbon source in nitrogen limited condition. Bacillus sp.2-11 produced maximum PHA and EPS yield of 42.15% and 50.80% respectively using sago liquid waste as carbon source. As maximum yield was observed with Sago liquid waste, it was considered as the optimum carbon source for both PHA and EPS production. This will help in utilizing the liquid waste from sago mill in a better way and also in reducing the polymer production cost.

The relationship of the growth rate with the polymer production was also studied. The time course of growth with PHA accumulation revealed that maximum PHA accumulation was attained at the log phase of  $2.93 \pm g/L$  and biomass dry weight (DCW) of 6.95g/L and reduces as the growth reduces. This indicates that PHA accumulation is growth associated as reported by Roland Durner *et al.*, 2000 [31]. Certain reports show that maximum PHA yield was reported in the late exponential phase [32]. The growth study with EPS shows that maximum EPS yield was observed in the late exponential phase of  $3.17 \pm 0.12$  g/L and biomass of 6.24 g/L (Fig2) as reported by Sandrine *et al.*, 2000 [33].



The EPS obtained by solvent extraction method from the isolate *Bacillus sp.* 2-11 was subjected to FT-IR analysis in the range 4000–400 cm. FTIR spectrum indicates the major functional groups and chemical bonds present in EPS. The IR spectra of EPS produced by the *Bacillus* 2-11 spp revealed two major peaks and few minor peaks (Fig 3). A major peak was observed at 3439.34 cm<sup>-1</sup> which is distinctive of OH band and indicates the presence of carboxylic acids. The adsorption peak at 1675.90cm<sup>-1</sup> shows the presence of carbonyl (C=O) stretching groups [34] that reveals the presence of polysaccharide.



Fig 3. The FT-IR spectrum of the EPS extracted from the isolate Bacillus sp. 2-11.

The GC-MS analysis of the extracted PHA showed a predominant peak with hexanal covering a maximum area of 62.73% and molecular weight 100 with retention time of 3.04 (Fig-4). A second major peak of 4-Methyl-3-(1-phenyl-3-methylenebutyloxy) pentanol molecular weight 262 covering an area of 18.13 with retention time of 3.31 was obtained and other minor components were also observed.



Fig 4. GC-MS analysis of PHA extracted from the isolate Bacillus 2-11 sp.

Many bacteria can produce PHA and EPS polymers both intracellularly and extracellularly using carbon substrate as energy source under nitrogen limited condition. Thus it is essential to understand the relation between endo-and exopolymers by the same microorganism. Sago liquid waste is the cheapest waste compared to other waste used in this study and utilization of agro-industrial waste will also favours in reducing the overall production cost.

# References

1. Thi Hang Pham,1 Jeremy S. Webb2 and Bernd H. A. Rehm. The role of polyhydroxyalkanoate biosynthesis by Pseudomonas aeruginosa in rhamnolipid and alginate production as well as stress tolerance and biofilm formation. *Microbiology* (2004), 150, 3405–3413

2. Uchechukwu U. Nwodo\*, Ezekiel Green and Anthony I. Okoh Bacterial Exopolysaccharides: Functionality and Prospects *International Journal of Molecular Sciences*. 2012, *13*, 14002-14015; doi:10.3390/ijms131114002

3. Baljeet Singh Saharan,1,2 Anita Grewal,3 and Pardeep Kumarl Biotechnological Production of Polyhydroxyalkanoates: A Review on Trends and Latest Developments *Chinese Journal of Biology* Volume 2014, Article ID 802984, 18 pages

4. Hazer B, Steinbuchel A. Increased diversification of polyhydroxyalkanoates by modification reactions for industrial and medical applications. *Applied Microbiology and Biotechnology*. 2007; 74: 1-12.

5. J. C. Quagliano and S. S. Miyazaki, "Biosynthesis of poly- $\beta$ -hydroxybutyrate and exopolysaccharides on *Azotobacter chroococcum* strain 6B utilizing simple and complex carbonsources," *Applied Biochemistry and Biotechnology A*, vol. 82, no.3, pp. 199–208, 1999.

6. Prashant Shukla\*, Nimesh Patel, Raja Mohan Rao, Jini Shukla, Seema Verma, Shobha Jha and Shobha Gawri Isolation and characterization of polyhydroxyalkanoate and exopolysaccharide producing Bacillus sp. PS1 isolated from sugarcanefield in Bhilai, India J Microbial Biochem Technol 2011, 3:2

7. Soma Pal, A. Manna and A. K. Paul, Production of [poly (B-hydroxybutyric acid ) and exopolysaccharide by Azotobacter beijerinckii WDN-01 *World Journal of Microbiology and Biotechnology* 15: 11-16,1999

8. Jin Wang, Han-Qing YU, Biosynthesis of polyhydroxybutyrate (PHB) and extracellular polymeric substances (EPS) by Ralstonia eutropha ATCC 17699 in batch cultures *Applied Microbiology and Biotechnology* 2007 75: 871-878

9. Halami PM. Production of polyhydroxyalkanoate from starch by the native isolate *Bacillus cereus* CFR06. *World Journal of Microbiology and Biotechnology*. 2008; 24: 805-812.

10. Artun Sukan, Ipsita Roy, Tajalli Keshavarz Agro-Industrial Waste Materials as

Substrates for the Production of Poly(3-Hydroxybutyric Acid) Journal of Biomaterials and Nanobiotechnology, 2014, 5, 229-240

11. Jyotsana Dalala, Priyangshu M. Sarmab, Meeta Lavaniab, Ajoy K. Mandalb, Banwari Lal b,\* Evaluation of bacterial strains isolated from oil-contaminated soil for production of polyhydroxyalkanoic acids (PHA). *Pedobiologia*. 54 (2010) 25-30

12. Zazali Alias, Irene K.P. Tan \* Isolation of palm oilutilising, polyhydroxyalkanoate (PHA)-producing bacteria by an enrichment technique. *Bioresource Technology*. 96 (2005) 1229–1234

13. Yu-Hong Wei,<sup>1,\*</sup> Wei-Chuan Chen,<sup>1</sup> Chin-Kuei Huang,<sup>1</sup> Ho-Shing Wu,<sup>2</sup> Yi-Ming Sun,<sup>1,2</sup> Chi-Wei

Lo,<sup>2</sup> and Om-Murugan Janarthanan Screening and Evaluation of Polyhydroxybutyrate-Producing Strains from Indigenous Isolate *Cupriavidus taiwanensis* Strains. *International Journal* of Molecular Sciences. 2011; 12(1): 252–265.

14.MADDALENA DEL GALLO, t MAHIMA NEGI, AND CARLOS A. NEYRA\* Calcofluor- and Lectin-Binding Exocellular Polysaccharides of Azospirillum brasilense and Azospirillum lipoferumt *Journal of Bacteriology*, June 1989, p. 3504-3510 Vol. 171, No. 6

15. Roberta Fusconi, Mirna Januaria Leal Godinho, Nelma Regina, Segnini BossolanCulture and exopolysaccharide production from sugarcane molasses by Gordonia polyisoprenivorans CCT 7137, isolated from contaminated groundwater in *Brazil World Journal of Microbiology and Biotechnology*. 24(7):937-943 · July 2008

16.R. C Ray and S. N. Moorthy Exopolysaccharide (pullulan) production from cassava starch residue by Aureobasidium pullulans strain MTCC 1991. *Journal of scientific and Industrial Research* Vol 66 2007 252-255

17. EmanZakariaGomaaProductionofpolyhydroxyalkanoates(PHAs)byBacillussubtilisand Escherichia coligrown on canemolasses fortifiedwithethanolBrazilianArchivesofBiologyTechnology.vol.57no.1CuritibaJan./Feb.2014

18. Daniel Heinrich1, Mohamed H Madkour2, Mansour A Al-Ghamdi2, Ibraheem I Shabbaj2 and Alexander Steinbüchel Large scale extraction of poly(3-hydroxybutyrate) from Ralstonia eutropha H16 using sodium hypochlorite *AMB Express* 2012, 2:59

19. São Paulo Rawia F. Gamal; Hemmat M. Abdelhady; Taha A. Khodair; Tarek S. El-Tayeb; Enas A. Hassan; Khadiga A. Aboutaleb Semi-scale production of PHAs from waste frying oil by *Pseudomonas fluorescens* S48. *Brazilian Journal of Microbiology*. vol.44 no.2 2013

20. Du, G., Chen, J., Yu, J. and Lun, S. 2001. Continuous production of poly-3-hydroxybutyrate by Ralstonia eutropha in a two stage culture system. *Journal of Biotechnology.*, 88: 59-65.

21. Zakaria, M. R., Ariffin, H., Johar, N. A. M., Aziz, S. A., Nishida, H., Shirai, Y. and Hassan, M. A. 2010. Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3hydroxybutyrate) copolymer from wild type Comamonas sp. EB172. *Polymer Degradation and Stability.*, 95: 1382-1386.

22. Silvia Messias Bueno; Crispin Humberto Garcia-Cruz ,Optimization of polysaccharides production by bacteria isolated from soil *Brazilian Journal of Microbiology* (2006) 37:296-301

23. Michel. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, Fred. Smith Colorimetric Method for Determination of Sugars and Related Substances *Analytical Chemistry*, 1956, 28 (3), pp 350–356

24. Yongqin Jiao,<sup>1</sup> George D. Cody,<sup>2</sup> Anna K. Harding,<sup>1</sup> Paul Wilmes,<sup>3,4</sup> Matthew Schrenk,<sup>4</sup> Korin E. Wheeler,<sup>1,§</sup> Jillian F. Banfield,<sup>3</sup> and Michael P. Thelen Characterization of Extracellular Polymeric Substances from Acidophilic Microbial Biofilm. *Applied and Environmental Microbiology*. 2010 May; 76(9): 2916–2922.

25. Muthusamy Ashok kumar1\*, Kanapathi Thangavel Kasirajan Anandapandian2 and Karuppiah Parthiban Production and Characterization of Exopolysaccharides (EPS) from Biofilm Forming Marine Bacterium Vol.54, n. 2: pp. 259-265, March-April 2011 ISSN 1516-8913. Brazilian Archives of Biology and Technology 26.S. E. Desouky1\*, H. H. El-Shiekh1,2, M. A. Elabd3 and A. M. Shehab1 Screening, Optimization and Extraction of Polyhydroxyalkanoates (PHAs) from *Bacillus thuringienesis Journal of Advances in Biology & Biotechnology* 1(1): 40-54, 2014; Article no. JABB.2014.004

27. Daniel C.O. Thornton1, Elizabeth M. Fejes, Steven F. DiMarco, and Kristen M. Clancy Measurement of acid polysaccharides in marine and freshwater samples using alcian blue *Limnology and Oceanography.: Methods* 5, 2007, 73–87 © 2007, by the American Soc

28. A. A. Khardenavis, M. Suresh Kumar, S. N. Mudliar, and T. Chakrabarti, "Biotechnological conversion of agroindustrial wastewaters into biodegradable plastic, poly  $\beta$ -hydroxybutyrate," *Bioresource Technology*, vol. 98, no. 18, pp. 3579–3584, 2007.

29. J. Yu, "Production of PHA from starchy wastewater *via* organic acids," *Journal of Biotechnology*, vol. 86, no. 2, pp. 105–112, 2001.

30. M. G. E. Albuquerque, M. Eiroa, C. Torres, B. R. Nunes, and M. A. M. Reis, "Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses," *Journal of Biotechnology*, vol. 130, no. 4, pp. 411–421, 2007.

31. Roland Durner, Bernard Witholt, and Thomas Egli Accumulation of Poly[(*R*)-3-Hydroxyalkanoates]

in*Pseudomonas oleovorans* during Growth with Octanoate in Continuous Culture at Different Dilution Rates. *Applied and Environmental Microbiology*. August 2000 vol. 66 no. 83408-3414

32. Valappil SP<sup>1</sup>, Rai R, Bucke C, Roy I. Polyhydroxyalkanoate biosynthesis in Bacillus cereus SPV under varied limiting conditions and an insight into the biosynthetic genes involved. *Journal of Applied Microbiology*. 2008 Jun;104(6):1624-35. doi: 10.1111/j.1365-2672.2007.03678.x. Epub 2008 Jan 7.

33. Sandrine Petry,<sup>1,\*</sup> Sylviane Furlan,<sup>1</sup> Marie-Jeanne Crepeau,<sup>2</sup> Jutta Cerning,<sup>1</sup> and Michel Desmazeaud<sup>1</sup> Factors Affecting Exocellular Polysaccharide Production by *Lactobacillus delbrueckii* subsp. *bulgaricus* Grown in a Chemically Defined Medium<sup>†</sup> Applied and Environmental Microbiology. 2000 Aug; 66(8): 3427–3431.

34. Rosa Palmeri\*, Francesco Pappalardo, Manuela Fragalà, Marco Tomasello, Arcangelo Damigella, Antonino F. Catara Polyhydroxyalkanoates (PHAs) Production Through Conversion of Glycerol by Selected Strains of *Pseudomonas Mediterranea* and *Pseudomonas Corrugata Chemical engineering transactions* Vol. 27, 2012