[P-I.116]

Increasing of tapioca flour by blending and forming with commercial PHBV and biopolymer obtained from fermented sugar cane juice for producing as bioplastic

Pakawadee Kaewkannetra[1,2,*], Jaruwat Soonthornchiya[1,2], Samart Moonamart[1]

[1] *Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand*
[2] *Fermentation Research Centre for Value added Agricultural Products (FerVAAP),Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*
Keywords: tapioca starch; PHBV (poly-3-hydroxybutyrate-*co*-hydroxyvalerate); sugar cane; blended films; bioplastic

The objective of this work is to increase value of tapioca flour by blending and forming biopolymer obtained from fermented sugar cane juice comparing to commercial biopolymer in solid form of Poly-3-hydroxybutyrate-*co*-hydroxyvalerate (PHBV). Typically, PHBV is known as biodegradable, biocompatible, semi-bacterial polyester and has similar properties to polypropylene (PE). Firstly, the commercial PHBV was preliminary investigated as sheet films with bio-based material of tapioca flour. The 3% (w/v) PHBV was dissolved with hot chloroform at about 60 °C while the 3% (w/v) flour was mixed with water. Then, different ratios (v/v) of PHBV and the flour were mixed (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 and 100:0). Two mixtures were blended and formed as bioplastic films using conventional solvent-casting techniques. The biopolymer obtained after fermentation of sugar cane juice was also done in the same way with the flour. The bioplastic films are characterized by various physical and chemical techniques such as polarized light microscopy, differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). The results revealed remarkably crystalline structure with cross-polarized light on optical microscope. The optimal blended films with a ratio of 40: 60 were obtained by observation of their morphology and immiscibility of the blends is gradually increased on increasing the starch portions. The blended films are mostly brittle and had specify glass temperatures (Tg) depended on the portion. Melting transition temperatures (Tm) of blended films are slightly higher than the bacterial synthesized biopolymer as examined by DSC. These results are correspondent to their highly crystallinity from diffractograms (XRD). In addition, microstructure of the blended films is reduced largely crystallinity by the solution casting.

References

J. Rodrigues, D.F. Parra, A.B. Lugao. Crystallization of film of PHB/PEG blends evaluation by DSC. J. Thermal Analysis and Calorimetry 79 (2005) 379–381.
S. Godbole, S. Gote, M. Latkar, T. Chakrabarti. Preparation and characterization of biodegradable poly-3-hydroxybutyrate-starch blend films. Bioresource Technology 86 (2003) 33–37.
Y. Song, Q. Zheng. Improved tensile strength of glycerol-plasticized gluten bioplastic containing hydrophobic liquids. Bioresource Technology 99 (2008) 7665–7661.
A. El-Hadi, R. Schnabel, E. Straube, G. Muller, S. Henning. Correlation between degree of crystallinity, morphology, glass temperature, mechanical properties and biodegradation of poly (3-hydroxyalcanoates) PHAs and their blends. Polymer testing. 21 (2002) 665–674.

doi:10.1016/j.jbiotec.2010.09.482

[P-I.117]

Evaluation of bacterial lysis procedures for the efficient release of cellular proteins and polymers of biomedical interest

E. García-Fruitós[1,2], R. Mendoza[1,2], A. Villaverde[2,3], E. Rodríguez-Carmona[1,2,*]

[1] *CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain*
[2] *Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, 08193 Bellaterra (Cerdanyola del vallès), Barcelona, Spain*
[3] *Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain*
Keywords: bacterial lysis; cellular proteins; heterologous protein; cell disruption

The development of metabolic engineering, protein engineering and production strategies has supported the success in heterologous protein production for the manufacture of pharmaceutical or industrial proteins. One of the major disadvantages of bacteria as hosts for protein production is that they generally do not excrete high levels of polypeptides to the medium. Therefore, lysis of microbial cells is often required for their complete isolation, release and recovery from the cells. Moreover, the new nanomedical and nanotechnological applications of recombinant proteins (and other bacterial molecules) including diagnostics, biosensors, regenerative medicine, drug delivery, etc. have forced the application of methods that assure safety and no risk of possible bacterial contamination. Many treatments are available for the disruption of bacterial cells, including mechanical, physical, chemical and enzymatic procedures.

The general aim of this work is understanding the effectiveness of cell lysis procedures by comparing the effects of four approaches based on physical strategies, one chemical procedure and two combined procedures, on *E. coli*, *S. aureus* and *P. aeruginosa* strains as bacterial models. For this purpose, the different methods were tested in terms of cell viability by measuring the change in viable bacteria (CFU) before and after the procedures and in terms of total cell protein in supernatant samples.

The obtained results showed that heat shock (100 °C) and heat shock+sonication combination methods were the most effective in terms of reducing cell viability for the three assayed bacteria. Moreover, the highest amount of released cell protein was achieved by different methods, depending on the bacterial species.

doi:10.1016/j.jbiotec.2010.09.483

[P-I.118]

Effect of auxotrophies on yeast growth in aerated fed-batch reactor

L. Paciello[1], C. Landi[1], E. de Alteriis[2], L. Brambilla[3], P. Parascandola[1,*]

[1] *University of Salerno, Italy*
[2] *University of Naples Federico II, Italy*
[3] *University of Milano-Bicocca, Italy*
Keywords: CEN.PK strains; auxotrophy; complementation; fed-batch culture

Mutant and deletion strains of the yeast *Saccharomyces cerevisiae* having one/several auxotrophies are largely used in the development of recombinant strains for heterologous protein production because they ensure maintenance of plasmids with selectable markers. The production is usually carried out by cul-

turing the recombinant strain in aerated fed-batch, where sugar limitation achieves high yields of biomass and product.

In a previous work, it was evidenced that growth of the auxotrophic *S. cerevisiae* BY4741 (*MATa, ura3Δ0, leu2Δ0, met15Δ0, his3Δ1*) engineered for human IL-1β production, and employed in aerated fed-batch, early arrested even in the presence of a correct nutritional complementation (being specific nutrients for genetically uncomplemented auxotrophies provided in no growth-limiting amounts). It was assumed that this behaviour may depend on the high number of auxotrophies, since the prototrophic strain S288C, from which BY4741 derives, showed a typical performance under the same cultivation mode.

Therefore, a systematic investigation on the effect of auxotrophies on yeast growth in aerated fed-batch was carried out. Four isogenic strains of the CEN.PK family, with a progressively increasing number of auxotrophies (from one to four) were assayed under fed-batch conditions and a proper nutritional complementation. Feeding to the reactor was exponentially increased imposing a specific growth rate below the critical one. The behaviour of the auxotrophic strains was compared with that of the isogenic prototrophic strain. by evaluating the capacity to keep the specific growth rate chosen. A clear correlation among optimum growth and number of auxotrophies has been found. Furthermore we have investigated the possible effect of the type of auxotrophy (*ura-* or *leu-*) on the strain performance, monitoring as well cell viability of each strain. The study is a contribution to know the phenotypic effects of auxotrophies in yeast and can have implications for biotechnological applications.

doi:10.1016/j.jbiotec.2010.09.484

[P-I.119]

Molecular Studies on Novel Thermostable Carboxyl Esterases

Nadia A. Soliman*, Yasser R. Abdel-Fattah

Mubarak City For Scientific Research and Technology Applications, Egypt
Keywords: thermophilic; thermostable; esterase; shuffling

Esterases are the most frequently used enzymes belong to the class of hydrolases (E.C.3), in industrial applications and in research. Most important in this class are hydrolytic enzymes cleaving carboxylic ester bonds. Lipases (EC 3.1.1.3), carboxylesterases (EC 3.1.1.1), as they accept a broad range of non natural substrates, are usually very stable in organic solvents and exhibit good to excellent stereo-selectivity, e.g. in the kinetic resolution of racemates or the desymmetrization of postereogenic compounds.

An increasing demand for new biocatalysts adapted to special condition is needed. Besides attempts to exploit natural diversity or to improve enzymes by rational design and directed evolution the various genome projects have provided researchers with an enormous amount of sequence information, waiting to be explored.

In this study we focused our attention to isolate large numbers of thermophilic bacteria (~300 isolate) from Saudi Arabian soil. The isolates were screened for lipase/esterase production, then the potent isolates (~30 isolates) were identified. Based on a phylogenetic analysis, they are clustered into different groups including *Bacilli* and *Geobacilli* spp. PCR cloning for different estersses of different species including *stearothermophilus, thermodenitrificans, thermocatenulatus, caldolyticus, caldovelox* and *thermoleovorans* was performed using degenerate primers. Sequence analysis was verified the relatedness of these fragments to different esterses with an identity percentage ranged (37-97%). Working are currently performed to express some of these genes to use for shuffling.

doi:10.1016/j.jbiotec.2010.09.485

[P-I.120]

Production of omega-3 Fatty acids by unicellular cyanobacterium *Synechococcus* sp.

Seyed Bagher Mosavi-Azam*, Mohammad Hossein Morowvat, Sara Rasoul-Amini, Younes Ghasemi

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Iran, Islamic Republic of
Keywords: cyanobacteria; *Synechococcus*; fatty acid; omega-3

Introduction: Microalgae have a large biotechnological potential for producing valuable substances for the feed, food, cosmetics and pharmacy industries as well as for biotechnological processes. They are a major source of essential long-chain, highly unsaturated fatty acids (HUFA), sterols, and other nutrients. Under certain conditions, microalgae have been reported to contain up to 85% of the dry weight as lipids. The range of potential applications for these microalgal fats and oils is very wide. During the last two decades the utilization of microalgae and other forms of microorganisms as sources of single cell protein (SCP) has gained increasing interest.

Methods: In this study, the fatty acids profiles of the unicellular cyanobacterium, ***Synechococcus* sp.** was examined. ***Synechococcus* sp.** was isolated During a screening program from paddy fields of Fars Province in the south of Iran. Total lipids from freshly freeze dried cultures of the strain was extracted and converted into fatty acid methyl esters and used for determination of different types of fatty acids by gas chromatography, mass spectrometry (GC/MS) method.

Results: The composition of fatty acids in the cyanobacterium was mainly, oleic acid, a mono unsaturated (ω9) fatty acid, 6,9,12-octadecatrienoic acid, a poly unsaturated (ω6) fatty acid, and 9,12,15-octadecatrienoic acid, a poly unsaturated (ω3) fatty acid.

Discussion: This method allowed us to discover several types of fatty acids, such as chained, mono unsaturated, poly unsaturated, in the studied naturally isolated cyanobacterium. The ***Synechococcus* sp.**, is a good and unstudied candidates to be used as SCP as human food or animal feed.

doi:10.1016/j.jbiotec.2010.09.486

[P-I.121]

Analysis of Transport Phenomena and Bioreaction in Fluidized Bed Reactor for Fructose Production

E. Ricca*, M. Massarotto, V. Calabro', S. Curcio, G. Iorio

Department of Engineering Modelling, University of Calabria, I-87030 Arcavacata di Rende (CS), Italy
Keywords: Enzyme hydrolysis; Fructose production; Modelling Mass Transport Resistances; Fluid-dynamics

In this work inulinase enzyme immobilized on Sepabeads® was used for continuous production of fructose from inulin. Among continuous bioreactors, fluidized bed bioreactors (FBBR) offer many advantages with respect to stirred and packed reactors in terms of: low microbial contamination due to continuous washing of undesired micro-organisms during biocatalysis, no clogging, low mass transport resistances and fluid-dynamic behaviour favourable to