Biodegradable polymers from whey

L.S. Serafim*, P.C. Lemos*, V. Martínez** M.A. Prieto**, M.A.M. Reis*

* REQUIMTE/CQFB, Chemistry Department, FCT/Universidade Nova de Lisboa, 2829-516 Caparica Portugal, luisa.serafim@dq.fct.unl.pt
** Department of Molecular Microbiology, Biological Research Center, CSIC, Madrid, Spain

ABSTRACT

Cheese whey, the major by-product of cheese industry, is rich in lactose and can serve as a raw material for industrial production of PHA. Only few microorganisms are able to convert directly lactose into PHA, e.g. recombinant *Escherichia coli* harboring PHA biosynthesis genes. PHA production cost hampers its wide utilization but these bacteria can contribute for the reduction of the downstream process costs and complexity since they can disrupt and release the internal PHA granules, when in very high content (*E. coli*). The results obtained showed that recombinant *E. coli* has a good potential for PHA producing from cheese whey. On going research is focused on the optimization of PHA production by the selected *E. coli* strains.

Keywords: PHA, lactose, cheese whey, *Escherichia coli*

1 INTRODUCTION

Whey is the major by-product of cheese industry in Europe. For each kilogram (Kg) of cheese, 9 Kg of whey are produced. In 2004, 40 420 800 tons of whey were produced in Europe and 13 500 000 containing 620 000 tons of lactose were discharged in the environment, causing a huge disposal problem [1]. The elimination of whey is a problem for the diary industry, since it has high biological oxygen demand, exhibiting high BOD5 (30000-50000 ppm) and COD contents (60000-80000 ppm). Lactose (4.5-5.0%) is the main responsible for this high organic matter content [2]. The need of finding processes to convert the lactose present in whey is vital.

Lactose can serve as carbon source for the production of polyhydroxyalkanoates (PHA). PHA are thermoplastics synthesized by bacteria that can be produced from renewable resources. Until now the main obstacle to the replacement of synthetic plastics by PHA was the great difference in cost. The major costs in industrial production are determined by the substrate price and the polymer extraction from the cells (downstream). Polyhydroxybutyrate (PHB) is the most common PHA. PHB homopolymer is very crystalline, stiff and brittle, making it difficult to process. On the other hand the introduction of other 3-hydroxyacid monomers, like 3-hydroxyvaleric acid (HV) in the PHB chains increases the malleability and the processability. Copolymers of P(HB/HV) can replace synthetic plastics in a wide range of applications.

In the last years, researchers are looking for microbial strains, genetically modified or not, able to utilize lactose from whey as carbon source for PHA production and, at the same time, with easy polymer extraction. Only few microorganisms are able to convert directly lactose into PHA, e.g. recombinant *Escherichia coli* harboring PHA biosynthesis genes. This bacterium, the most utilized microorganism for cheese whey valorization, is able to accumulate PHB till 90% of cellular dry weight [3]. Some *E. coli* strains become fragile and can disrupt when accumulating high amounts of PHB, making the downstream process easy [4].

In this work, recombinant *E. coli* strains were tested for PHA production from lactose present in cheese whey.

2 MATERIALS AND METHODS

2.1 Bacterial strains and plasmid construction

Six *Escherichia coli* strains, ET8000, B, C, MG1655, BL21DE3 and W3110 supplied by CSIC-CIB (Spain) were tested for their ability in growing in lactose. The strains that showed the highest growth rate (ET8000, C and MG1655) were genetically modified through the introduction of the plasmid pAV1 .

DNA manipulations and plasmid pAV1 construction were performed according to [5, 6, 7, 8]. The plasmid pAV1 contains the complete *phbA*, *phbB* from *Cupriavidus necator* H16 encoding the β-ketothiolase, and NADP-specific acetoacetyl-CoA reductase, respectively, and *phbC* gene coding for the polyhydroxybutyrate polymerase .

2.2 Culture conditions

Seed cultures were prepared in a 250-ml flask containing 130 ml of MR medium (see below) in a shaking incubator at 37 ºC and 200 rpm. The MR medium [9] contains per liter (pH 6.9): 20 g of lactose, 13.5 g KH_2PO_4, 4 g $(NH_4)_2HPO_4$, 1.4 g $MgSO_4 \cdot 7H_2O$, 1.85 g $C_6H_8O_7 \cdot H_2O$, 20 mg proline, 337 mg of thiamine-HCl and 10 ml trace metal solution. The trace metal solution in 1 M HCl

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contains per liter 5 g $FeSO_4 \cdot 7H_2O$, 1 g $CaCl_2$, 1.1 g $ZnSO_4 \cdot 7H_2O$, 0.19 g $MnSO_4 \cdot H_2O$, 0.5 g $CuSO_4 \cdot 5H_2O$, 0.05 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0.01 g $Na_2B_4O_7 \cdot 10H_2O$.

2.3 Fed-batch cultures

Using the recombinant *E. coli* C (pAV1), fed-batch cultures were carried out at 37ºC in a 2-l jar fermentor (BioStat B-Plus, Sartorius, Germany) containing 1.5 l of MR medium plus 20 g/l of lactose. The culture pH was controlled at 6.95 by the automatic addition of NaOH. Foam formation was suppressed by adding Antifoam A (Fluka). The dissolved oxygen concentration (DOC) was controlled at 30% by automatically increasing the agitation and varying the air flow rate.

When the lactose was depleted, a new pulse of lactose was added in order to obtain a concentration of 20 g/l.

2.4 Analytical procedures

Cell growth was monitored by measuring the turbitiy at 600 nm. PHB concentration was determined by gas chromatography and ammonia concentration was determined using an electrode as described in [10]. Cell concentration, defined as cell dry weight per liter of culture broth, was determined as previously described [9]. The residual cell concentration was defined as the cell concentration minus PHB concentration. The PHB content (%PHB) was defined as the percentage of the ratio of PHB concentration to cell concentration. The concentrations of lactose were measured by HPLC with a CarboPac PA10 column (Dionex), equipped with an amperometric detector. The analysis was preformed at 30ºC, with sodium hydroxide (NaOH 52 mM) as eluent, at a flow rate of 0.9 ml/min. Nile blue staining was preformed according to Rees et al. [11].

3 RESULTS AND DISCUSSION

In this work, recombinant *E. coli* strains were utilised for PHA production from cheese whey. Six E. coli strains (B, C, BL21DE3, ET8000, MG1655, W3110) were screened for growth ability on lactose. All of the six strains were grown in 250 ml flasks containing MR medium with 20 g/l of lactose. All the six strains were able to grow in lactose but at different specific growth rates and different maximum cell concentrations as shown in Table 1 and Figure 1.

Strains C and ET8000 were selected for modification because of the highest values of specific growth rate showed, 0.50 h-1 and 0.48 h-1, respectively. MG1655 was also selected because of the highest cell concentration, 0.94 g/l.

Strain	μ (h^{-1})	X (g/l)
ET8000	0.48	0.73
MG1655	0.34	0.94
W3110	0.34	0.69
B	0.24	0.52
BL21DE3	0.34	0.48
C	0.50	0.61

Table 1. Specific growth rates and cell concentration for the *E.coli* strains tested

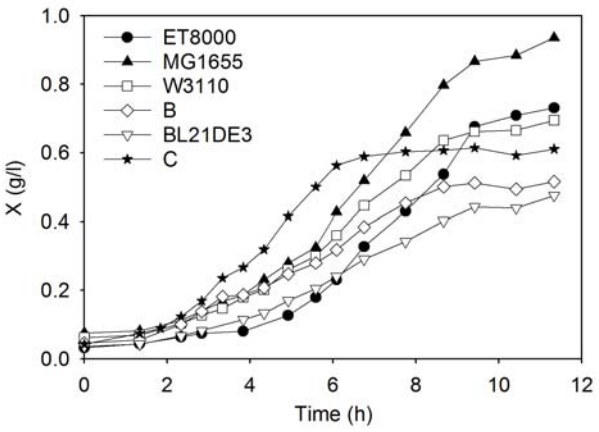

Figure 1: Growth evolution of the six *E. coli* strains

The three *E. coli* strains were modified through the inclusion in a plasmid of *Cupriavidus necator* genes for the expression of enzymes responsible for PHA production (pAV1). The preliminary results of tests performed in shaken flasks with the modified strains are shown in Table 2 and in Figure 2. The three strains possess the ability to utilize lactose to grow and produce PHB however with different results.

Strain	μ (h^{-1})	%PHB
ET8000 (pAV1)	0.30	48.4
MG1655 (pAV1)	0.30	39.3
C (pAV1)	0.42	53.9

Table 2. PHB production and specific growth rate on lactose by the recombinant *E. coli* strains

Strain C (pAV1) showed the highest specific growth rate (0.42 h^{-1}) and the highest PHB cell content (53.9%) being chosen to scale up the system. After 30 h and two spikes of lactose (20 g/l), the culture was able to grow to 7.4 g/l and accumulated 44% of PHB as shown in Figure 2. The storage only started when the growth became limited, after the depletion of the ammonia, around 7 h. The storage yield during the second spike of lactose corresponded to

0.10 gPHB/g lactose. This yield is slightly lower than the values reported in the literature for recombinant *E. coli* by Wong and Lee [3], between 0.17 and 0.42 g PHB/g lactose. However this yield value is being optimized.

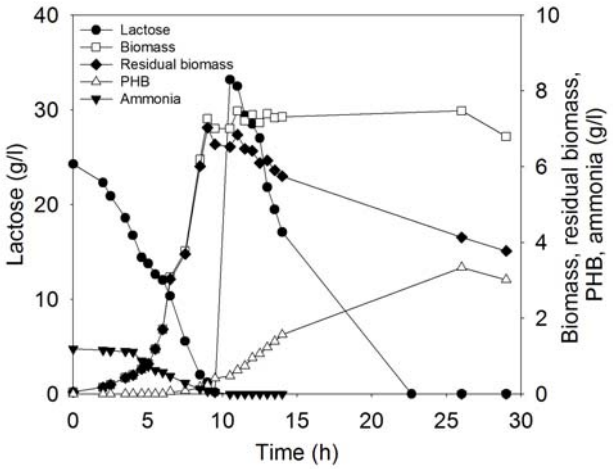

Figure 2: PHB production and cell growth on lactose by *Escherichia coli* C (pAV1)

The decrease of the residual biomass when the PHB storage started resulted probably from the ammonia limitation. In the work of Wong and Lee [3], this decrease was also visible when the growth became limiting and the PHB storage started.

At the end of the experiment Nile blue staining was preformed and the result is shown on Figure 3: all the biomass was involved in PHB production.

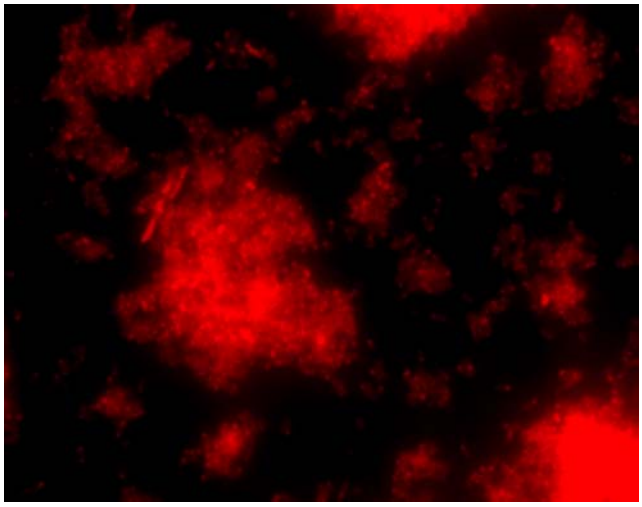

Figure 3: Nile blue staining of the *E. coli* C (pAV1) biomass after 30 h of experiment

4 CONCLUSIONS

The use of cheese whey for PHA production not only allows for the diminishing of the high organic content of this effluent but also contributes for the decrease of the final price of PHA. These results showed the potential of the selected modified *Escherichia coli* strains for PHB production from cheese whey.

5 FUTURE WORK

On going research is focused on the optimization of PHA production by the selected *E.coli* strains. The scale up of the process for higher reactor volume is being performed as well as tests with direct utilization of cheese whey. The work will also focus on the optimisation of the productivity and quality of polymer produced.

The conditions that favors the formation of copolymers P(HB/HV), in detriment of PHB production from whey, will be analyzed.

The optimization of downstream process and characterization of polymers is also an objective of this project.

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