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Poly – β – Hydroxybutyrate (PHB) - Plastics from bacteria and for bacteria

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Keywords

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Abstract

Bacillus subtilis was found to produce PHB and the amount of PHB produced was estimated under various conditions like pH, temperature, and also using different substrates. PHB was also produced using various inexpensive substrates like arrowroot powder; rice water: and sago water and the amount of PHB produced were estimated by reading the absorbance at 235nm.

1. Introduction

Humans in developed countries have grown accustomed to life in a "plastic society". Most plastics end up in landfills on our shores or in the ocean. Bacteria from the genera *Alcaligenes, Bacillus, Azospirillum, Pseudomonas* etc produces polymers in the PHA family which are used for energy and as a storage form of cellular carbon. These microorganisms can accumulate from 30-80% of their dry weight in PHA. The various inexpensive substrates that are used for the production of PHB include whey, starch containing substances like arrowroot, rice water and sago water.

2. Materials and Methods

Isolation of organism from soil Soil samples were collected from the sugarcane factory, Sakaravayil, Vellore. The soil samples were serially diluted and inoculated into PHB medium and incubated for 48hours.

Identification of PHB granules in the isolated organism

The isolated organism was stained with Sudan black B and safranine and observed under oil immersion(100x) objective.

Isolation of plasmid and transformation from the isolated organism

The plasmid was isolated from the organism by alkaline lysis method.Competent cells were prepared using DH5α strain of *E. coli* and transformation was carried out using calcium chloride method of transformation.

Identification of transformed cells for PHB granules and isolation of PHB granules

The transformed cells were inoculated into PHB medium and incubated for 48 hours. After incubation, the transformed cells were stained with Sudan black B for the presence of PHB granules.The PHB granules were isolated by gravimetric method using sodium hypochlorite, chloroform and precipitated using ice cold methanol.

Standardization of PHB

The standard PHB was obtained from Malaysia and it was serially diluted using sulphuric acid and absorbance was read at 235nm.

Production of PHB using various substrates, temperature and pH

Various substrates like glucose, fructose, maltose and sucrose was used for the production of PHB. Various temperatures like 25 C, 37 C, and 45 C were used for the production of PHB and the amount of PHB produced was estimated. Various pH like pH 1-3, pH 6.5-7, pH 8- 9 were used for the production of PHB and the amount of PHB produced was calculated.

Production of PHB using inexpensive substrates

PHB was produced using various inexpensive substrates like arrowroot powder; whey; rice water and sago water and the amount of PHB produced was estimated using spectrophotometric assay. Nitrate reduction test, Citrate utilization test, Voges- proskauer test was used to confirm the organism to be *Bacillus subtilis*

Isolation of plasmid from the isolated organism

The plasmid was isolated from *Bacillus subtilis* by alkaline lysis method. Transformation was carried out using calcium chloride method. After transformation, the recombinant plasmids were mainly identified using X-gal and IPTG. a)

Colonies that carry the recombinant plasmid appeared blue in colour. b)The non- transformed colonies, which does not contain the PHB biosynthesis genes appeared white in colour.

3. Results and Discussion

Isolation of PHB producing organism from soil

The soil samples which were used for the isolation of PHB producing organism was collected from the sugarcane factory in Vellore. The serially diluted samples were inoculated into PHB medium. Growth occurred after 48 hours of incubation. The PHB medium which was initially colourless turned cloudy white in colour. Presence of pellicle formation was also observed.

Identification of PHB granules in the isolated organism by PHB staining method

The isolated organism was stained with Sudan black B and observed under oil immersion. Under oil immersion microscopy, blue droplets of PHB were observed, while the cytoplasm appeared pink in colour.

Identification of the isolated organism

The isolated organism was stained with Gram's stain and to get gram positive bacilli observed and the identification was done by PHB staining, green in colour and the vegetative cells pink in colour.

Identification of transformed cells for PHB granules

The transformed *E. coli* (DH5 α) cells containing the PHB biosynthesis genes were grown in the PHB medium for its expression. The PHB medium containing the growth of the transformed organism turned yellowish white in colour. The transformed cells were stained with Sudan black B and the PHB granules appeared as blue droplets and the cytoplasm appeared pink in colour.

Isolation and Standardisation of PHB granules

The PHB granules were extracted from *Bacillus subtilis* as well as transformed *E. coli* by hot chloroform extraction method.The standard PHB was diluted sixteen times and the optical density was read at 235nm in a UV spectrophotometer.

Production of PHB using various substrates, temperatures and various pH

It was found that in glucose the transformed cells produce more PHB than the amount which was produced by *Bacillus subtilis*. In sucrose both *Bacillus* and transformed *E. coli* has the capacity to utilize sucrose for the production of PHB. Both *Bacillus* and the transformed *E. coli* were not able to utilize fructose and maltose to a maximum level. (Table 1)

It was found out that maximum production of PHB was produced when the temperature was maintained at 37ºC. (Table 2).

It was found that maximum PHB was produced at pH 6.5-7. (Table 3)

Table 1 – Production of PHB using various substrates

Substrate	Organism	Concentration of PHB (mg/ml)
Glucose	*Bacillus subtilis*	0.028
	Transformed *E. coli* (DH5α)	0.034
Sucrose	*Bacillus subtilis*	0.031
	Transformed *E. coli* (DH5α)	0.032
Fructose	*Bacillus subtilis*	0.010
	Transformed *E. coli* (DH5α)	0.012
Maltose	*Bacillus subtilis*	0.018
	Transformed *E. coli* (DH5α)	0.024

Table 2 – Production of PHB at various temperatures

Organism	Temperature (°C)	Concentration of PHB (mg/ml)
Bacillus subtilis	25	0.016
Transformed *E. coli* (DH5α)	25	0.020
Bacillus subtilis	37	0.034
Transformed *E. coli* (DH5α)	37	0.026
Bacillus subtilis	45	0.030
Transformed *E. coli* (DH5α)	45	0.045

Table 3 – Production of PHB at various pH

Organism	pH	Concentration of PHB (mg/ml)
Bacillus subtilis	1 – 3	0.010
Transformed *E. coli* (DH5α)	1 – 3	0.014
Bacillus subtilis	6.5 – 7	0.026
Transformed *E. coli* (DH5α)	6.5 – 7	0.030
Bacillus subtilis	8 –9	0.010
Transformed *E. coli* (DH5α)	8 –9	0.008

Table 4 – Production of PHB using inexpensive substrates

Substrate	Organism	Concentration of PHB (mg/ml)
Arrow root powder	*Bacillus subtilis*	0.026
	Transformed *E. coli* (DH5α)	0.029
Rice Water	*Bacillus subtilis*	0.028
	Transformed *E. coli* (DH5α)	0.025
Sago water	*Bacillus subtilis*	0.030
	Transformed *E. coli* (DH5α)	0.034
Whey	*Bacillus subtilis*	0.024
	Transformed *E. coli* (DH5α)	0.033

Production of PHB using inexpensive substrates

From the results obtained it was found that maximum production of PHB was obtained when sago water was used as a substrate. Further the transformed *E. coli* was able to utilize sago water more rapidly than *Bacillus subtilis*, and thereby it gives maximum amount of PHB. (Table 4)

References

Alexander N. Glazer, Hiroshi Nikaido, Microbial Biotechnology, Freeman Publication, 283 – 295.

Anderson AJ & Dawes EA,(1990) Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates, Microbiological Reviews 54: 450 – 472.

David R. Walt, Applied Biochemistry and Biotechnology, (1997)Spring Vol. 63 – 65, 627 – 635.

H. Kleinkauf, H. Von Dohren, Biotechnology, Products of secondary metabolism, Vol. VII, A Wiley Company, 177 – 180.

H.J. Rehm & G. Reed, Biotechnology, Products of primary metabolism, A Wiley Company, 405 – 464.

Oliver P. Peoples & Anthony J. Sinskey, (1989)PHB Biosynthesis in Alcaligenes eutrophus H16, The Journal of Biological Chemistry 264: 15298 – 15303.