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Effect of various carbon sources on poly-β-hydroxybutyrate production by screened strains of *Wautersia eutropha*

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Abstract

Selection of suitable substrate and optimizing the substrate concentration are pivotal parameters for biopolymer production in order to make it economically acceptable. Therefore, fructose, dextrose, propionate and methanol were taken under consideration to evaluate the effect of various carbon sources on poly-β-hydroxybutyrate [P(3HB)] production by screened strains of *Wautersia eutropha* (two mutagenized strains and wild-type organism). *Wautersia eutropha* M2 was determined to render high P(3HB) content at relatively low fructose concentration than wild-type organism. Maximum P(3HB) yields by wild-type *Wautersia eutropha* and *Wautersia eutropha* M2 utilizing fructose were 59.58% and 60.28% respectively. P(3HB) yield by wild-type organism at 20 gL^{-1} fructose concentration (59.58%) was similar to that of P(3HB) yield by *Wautersia eutropha* M2 at 10 gL^{-1} fructose concentration (59.22%). A shift in degree of substrate specificity due to random mutagenesis was found in *Wautersia eutropha* M5 that utilized dextrose more efficiently than fructose (unlike wild-type *Wautersia eutropha*), rendering highest P(3HB) content (52.52%) during dextrose feeding. Increasing concentration of propionate showed inhibitory effect on bacterial growth and *in vivo* polymer accumulation in all the strains with highest yield of 36.76% at 8 gL^{-1} concentration by *Wautersia eutropha* M2. Methanol supported scanty growth and thus insignificant P(3HB) yields were reported by all the strains.

Keywords: Bioplastic; *Wautersia eutropha*; poly-β-hydroxybutyrate; carbon source

INTRODUCTION

Reasonable alternative to petrochemically-based recalcitrant plastics are the biodegradable polymers [1,2] whose molecular structures and chemical bond sequences are recognized by existing degradative enzymes. Microbiologically they can be produced from renewable resources unlike polypropylene and polyethylene that are derived from finite reserve of coal and oil [3,4,5,6,7,8]. Among all biodegradable polyesters, polyhydroxyalkanoates (PHAs) are of central interest, as they possess mechanical properties proximal to synthetic thermoplastics [9, 10; 11]. P(3HB) and other PHAs are produced *in vivo* by different groups of bacteria, cyanobacteria, actinomycetes under a critical nutrient limitation [12], providing that surplus carbon source remains available [13].

The fact that PHAs are much more expensive than petrochemical plastics restrict the replacement of broad-spectrum applications of recalcitrant plastics by PHAs. Selection of a suitable substrate is a pivotal factor for optimizing the P(3HB) production. Salehizadeh and Loosdrecht (2004) [14] reported that over 40% of the total operating expense of PHA production is related to the raw materials, and more than 70% of this cost is attributed to the carbon source. *Hence, present investigation involved detecting the level of P(3HB) accumulated with different carbon sources at varying concentrations by Wautersia eutropha and its mutagenized strains. Objective was to find an Wautersia eutropha strain that can convert a wide range of carbon substrates into P(3HB) and produce P(3HB) at higher amount depleting comparatively lower carbon content than wild-type Wautersia eutropha, thus aiming better economy.*

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MATERIALS AND METHODS

Bacterial strains: Wild-type *W. eutropha* (MTCC 1285) obtained from IMTECH, Chandigarh was used in the experiments. Mutagenised *W. eutropha* strains obtained by random mutagenesis using UV irradiation were also used in the study.

All cultures were maintained on nutrient agar slants at 4 °C and the cultures were subcultured once in 20 days.

Media: Nutrient agar medium, containing beef extract-2.0 gL^{-1}, peptone-5.0 gL^{-1}, NaCl-5.0 gL^{-1} and agar-15.0 gL^{-1} was used as growth medium for all the cultures. To compare P(3HB) production with different carbon sources, mineral salt broth , containing KH_2PO_4-3.0 gL^{-1}, Na_2HPO_4-6.0 gL^{-1}, NaCl-5.0 gL^{-1}, NH_4Cl- 1.5 gL^{-1}, $MgSO_4$-0.1gL^{-1}, yeast extract-0.1gL^{-1} supplemented separately with four different carbon sources (fructose/dextrose/propionate/methanol) at varying concentrations (0.8%, 1%, 2%, and 3%) was used. The pH of all media was adjusted to 7.0 with 0.2 N NaOH/0.2 N HCl and all the cultures were incubated at 30 °C for 24-48 hours.

Analytical methods

Cell dry weight determination: 10 ml of each broth culture was taken and cell pellet was obtained by centrifugation at 10,000 rpm for 10 minutes. Supernatant was discarded and cell pellet was dried at 90 °C till constant weight was reached [15].

Extraction and quantification of P(3HB): Bacterial cells of both *W. eutropha* and its mutants were harvested by centrifugation at 10,000 rpm for 10 minutes and P(3HB) was extracted in chloroform following the method stipulated by Green *et al.* [16].

Extracted P(3HB) was treated with concentrated sulfuric acid with heating for quantitative conversion to crotonic acid. The amount of P(3HB) was determined by measuring the concentrations of crotonic acid with SL164 double-beam UV-VIS spectrophotometer at 235 nm using ~ 98% sodium -DL-β-hydroxybutyric acid (Sigma chemical company) as a standard [17]. P(3HB) content was defined as the percentage of the ratio of P(3HB) to dry cell weight.

RESULTS AND DISCUSSIONS

Wild-type *Wautersia eutropha* and its screened mutants *W. eutropha* M2, *W. eutropha* M5 were tested with four different carbon sources to examine P(3HB) productivity. It can be concluded from Table 1 to Table 4 that *W.eutropha* M2 rendered higher P(3HB) yield than wild-type *W. eutropha* in fructose, dextrose and propionate. In methanol, the highest P(3HB) yield was gained by wild-type *W. eutropha*. P(3HB) yield given by *W. eutropha* M5 was lower than the wild-type *W. eutropha* in fructose, propionate and methanol, but higher in dextrose. *Hence, W.eutropha M2 was found to be capable of converting a wide range of carbon substrates into P(3HB).*

P(3HB) content increased with increasing concentration of fructose with 20 gL^{-1} concentration being optimum. At 20 gL^{-1} fructose concentration, P(3HB) yields by wild-type *W. eutropha* and *W. eutropha* M2 were 59.58% and 60.38% respectively.

An economically interesting finding was that P(3HB) yield by wild-type organism at 20 gL^{-1} fructose concentration (59.58%) was similar to that of P(3HB) yield by *W. eutropha* M2 at 10 gL^{-1} fructose concentration (59.22%) and noticeably biomass production rate was less in *W. eutropha* M2 than wild-type organism. This observation indicates that carbon flow is directed towards P(3HB) biosynthetic pathway rather than toward residual cell growth in mutant strain. In other words, the partial blockage of TCA cycle through mutagenesis might have induced carbon flow for the P(3HB) biosynthetic pathway rather than TCA cycle, which is closely associated with residual cell growth.

So, W. eutropha M2 was found to be capable of rendering higher P(3HB) content at a relatively low fructose concentration than wild-type organism. Park and Lee (1996) [18] reported similar type of result during screening of isicitrate dehydrogenase leaky mutant.

W. eutropha M5 showed relatively lower P(3HB) content when compared with other two strains. It showed more P(3HB) content at 30 g/l fructose concentration (56.70%) than 20 gL^{-1} (56.50%). So, 20 gL^{-1} fructose concentration was not found to be optimum for P(3HB) production by this strain.

Dextrose was more efficiently utilized by W. eutropha M5 rather than wild-type W. eutropha and W. eutropha M2. Probable reason for this might be change in degree of substrate specificity due to random mutagenesis. But all three strains displayed increasing P(3HB) content with increasing concentration of dextrose with the highest P(3HB) yield (52.52%) by *W. eutropha* M5 at a dextrose concentration of 30 gL^{-1}.

Propionate did not support bacterial growth as well as P(3HB) accumulation for all three test strains. *W. eutropha* M2 showed comperatively higher P(3HB) yield than wild-type *W. eutropha* and *W. eutropha* M5, with the highest yield of 38.76% at 8 gL^{-1} propionate concentration. Cell dry weight and P(3HB) content decreased with increasing concentration of propionate. Anderson and Dawes [19], Khanna and Srivastava [15], Madison and Huisman [20] reported similar inherent inhibitory effect of higher concentration of propionate on bacterial growth and P(3HB) production.

Methanol was reported not to support growth and P(3HB) accumulation for all three strains. *W. eutropha* M2 showed comparatively higher P(3HB) yield than wild-type *W. eutropha* and *W. eutropha* M5, with the highest yield of 25.27% at 8 ml/l methanol concentration.

Table 1: Effect of various concentrations of fructose on P(3HB) accumulation by screened strains of *Wautersia eutropha*

Fructose concentration (g/l)	*Vautersia eutropha*			*'autersia eutropha* M2			*Wautersia eutropha* M5		
	ry wt. (g/l)	3HB) (g/l)	(3HB) content %)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)
8	2.13	1.20	56.33	1.97	1.12	56.85	2.22	1.22	54.95
10	2.76	1.58	57.24	2.06	1.22	59.22	2.31	1.29	55.84
20	2.87	1.71	59.58	2.09	1.26	60.28*	2.46	1.39	56.50
30	2.91	1.72	59.10	2.14	1.27	59.34	2.61	1.48	56.70

All values – Mean of triplicates

Table 2: Effect of various concentrations of dextrose on P(3HB) accumulation by screened strains of *Wautersia eutropha*

Dextrose concentration (g/l)	*Wautersia eutropha*						*Wautersia eutropha* M5		
	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)
8	2.11	0.95	45.02	1.90	0.87	45.78	2.20	1.02	46.36
10	2.56	1.18	46.09	2.0	0.94	47.00	2.67	1.26	47.19
20	2.79	1.40	50.17	2.07	1.06	51.20	2.81	1.45	51.60
30	2.85	1.45	50.87	2.12	1.10	51.88	2.97	1.56	52.52*

All values – Mean of triplicates

Table 3: Effect of various concentrations of propionate on P(3HB) accumulation by screened strains of *Wautersia eutropha*

Propionate concentration (g/l)	*Wautersia eutropha*			*Wautersia eutropha* M2			*Wautersia eutropha* M5		
	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)
8	1.96	0.71	36.22	1.78	0.69	38.76*	1.60	0.55	34.37
10	1.26	0.43	34.12	1.32	0.47	35.60	1.13	0.37	32.74
20	0.75	0.23	30.66	0.88	0.29	32.95	0.74	0.21	28.37
30	0.32	0.09	28.13	0.56	0.17	30.35	0.37	0.10	27.03

All values – Mean of triplicates

Table 4: Effect of various concentrations of methanol on P(3HB) accumulation by screened strains of *Wautersia eutropha*

Methanol concentration (ml/l)	*Wautersia eutropha*			*Wautersia eutropha* M2			*Wautersia eutropha* M5		
	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)	Dry wt. (g/l)	P(3HB) (g/l)	P(3HB) content (%)
8	0.96	0.24	25.00	0.91	0.23	25.27*	0.94	0.21	22.34
10	0.91	0.21	23.07	0.83	0.19	22.89	0.81	0.16	19.75
20	0.62	0.12	19.35	0.56	0.11	19.64	0.62	0.07	11.29
30	0.37	0.06	16.21	0.42	0.07	16.66	0.40	Not detectable	Not detectable

All values – Mean of triplicates

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