

Tailoring PHBV using variable propionate-glucose media to produce high valerate content copolymers in *Chromobacterium violaceum* cultures

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Abstract – Copolymers of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) were produced by *Chromobacterium violaceum* to study the degree of incorporation of valerate units using glucose and propionate. Experiments were carried out in shaker under nitrogen limitation using different pH and glucose and propionate concentration. Results showed that substrate concentration, pH and addition of propionate affect the total amount of PHBV produced. Using glucose as sole carbon source, *C. violaceum* was able to incorporate 3HV units up to a maximum of 8.7%. The use of propionate improved the fraction of valerate units in the PHBV, reaching 17.6% of 3HV, but decreased the amount of biomass and copolymer production.

Polyhydroxyalkanoates (PHAs) are natural polyesters synthesized and accumulated by many bacteria as intracellular carbon and energy storage compounds [1,2]. *Chromobacterium violaceum* is a Gram-negative bacterium that is able to accumulate PHAs. It is capable of producing the homopolymer of polyhydroxyvalerate (PHV), and the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), that incorporates units of 3-hydroxybutyrate (3HB) [3,4]. This study focused on the degree of incorporation of valerate units in the PHBV produced by *C. violaceum*, what determines copolymer properties.

Copolymers of PHBV were produced by *C. violaceum* in shaker, at 30°C and 48 h, under nitrogen limitation, using different pH (6 and 7), concentration of glucose (10 and 30 g·l⁻¹) and propionate (0 and 10 mM). Biomass produced was determined by gravimetry and the fraction of monomers (3HB and 3HV) incorporated into the copolymer was determinate by gas chromatography (GC) [5].

Results showed that substrate concentration, pH and addition of propionate affect the total amount of PHBV produced (Table 1). A 70 wt% accumulation was obtained under nitrogen limitation in the shaker culture at pH 7, 10 g·l⁻¹ glucose, without addition of propionate. We found that *C. violaceum* was able to incorporate units of propionate using glucose as sole carbon source, obtaining 8.7% of 3HV at pH 6 and 30 g·l⁻¹ glucose. The use of 10 mM propionate improved the incorporation of valerate units in all the conditions tested, reaching 17.6% of 3HV, a 100% increase in selectivity, at the expense of biomass and copolymer amounts. The variable incorporation of valerate units by propionate co-fed glucose cultures opens up the possibility towards producing copolymers with tailored properties.

Table 1: Cultivation conditions, biomass (g·l⁻¹), PHBV (g·l⁻¹) and fraction of butyrate and valerate in 100 g of PHBV (g·g⁻¹) produced by *Chromobacterium violaceum* under nitrogen limitation in shaker at 30°C and 48 h.

pH	Glucose (g·l ⁻¹)	Propionate (mM)	Biomass (g·l ⁻¹)	PHBV ^a (g·l ⁻¹)	% 3HB ^b (g·g ⁻¹)	% 3HV ^b (g·g ⁻¹)
6	10	0	0.607	0.34	93.6	6.4
		10	0.259	0.16	83.3	16.7
	30	0	0.652	0.27	91.3	8.7
		10	0.296	0.16	82.4	17.6
7	10	0	2.437	1.65	98.3	1.7
		10	0.652	0.46	91.7	8.3
	30	0	2.719	1.76	98.4	1.6
		10	0.844	0.33	84.6	15.4

^a PHBV – poly(3-hydroxybutyrate-co-3-hydroxyvalerate).

^b g of 3HB or 3HV in 100g of PHBV.

References

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