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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

The impact of glycerol and some carbohydrates on antibiotic production by *Streptomyces hygroscopicus* CH-7

Slavica B. Ilić · Sandra S. Konstantinović · Gordana Đ. Gojgić Cvijović · Dragiša S. Savić · Vlada B. Veljković

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Abstract The impact of different carbon sources on the antibiotic production by *Streptomyces hygroscopicus* CH-7 was studied with the main goal to increase the yield of antibiotics hexaene H-85 and elaiophylin. Glucose, as a basic carbon source in the nutrition medium, was replaced with glycerol, xylose, sorbose, melibiose, inulin, and mannitol (15 g/dm^3). Insuring the maximum yields of hexaene and elaiophylin of 192 and 88 μg/cm^3, respectively, glycerol was shown to be the best carbon source among the investigated ones.

Keywords Antibiotics · Carbon source · Glycerol · Hexaene · Elaiophylin · Streptomyces · *Streptomyces hygroscopicus* CH-7

Introduction

Streptomyces hygroscopicus, aerobic bacteria from the actinomycetes group, is known as a producer of different polyenic antibiotics depending on the environmental and nutritional conditions (Vučetić *et al*., 1994; Karadžić *et al*., 1991). A way to increase the antibiotic formation usually involves a search for the optimal medium. This is achieved by a systematic study of the suitability of large number of carbon and nitrogen sources (Pandey *et al*., 2005). *S. hygroscopicus* CH-7, a strain isolated from a soil sample, was shown to produce three antibiotics: polyenic hexaene H-85, polyetheric nygericine, and macrodiolide elaiophylin [Vučetić *et al*., 1994; Karadžić *et al*., 1991].

Intensive studies of the antibiotic production on different carbon sources have shown different results depending primarily on the strain of microorganism. For example, strain *S. hygroscopicus* 111-81, which was isolated from a soil sample in Bulgaria (Gesheva *et al*., 2005), produces a similar group of antibiotics as well as the *S. hygroscopicus* strain CH-7. Gesheva *et al*. showed that mixtures of carbohydrates (glucose, lactose, and glucose as well as glucose and sucrose) and glycerol stimulated a biosynthesis of azalomycine B (elaiophylin) by *S. hygroscopicus* 111-81, while glucose, glycerol, sucrose, fructose, and starch stimulated the production of polyetheric antibiotics and azalomycine B (elaiophylin) (Gesheva *et al*., 2005). The same research group showed that the best carbon source for the biosynthesis of macrolide antibiotics from *S. hygroscopicus* 111-81 was lactose (Gesheva *et al*., 2005). Vinogradova *et al*. (1985) detected high content of heliomicin with lactose as a carbon source in the culture medium of *S. hygroscopicus* 111-81 (Vinogradova *et al*., 1985). Sanchez and Demain (2002) also confirmed the positive effect of lactose on the biosynthesis of penicillin, erythromycin, and eniatina by *Streptomyces* (Sanchez and Demain, 2002). Bhattacharyya *et al*. reported that glycerol and glucose are the best carbon sources for growth and antibiotic production by *S. hygroscopicus* (Bhattacharyya *et al*., 1998). The medium with lactose as a carbon source has a great influence on production of elaiophylin by *S. hygroscopicus* strain CH-7, compared to the basal medium with glucose (Ilić *et al*., 2010a, b).

S. B. Ilić · S. S. Konstantinović (✉) · D. S. Savić · V. B. Veljković
Faculty of Technology, University of Niš, Bulevar oslobodjenja 124, Leskovac, Serbia
e-mail: sakisandra12@yahoo.com

S. B. Ilić
e-mail: ilicslavica@yahoo.com

G. Đ. Gojgić Cvijović
Department of Chemistry, University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Nemanjina 12, Belgrade, Serbia

Glycerol is known to be of low toxicity, low price, large availability, and large renewability. It is obtained as a byproduct in biodiesel production in large amounts. It is interesting to find new possibility for using glycerol such as a carbon source for antibiotic production by *S. hygroscopicus*.

The present paper describes the utilization of different carbon sources such as glucose, xylose, sorbose, melibiose, inulin, glycerol, and mannitol for antibiotic production by *S. hygroscopicus* CH-7. The main goal was to compare the suitability of glycerol as a carbon source for the antibiotics hexaene H-85 and elaiophylin with the compound commonly used in production of antibiotics.

Materials and methods

Strain and nutrition media

The used strain *S. hygroscopicus* CH-7 was gained from Collection of Microorganisms of Faculty of Chemistry in Belgrade. The producing microorganism was grown on the basic and modified nutrition media. The former contained (in g/dm^3): glucose 15.0 (Merck, Germany), $CaCO_3$ 3.0 (Alkaloid, Skopje), NaCl 3.0 (Alkaloid, Skopje), $MgSO_4$ 0.5 (Merck, Germany), $(NH_4)_2HPO_4$ 0.5 (Merck, Germany), K_2HPO_4 0.5 (Merck, Germany), and soybean 1.0 (Banat, Vršac), and the latter ones had the same composition as the basic one except that glucose was replaced with glycerol, xylose, sorbose, melibiose, inulin, and mannitol (15 g/dm^3) (Merck, Germany). The nutrition medium was autoclaved in 20 min at 121 °C before use.

Antibiotic production

A nutrition medium (100 ml) was poured in the sterile Erlenmeyer flasks (1 l). The sterile medium was inoculated with a 48 h culture (5 ml) under sterile conditions. The culture flasks were fixed on a rotary shaker (200 rpm; rotation diameter: 2.0 cm) placed in a thermostated cabinet at 28 °C. Samples (10 ml) were taken periodically during the 7 days fermentation process (Ilić *et al.*, 2008, 2010a, b). Hexaene H-85 and elaiophylin were first extracted from the fermentation broth by *n*-butanol and ethyl acetate, respectively.

Analytical methods

The microbial growth was followed by measuring the dry biomass weight. The fermentation broth was centrifuged at 4,000 rpm for 15 min to separate the mycelial biomass. The biomass was then dried at 105 °C to constant weight (Ilić *et al.*, 2008, 2010a, b).

Concentrations of hexaene H-85 and elaiophylin were spectrophotometrically (Perkin–Elmer Lambda 15 UV/VIS spectrophotometer) determined by measuring the absorbance at $\lambda_{max} = 364$ and 252 nm (Vučetić *et al.*, 1994; Karadžić *et al.*, 1991; Ilić *et al.*, 2008, 2010a, b).

Results and discussion

Figure 1 shows the variations of the dry biomass with the progress of the fermentation. The concentration of dry biomass increased until the third to fourth day of the fermentation and then decreased. Values of the maximum dry biomass concentration achieved during the growth on different carbon sources are given in Table 1. As it can be seen, the highest dry biomass concentration was reached in the nutrition medium with glycerol (10.3 g/dm^3). In the media with monosaccharides, a maximum concentration of dry biomass was lower than that in the basic medium with glucose (Sanchez and Demain, 2002). Even comparing with the results gained from carbohydrates used in previous work (Sanchez and Demain, 2002), such as fructose, ribose, trehalose, and lactose, the glycerol was the best carbon source for producing both antibiotics. The carbon source needed for maximal yield of the antibiotic production seems to be different among bacterial strains. Lactose was shown to be the best carbon source for production of nonpolyenic macrolide antibiotic, antifungal peptide and biosynthesis of penicillin and erythromycin from strain *S. hygroscopicus* (Gesheva *et al.*, 2005; Vinogradova *et al.*, 1985; Sanchez and Demain, 2002). Lactose as a carbon source has a great influence on production of elaiophylin which is macrodiolide antibiotic by *S. hygroscopicus* CH-7, compared to the basal medium with glucose (Ilić *et al.*, 2010a, b). Lactose also has a positive effect on production of polyenic antibiotic hexaene H-85 but less than glucose and glycerol (Ilić *et al.*, 2010a, b). The microbial growth on the medium containing disaccharides was also reduced relative to that achieved on the base substrate (Fig. 1). The biomass formation was significantly reduced when mannitol or inulin was replaced by glucose in the nutrition medium.

In Fig. 2, variations of the hexaene H-85 production with time are shown, and values of its maximum concentration achieved are given in Table 1. Depending on the carbon source, the production of hexaene H-85 was in the range between 73 μg/cm^3 (inulin) and 192 μg/cm^3 (glycerol). In the basic nutrition medium, with glucose as the carbon source, the maximum concentration of hexaene H-85 (169 μg/cm^3) was reached in the second day of fermentation. The lowest amount of produced hexaene was obtained in the media with sorbose, melibiose, inulin, and mannitol. The reduction of the hexaene H-85 yield on the substrate with melibiose and mannitol were 19 % (14.4 μg/g

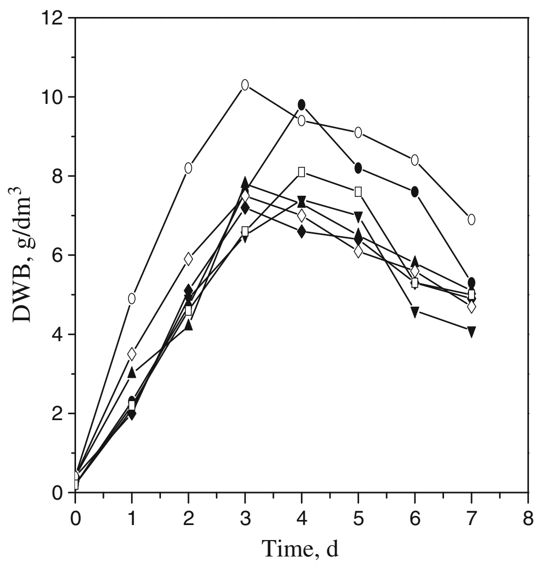

Fig. 1 Change of concentration of dry weight biomass (DWB) in the basal medium (*filled circle*) and in the media with xylose (*open square*), sorbose (*filled down triangle*), melibiose (*filled up triangle*), inulin (*filled diamond*), glycerol (*open circle*), and mannitol (*open diamond*)

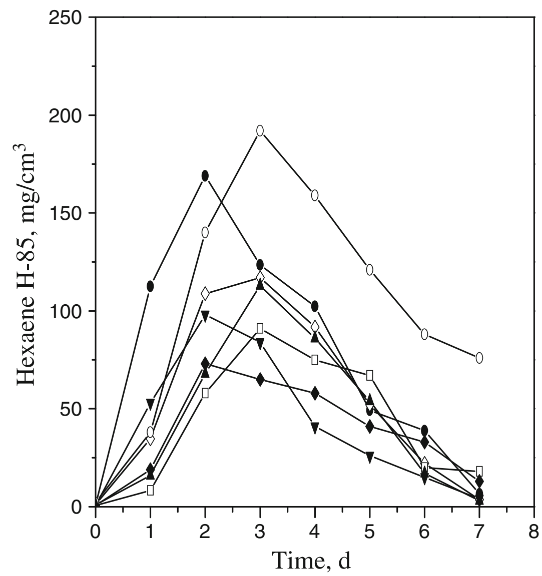

Fig. 2 Change of concentration of hexaene H-85 in basal medium (*filled circle*) and media with xylose (*open square*), sorbose (*filled down triangle*), melibiose (*filled up triangle*), inulin (*filled diamond*), glycerol (*open circle*), and mannitol (*open diamond*)

Table 1 The influence of different carbon sources on the maximum dry biomass concentration (X_{max}) and the maximum antibiotic concentration (C_{max}) and yield (Y_{max})

Carbon source	X_{max}	Hexaene H-85		Elaiophylin	
		C^{H}_{max}	Y^{H}_{max}	C^{E}_{max}	Y^{E}_{max}
	g/dm^3	μg/cm^3	μg/g$_{d.b}$	μg/cm^3	μg/g$_{d.b}$
Glucose (Ilić *et al*., 2010b)	9.8	169	17.2	64	6.5
Xylose	8.1	91	11.2	21	2.5
Sorbose	7.4	98	13.2	23	3.1
Melibiose	7.8	113	14.4	20	2.5
Inulin	7.2	73	10.1	14	1.9
Glycerol	10.3	192	18.6	88	8.5
Mannitol	7.5	117	15.6	37	4.9

C^{H}_{max} maximum concentration of hexaene H-85, Y^{H}_{max} maximum yield of hexaene H-85, C^{E}_{max} maximum concentration of elaoiphylin, Y^{E}_{max} maximum yield of elaoiphylin, and $g_{d.b}$ gram of dry biomass

per dry biomass) and 10 % (15.6 μg/g per dry biomass), respectively, compared to that achieved in the basic medium (17.2 μg/g per dry biomass). The minimum concentration of hexaene (73 μg/cm^3) was obtained when inulin was used as carbon source, the yield being 1.7 times smaller than that obtained in the medium with glucose. The highest concentration of hexaene H-85 of 192 μg/cm^3 was achieved with the medium containing glycerol at the third day of fermentation, when an increase in the yield, compared to that in the basic medium, was 8 %. The mechanism for catabolite repression in *Streptomyces* is not clearly understood, but appears to be unique in the way it regulates the rate of carbon consumption (Jonsbu *et al*., 2002). *Streptomyces* as chemoautotrophic organism requires synthetic medium for their cultivation. Glycerol, as a carbon source, was found to support growth and antibiotic production most convincingly (El-Banna, 2006). Glycerol supported a better antibiotic production by *S. hygroscopicus* D 1.5 than glucose (El-Banna, 2006).

Figure 3 illustrates changes in the elaiophylin production with time during the fermentation at different carbon sources, while maximum values of the elaiophylin concentration are presented in Table 1. The maximum concentration of elaiophylin (64 μg/cm^3) in the basic medium was reached in the third day of fermentation. The highest production of elaiophylin was also achieved with glycerol as a carbon source. This is in accordance to the results obtained for production of elaiophylin by *S. hygroscopicus* 111-81 (Gesheva *et al*., 2005). Its maximum concentration reached in the third day of fermentation was 88 μg/cm^3 (31 % yield increase compared to basic medium). The lowest production was gained when glucose was replaced with inulin, melibiose, xylose, and sorbose (yield 2.1–3.4 times lower compared to basic medium).

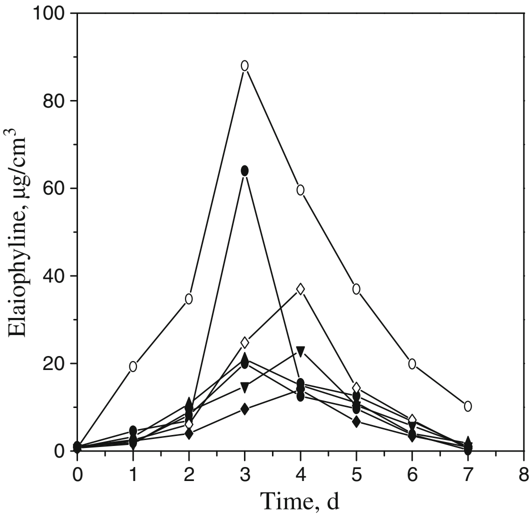

Fig. 3 Change of concentration of elaiophylin basal medium (*filled circle*) and media with xylose (*open square*), sorbose (*filled down triangle*), melibiose (*filled up triangle*), inulin (*filled diamond*), glycerol (*open circle*), and mannitol (*open diamond*)

Conclusion

This study showed that glycerol, xylose, sorbose, melibiose, inulin, and mannitol as carbon sources have a different effect on antibiotic production by *S. hygroscopicus* CH-7. Glycerol was shown to be the best carbon source among the investigated ones. The maximum yields of hexaene and elaiophylin obtained with glycerol were 192 and 88 μg/cm^3, respectively. These results indicate the need for further investigations considering the applications of glycerol from biodiesel production where it is a by-product.

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