

Intensification of microbial exopolysaccharide ethapolan synthesis under *Acinetobacter* sp. IMV B-7005 cultivation on sunflower oil

Mykola Ivahniuk, Tetyana Pirog

National university of food technologies, Kyiv, Ukraine

Abstract

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Corresponding author:

Mykola Ivahniuk
E-mail:
Ivahniuk@mail.ru

Introduction. Microbial exopolysaccharides (EPS) by the ability of their solutions to change the rheological properties of aqueous systems are widely used in various industries. In recent years, research on the use of industrial waste (including oil-containing) to obtain practically valuable microbial metabolites intensified.

Materials and methods. Cultivation of *Acinetobacter* sp. IMV B-7005 strain was performed in liquid medium, containing as a carbon source sunflower oil (1–5 %, v/v), a source of nitrogen – ammonium nitrate (0.4–0.8 g/l), a source of pantothenate – multivitamin complex «Complevit» (0.00085 and 0.00095 %). EPS concentration was determined gravimetrically after precipitation with isopropanol, EPS-synthesizing ability – as a ratio of EPS concentration to biomass concentration, which was expressed as g EPS / g biomass.

Results and discussions. It was established that increasing the concentration of sunflower oil in basic medium for *Acinetobacter* sp. IMV B-7005 cultivation to 4–5% was accompanied by decrease of ethapolan synthesis compared with those in the medium containing lower (2–3 %) substrate concentration. Increasing ammonium nitrate content to 0.6 g/l and/or pantothenate concentration to 0.00095% in a medium with 5% sunflower oil allowed to increase the amount of ethapolan synthesized up to 6.6–6.7 g/l, that is in 1.3–1.4 times higher than in the basic medium with the same concentration of the substrate but lower NH_4NO_3 (0.4 g/l) and pantothenate (0.00085 %).

Conclusion. The obtained results indicate the possibility of microbial polysaccharide ethapolan synthesis under *Acinetobacter* sp. IMV B-7005 cultivation in the medium with a high content of sunflower oil. These data are the basis for the development of ethapolan technology using as a substrate fried oil.

Introduction

Microbial exopolysaccharides (EPS) due to the ability of their solutions to gelation, emulsification, suspending and changing rheological properties of aqueous systems are widely used in various industries, agriculture and medicine [1, 2].

The vast majority of known microbial EPS are obtained from carbohydrate substrates. Usually, products derived from sugar beet: molasses, sugar syrup, sucrose or corn: starch, hydrolyzed starch, glucose syrup, glucose, maltose are used as substrates in the industrial production of EPS [3]. But studies conducted in the 70-80s of the twentieth century demonstrated the possibility of expanding the resource base of microbiological production of EPS by using of non-food substrates (methane, methanol, ethanol, ethylene glycol, hydrocarbons) [3]. However, the concentration of polysaccharides obtained on non-carbohydrate substrates remains low for today.

Our studies have shown that a wide range of mono- and mixed C₂-C₆-substrates (ethanol, acetate, propanol, pyruvate, C₄-dicarboxylic acids, carbohydrates – mono- and disaccharides, starch, molasses, etc.) can be used for the synthesis of ethapolan – complex exopolysaccharide preparation (producer is *Acinetobacter* sp. 12S, deposited in the Depository of the Institute of Microbiology and Virology, National Academy of Sciences of Ukraine by the number of IMV B-7005) [3]. The ability of *Acinetobacter* sp. IMV B-7005 to form EPS on C₂-C₆ compounds allows to develop a flexible universal technology of polysaccharide production from a wide set of carbon substrates, or complex of different technologies, each of which can be realized depending on the economic feasibility, availability and accessibility of a substrate necessary to obtain the EPS with certain physical and chemical properties.

Last years the researches of using industrial waste have been activated to obtain a practically valuable microbial metabolites [4]. Replacing traditional substrates for microbial synthesis by industrial waste will allow to reduce the cost of technology in several times, and recycle unwanted waste, to solve the problem of storage or destruction of large masses of waste in food industry, agricultural sector and in companies that produce biodiesel, as it needs a lot of energy and money. Oil-containing waste are promising for using in microbial technologies [5, 6].

The world production of sunflower oil is about 2.5–3 million tons, 75 % of which is obtained mainly from plant raw materials [6]. Significant amount of waste produces on the enterprises which recycling such materials, and its getting into the environment is extremely dangerous [4, 5]. Oil-containing waste are cheap and available in necessary quantities for using in microbial technologies, but still there are only a few reports in the literature about the possibility of its using as substrate for the biosynthesis of microbial polysaccharides. Thus, there is information concerning of use of waste water from plants of processing oils for the synthesis of EPS [7]. In recent years *Cellulomonas flavigena* UNP3 was described as the strain, which is able to synthesize kurdlan-like EPS in the medium with vegetable oil or appropriate waste [8].

Previously, we have established the possibility to use sunflower oil as a source of carbon and energy for the synthesis of microbial polysaccharide ethapolan [9]. However, in earlier studies, the concentration of oil in the cultivation medium was low (only 1 % v/v). As for the synthesis of ethapolan we supposed to use fried oil as a substrate, volume of which is extremely large, so its content in the medium has to be more higher.

The purpose of this work – to research intensification of microbial polysaccharide ethapolan synthesis in medium with the maximum concentration of sunflower oil.

Materials and methods

EPS-synthesized strain of bacteria *Acinetobacter* sp. 12S, which is deposited in the Depository of Institute of Microbiology and Virology, National Academy of Sciences of Ukraine by the number of IMV B-7005 was used as the object of research.

Cultivation of *Acinetobacter* sp. IMV B-7005 was carried out in a liquid mineral medium of such composition (g/l): KH_2PO_4 – 6.8; KOH – 0.9; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ – 0.4; $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ – 0.1; NH_4NO_3 – 0.4; $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ – 0.001. In one variant, the concentration of ammonium nitrate in the medium was increased to 0.6 and 0.8 g/l.

Sunflower oil (1–5 %, v/v) was used as a source of carbon and energy. In additionally yeast autolysate (0.5 %, v/v) and multivitamin complex "Complevit" (0.00085 and 0.00095 %) were added to the medium as growth promoter and source of pantothenate, respectively.

Culture from the exponential phase, grown in the medium with 0.5 % of sunflower oil was used as the inoculum. Quantity of inoculum was 10 % from the volume of the medium.

Cultivation of *Acinetobacter* sp. IMV B-7005 was carried out in flasks (750 ml) with 100 ml of medium in shaker (320 rpm) at 30 °C for 120 hours.

Growth of the strain and EPS synthesis were evaluated by the following parameters.

Biomass concentration was determined by optical density of the cell suspension with the following recalculation on the absolutely dry biomass (ADB) according to the calibration curve. Quantity of synthesized ethapolan was determined gravimetrically. For this, 1.5–2 volumes of isopropanol were added to a certain amount of culture liquid (usually 10–15 ml), the precipitate of EPS was washed by clean isopropyl alcohol and dried at room temperature for 24 h. EPS-synthesizing ability was determined as the ratio of the EPS concentration to the concentration of ADB and was expressed in g EPS/g ADB.

The results of the experiment in accordance with the Student t-test were statistically significant at the 5 % significance level.

Results and discussions

Note, that the literature data about synthesis of microbial EPS on any industrial waste (not just oil-containing) is extremely limited. So, it is known that *Xanthomonas campestris* ATCC 13951 synthesized 28 g/l of xanthan under cultivation in reactor (2 l) during 96 h in the medium containing partially hydrolyzed molasses (the concentration of lactose, galactose, glucose was 4.7; 17.8; 17.8, respectively) as the carbon source [10]. It was determined that *Pseudomonas oleovorans* NRRLB-14682 synthesized EPS (12.18 g/l) on the medium with crude glycerol (by-product of biodiesel production) [11]. *Acinetobacter* sp. DR1 under cultivation in the medium with diesel oil (2 %) synthesized about 5 g EPS/g biomass [12]. It is known about synthesis of scleroglucan by fungi *Sclerotium rolfsii* from plant biomass [13]. Strain *C. flavigena* UNP3 synthesized 1 g/l of polysaccharide with high emulsifying properties in the medium containing 1 % of peanut oil after 192 h cultivation [8]. Parameters of EPS synthesis slightly decreased in case of replacement peanut oil with coconut, olive, castor, sesame, mustard and cotton oils. It should be noted, that until now in the available literature we couldn't find information about the synthesis of microbial EPS on sunflower oil.

Our previous data [9] have shown that during *Acinetobacter* sp. IMV B-7005 growth in medium with 1 % of sunflower oil, 5 g/l of EPS were synthesized. Further studies demonstrated that increasing sunflower oil content in the medium of IMV B-7005 strain to 2-3% was accompanied by increasing of synthesized ethapolan concentration to 5.8-6.3 g/l, but the EPS-synthesizing ability was slightly decreased (Table 1). Indices of EPS synthesis

decreased with the higher substrate concentration (4–5 %) and the highest EPS-synthesizing ability (5 g EPS/g ADB) was observed under *Acinetobacter* sp. IMV B-7005 cultivation in the medium with 1 % of sunflower oil (Table 1).

Table 1
Depending ethapolan synthesis on the concentration of sunflower oil in the cultivation medium of *Acinetobacter* sp. IMV B-7005

Concentration of sunflower oil in the medium, %	EPS, g/l	EPS-synthesizing ability, g EPS/g ADB
1	5.0±0.25	5.0±0.25
2	5.8±0.29	4.7±0.23
3	6.3±0.31	4.0±0.20
4	5.0±0.25	3.7±0.19
5	4.9±0.24	3.6±0.18

Note. The concentration of pantothenate in the medium was 0.00085 %, ammonium nitrate – 0.4 g/l.

As in case of increasing of carbon's concentration in the medium, C/N ratio changes, that significant impacts on synthesis of microbial polysaccharides [3], so on the next stage we increased concentration of nitrogen source simultaneously with enhancing of oil content (Table 2).

Table 2
The influence of the nitrogen source concentration on the synthesis of ethapolan under *Acinetobacter* sp. IMV B-7005 cultivation on sunflower oil

Concentration of ammonium nitrate, g/l	Concentration of sunflower oil in the medium, %	EPS, g/l	EPS-synthesizing ability, g EPS/g ADB
0.6	3	4.6±0.23	4.1±0.21
	4	5.6±0.28	4.2±0.21
	5	6.4±0.32	3.9±0.19
0.8	3	3.2±0.16	3.0±0.15
	4	3.4±0.17	2.9±0.14
	5	3.6±0.18	2.7±0.13

Note. The concentration of pantothenate in the medium was 0.00085 %.

Results presented in Table 2, show that increasing ammonium nitrate concentration to 0.8 g/l in a medium containing 3–5 % of sunflower oil promotes decrease of synthesized ethapolan concentration and EPS-synthesizing ability compared with those in the medium with lower (0.4 g/l) concentration of nitrogen sources (see Table 1 and 2). However, concentration of synthesized ethapolan in the medium with 4 and 5 % of sunflower oil and 0.6 g/l of NH_4NO_3 was 5.6 and 6.4 g/l, respectively. That is higher than in medium with 0.4 g/l of ammonium nitrate (5.0 and 4.9 g / l, see. Table. 1 and 2). EPS-synthesizing ability also increased under such cultivation conditions of IMV B-7005 strain. Thus, parameters of ethapolan synthesis were improved by increasing NH_4NO_3 concentration to 0.6 g/l with increase of oil content to 4–5 % in the medium.

The concentration of pantothenate in the medium is another factor that may affect on synthesis of ethapolan, as *Acinetobacter* sp. IMV B-7005 is auxotroph for calcium pantothenate [3]. Therefore, on the next stage concentration of pantothenate in the cultivation medium of IMV B-7005 strain was increased with enhancing sunflower oil and nitrogen source content (Table 3).

Thus, increasing of pantothenate content to 0.00095 % in medium with 0.4 g/l of ammonium nitrate and 5 % of sunflower oil allowed to enhance the concentration of EPS in 1.4 times, comparing with results in the medium with lower amount of pantothenate.

Table 3
Synthesis of ethapolan depending on the concentration of pantothenate in *Acinetobacter* sp. IMV B-7005 medium with sunflower oil

Concentration in the medium			EPS, g/l
of ammonium nitrate, g/l	of pantothenate, %	of sunflower oil, %	
0.4	0.00085	4	4.8±0.24
		5	4.9±0.24
	0.00095	4	5.6±0.28
		5	6.7±0.33
0.6	0.00085	4	5.6±0.28
		5	6.4±0.32
	0.00095	4	5.5±0.27
		5	6.6±0.33

However, no positive effect on the synthesis of ethapolan with higher concentrations of pantothenate and NH₄NO₃ (0.6 g/l) in the medium was observed (Table 3).

Conclusions

As a result of this work cultivation's conditions were established for producer of microbial exopolysaccharide ethapolan. They provide synthesis of 6.6–6.7 g/l of EPS in the medium with a high content of sunflower oil (4–5 %). These results were achieved in the case of both increasing of nitrogen sources content to 0.6 g/l and/or pantothenate – up to 0.00095 % with increasing of the substrate concentration for ethapolan synthesis. The experimental data are basic for the development of this polysaccharide technology in the medium with fried sunflower oil or other oil-containing industrial waste.

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