POTENTIAL OF *Xanthomonas* STRAINS ISOLATED IN SERBIA FOR XANTHAN PRODUCTION FROM CRUDE GLYCEROL

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Abstract
Xanthan is a natural polysaccharide that is biosynthesized by metabolic activity of Gram-negative bacteria from the genus *Xanthomonas*. Commercial production of xanthan is mainly performed as aerobic submerged batch cultivation of the reference strain *X. campestris* ATCC 13951 on a medium of appropriate composition and under optimal conditions. The success of xanthan production is largely influenced by the selection of producing strains, formulation of cultivation medium composition, and control and regulation of bioprocess parameters. The aim of this study was to examine xanthan production on medium containing crude glycerol from domestic biodiesel industry using reference strain *Xanthomonas campestris* ATCC 13951 and *Xanthomonas* strains locally isolated from different vegetable cultures. Xanthan was produced by submerged cultivation of examined producing strains at a laboratory level under aerobic conditions at 30°C and 150 rpm for 168 h. Bioprocess efficacy was estimated based on the xanthan concentration in media at the end of biosynthesis. According to the obtained results, xanthan production on crude-glycerol based medium was possible by all applied *Xanthomonas* strains. The highest potential for successful xanthan production has PL4 strain, while strain Xp 3-1 has the lowest ability to biosynthesize xanthan in applied experimental conditions.
Keywords: Biodiesel industry, crude glycerol, biotechnological production, xanthan, *Xanthomonas* isolates

Introduction
Biopolymers are attracting enhanced attention due to environmental concerns and their outstanding properties. Among them, microbial biopolymers are a promising alternative for existing polymers. Xanthan represents most commercially successful example of a microbial exopolysaccharide, which is produced by metabolic activity of Gram-negative bacteria from the genus *Xanthomonas* [1]. Because of its unique chemical structure, outstanding rheological properties, biodegradability, non-toxic nature and biocompatibility, this biopolymer is widely used as a thickener, rheological modifier, stabilizer, and emulsifier in the food, biomedical, pharmaceutical, petrochemical, chemical, and textile industry [2-4]. Xanthan occurs as a white or cream-colored free-flowing powder of neutral smell and taste [5] and has been classified as a food additive number E 415 by the European List of Permitted Food Additives, while the United States Food and Drug Administration has given the GRAS status (Generally Recognized as Safe) to an ethanol precipitate of xanthan [6]. Xanthan production is mainly performed as aerobic submerged batch cultivation of reference strain *X. campestris* ATCC 13951 on the medium of appropriate composition and under optimal conditions. Sucrose and glucose are mostly used as carbon sources in medium for xanthan production [7]. Taking into account that the cost of substrate is an important factor for commercial xanthan production and that an actual rise in prices of aforementioned sugars is present, it is clear that there is a need for exploitation of carbon sources of lower price in order to reduce the overall production costs [8]. Use of waste streams and by-products from different industries which contain aforementioned carbon sources can reduce the total costs of xanthan production, as well as reduce the environmental

pollution caused by their disposal into environment [9]. Since the previous research have confirmed that *Xanthomonas* strains have the ability to metabolize glycerol it can be concluded that crude glycerol from the biodiesel industry have a great potential to be one of the promising alternative substrates of lower market value which may be used as a carbon source in the cultivation medium for xanthan production [8, 10].
The aim of this study was to examine xanthan production on media containing crude glycerol from domestic biodiesel industry by reference strain *Xanthomonas campestris* ATCC 13951 and *Xanthomonas* strains locally isolated from different vegetable cultures. The bioprocess efficacy was estimated based on xanthan concentration in media at the end of biosynthesis.

Experimental

Producing microorganisms

The reference strain *X. campestris* ATCC 13951, eight *Xanthomonas* strains isolated from crucifers (Am, CF, CB, KA, Xp 3-1, Xp 7-2, Mn 7-2, 12-2) and five *Xanthomonas* strains isolated from pepper leaves (PL1, PL2, PL3, PL4, PL5) were used as the producing microorganisms in these experiments. All strains were stored at 4ºC on agar slant (Yeast Maltose Agar, HiMedia, India) and subcultured every four weeks. *Xanthomonas* strains isolated from infected crucifers were stored in the Microbial Culture Collection of the Faculty of Technology Novi Sad, Serbia and *Xanthomonas* strains isolated from infected pepper leaves were stored in the Microbial Culture Collection of the Faculty of Agriculture in Novi Sad, Serbia.

Cultivation media

Agar slant was used for refreshing of producing microorganisms, and commercial liquid medium (Yeast Maltose Broth, HiMedia, India) was used for incubation of producing microorganisms in inoculum preparation procedure. Xanthan biosynthesis was performed on medium containing crude glycerol from the biodiesel production in the Republic of Serbia. Glycerol content in the medium was 20.00 g/L. The cultivation medium also contained yeast extract (3.0 g/L), $(NH_4)_2SO_4$ (1.5 g/L), K_2HPO_4 (3.0 g/L) and $MgSO_4 \cdot 7H_2O$ (0.3 g/L). The pH value of all used media was adjusted to 7.0±0.2 and then sterilized by autoclaving (121°C, 2.1 bar, 20 min).

Inoculum preparation

Xanthomonas strains were subcultured on agar slant and incubated at 25°C for 48 h. Further, inoculum preparation procedure was included suspending of producing microorganism cells in commercial liquid medium. The prepared suspension was then incubated in aerobic conditions at 25°C and 150 rpm (laboratory shaker KS 4000i control, Ika® Werke, Germany) for 48h.

Xanthan production

The xanthan production was carried out in 300 mL Erlenmeyer flasks with 100 mL of the cultivation medium. Inoculation was performed by adding 10% (v/v) of inoculum prepared as previously described. The biosynthesis was performed under aerobic conditions at 30°C and 150 rpm (laboratory shaker KS 4000i control, Ika® Werke, Germany) for 168 h.

Xanthan separation

At the end of biosynthesis, the xanthan was separated from the supernatant of cultivation medium by precipitation with cold 96% (v/v) ethanol, as described in previous research [11].

Results and discussion

In accordance with the defined aim of this research, xanthan was produced by reference strain *X. campestris* ATCC 13951 and local *Xanthomonas* isolates on medium prepared with crude glycerol generated in domestic biodiesel facility. In order to conduct mutual comparison of the obtained results and to easier interpret them, experimental data for xanthan concentration in media at the end of biosynthesis were relativized by assigning the largest obtained value to be maximally successful (100%) while the other values were expressed in a proportion of the maximum. The relativized values of xanthan concentration in media at the end of biosynthesis are shown in Figure 1.

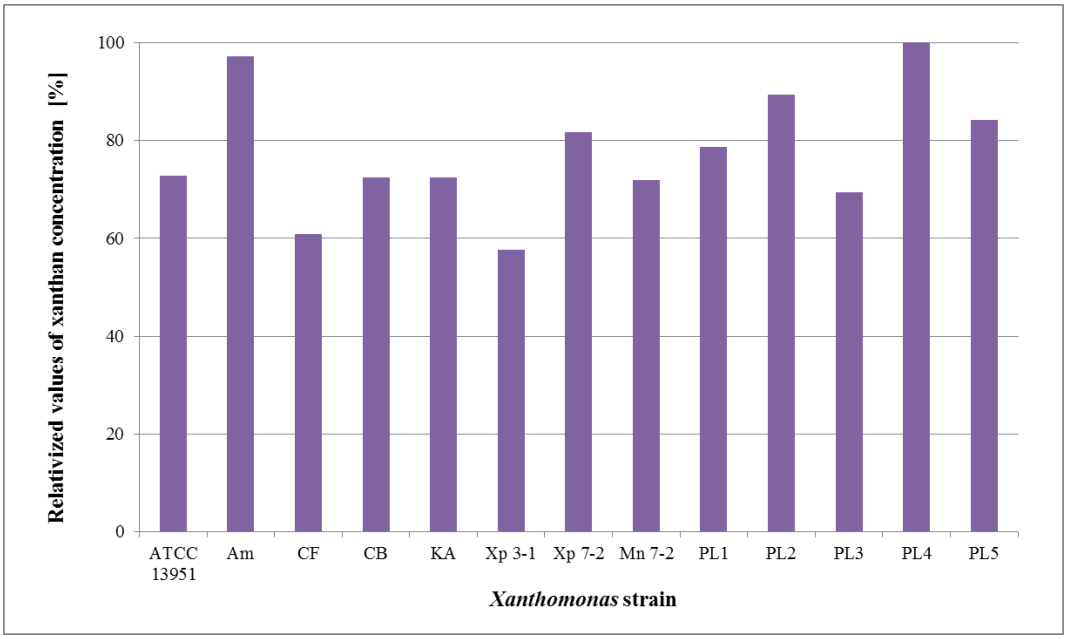

Figure 1. Relativized values of xanthan concentration in medium at the end of biosynthesis by different *Xanthomonas* strains

From the graphically presented results (Figure 1) it can be noticed that cultivation of strain PL4 exhibited the maximal xanthan production, hence it is considered as 100% successful, and in relation to this, the other values of xanthan concentration in media were relativized. Xanthan production by *Xanthomonas* strain Am was also very successful since the relativized value of xanthan concentration in medium amounted 97.25%. More than 80% of success was achieved by strains PL2, PL5 and Xp 7-2, based on the relativized values of xanthan concentration in medium of 89.40%, 84.09% and 81.66%, respectively. PL1 strain, reference strain *X. campestris* ATCC 13951, as well as strains CB, KA and Mn 7-2 showed somewhat lower ability to produce xanthan on crude glycerol containing medium considering that the success of xanthan production by these strains was 78.60%, 72.74%, 72.50%, 72.34% and 71.80%, respectively. The obtained results show that success of xanthan production by strains PL3, 12-2 and CF was 69.35%, 63.73% and 60.77%, respectively, indicating that these strains have low ability to produce xanthan on crude glycerol containing medium. However, Xp 3-1 strain exhibited the lowest ability to produce xanthan on medium with crude glycerol in applied experimental conditions since the success of this bioprocess was 57.57%.

The results obtained in this study indicate that all the used *Xanthomonas* strains isolated in Serbia have ability to biosynthesize xanthan on medium containing crude glycerol from domestic biodiesel industry. The highest potential for successful xanthan production on crude glycerol-based medium have PL4 strain, while strain Xp 3-1 has the lowest ability to produce xanthan in applied experimental conditions. The difference in productivity among previously discussed *Xanthomonas* strains is probably due the fact that different *Xanthomonas* species possess different metabolic pathways and cycles [12].

Conclusions

The results of this study have confirmed that reference strain *X. campestris* ATCC 13951, as well as *Xanthomonas* strains isolated from crucifers and pepper leaves have the ability to produce xanthan on medium containing crude glycerol from the biodiesel industry of the Republic of Serbia. This is very important from economic aspect considering that crude glycerol can substitute commonly used carbon sources in cultivation media for xanthan and reduce the costs of cultivation media preparation which leads to the reduction in the total production costs. Results of this study have also a great importance from an ecological aspect, since the biotechnological production of xanthan on crude glycerol-based media represents a promising solution for valorisation of this effluent. Results obtained in this study represent valuable information that can be used in further investigation related to development of biotechnological production of xanthan on crude glycerol-based media.

Acknowledgments

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