

Screening of Local Wild *Xanthomonas* Species for Xanthan Production on Crude Glycerol-based Medium

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Abstract

In this study, the effect of cultivation time on xanthan biosynthesis by different *Xanthomonas campestris* and *Xanthomonas euvesicatoria* strains, isolated from crucifers and pepper leaves, respectively, was examined. Xanthan was produced by submerged cultivation on crude glycerol-based medium at a laboratory level under aerobic conditions at 30 °C and 150 rpm for 168 h and 240 h. Bioprocess efficacy was estimated based on the xanthan concentration in media at the end of bioprocess and its average molecular weight. According to the obtained results, *Xanthomonas* strains have statistically significant effect on xanthan concentration in cultivation media when biosynthesis is performed by *X. euvesicatoria* strains, and cultivation time has significant effect on this parameter only when bioprocess is performed by *X. campestris* strains. The combination of *Xanthomonas* strains and cultivation time has a statistically significant effect on xanthan concentration in medium for both groups of isolates. The obtained results show that all applied *Xanthomonas* strains and cultivation time as well as their combination have statistically significant effect on average molecular weight of xanthan produced in applied experimental conditions. It is found that *X. euvesicatoria* strains produce higher amount of xanthan in a shorter period of time (168 h) when compared to the *X. campestris* strains. Xanthan of higher average molecular weight was produced when cultivation of both groups of isolates was performed for 240 h in applied experimental conditions. Results obtained in this research suggest that *X. euvesicatoria* strains have the greatest potential for application in biotechnological production of xanthan on crude glycerol-based medium.

Keywords

biotechnological production, xanthan, *Xanthomonas* isolates, crude glycerol, cultivation time

1 Introduction

The enormous usage of fossil fuels has led to the necessity for exploitation of alternative, renewable energy sources [1]. Among others, biodiesel proved to be the most promising, since it presents renewable and sustainable energy source that is safe for the environment [2]. Hence, the biodiesel industry has become one of the most rapidly growing industries in the world and enlarged biodiesel production results in the generation of significant amounts of effluents such as unused catalyst, glycerol, methanol, soaps, proteins and phospholipids. Crude glycerol is formed in the amount of 10% to 20% in relation to the volume of produced biodiesel, which indicates that significant amounts of this effluent are accumulated during

biodiesel production [3, 4]. Since the disposal of crude glycerol in the environment is unacceptable and purification costs are high, it is necessary to find an adequate solution for its utilization in crude form. One of economically and environmentally acceptable solution is potential use of crude glycerol as a raw material in biotechnological production [5, 6]. Several studies indicate that certain strains of bacteria of the genus *Xanthomonas* possess the ability to biosynthesize xanthan on a medium containing crude glycerol as the only carbon source [7–9].

Xanthan presents one of the most widely examined microbial exopolysaccharides which is produced by metabolic activity of bacteria of the genus *Xanthomonas* [7].

Due to its non-toxicity, biocompatibility and special rheological characteristics, xanthan is widely used in food, cosmetics, pharmaceutical, paper, textile and other industries [5, 10]. The chemical structure of this biopolymer is composed of glucose, mannose and glucuronic acid units and its molecular weight usually ranges from 2×10^5 g/mol to 2×10^7 g/mol [11, 12]. Commercial production of xanthan is generally conducted by aerobic submerged batch cultivation of reference strain *Xanthomonas campestris* ATCC 13951 on the appropriate medium under optimal conditions [13]. Glucose and sucrose are most commonly used carbon sources in cultivation media for xanthan production but rise in prices and the growing demand for mentioned sugars indicate that more economical carbon sources are needed in order to reduce the overall production costs [14]. Special characteristics and low cost of crude glycerol suggest that this effluent from biodiesel industry may be a suitable substrate for xanthan production [14]. However, there is a lack of intensive application of crude glycerol in xanthan production by reason of difficulty of the reference strain to successfully metabolize glycerol [6]. This indicates that there is a need for isolation of new *Xanthomonas* strains able to metabolize glycerol and produce xanthan. Due to this reason, other *Xanthomonas* strains, rather than reference strain, were used in previous studies focused on xanthan production on glycerol-based media [8, 9, 15, 16].

Besides the producing strain, cultivation time also has significant effect on xanthan quantity and quality [17]. Generally, fermentation time for xanthan production on glycerol containing media ranges from 48 h to 168 h [14, 18, 19]. The results from earlier studies show that after the cultivation of certain *Xanthomonas* strains on glycerol-based media for 96 h [14] and 120 h [18], glycerol conversion was about 50% or less. The applied producing strains did not metabolize all available amount of glycerol probably due to lack of time to adapt to glycerol and produce xanthan in a sufficient quantity. This indicates that there is a need for increase of cultivation time during the xanthan production on glycerol-based media in the interest of achieving higher productivity and higher degree of glycerol conversion.

The aim of this study was screening of reference strain *X. campestris* ATCC 13951 and *Xanthomonas* strains isolated from different vegetable cultures for xanthan production on crude glycerol-based medium for different cultivation time. The bioprocess efficacy was estimated based on xanthan concentration in media at the end of biosynthesis and biopolymer molecular weight.

2 Materials and methods

2.1 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. *Xanthomonas* strains isolated from crucifers were characterized according to their morphological and ecological characteristics as the members of *X. campestris* species [20] and strains isolated from pepper leaves were identified as *X. euvesicatoria* [21]. All strains were stored at 4 °C on agar slant (Yeast Maltose Agar, HiMedia, India) and subcultured every four weeks. *X. campestris* strains were isolated from infected crucifers and stored in the Microbial Culture Collection of the Faculty of Technology Novi Sad, Serbia and *X. euvesicatoria* strains were isolated from infected pepper leaves and stored in the Microbial Culture Collection of the Faculty of Agriculture in Novi Sad, Serbia.

2.2 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. Xanthan production was performed on medium containing crude glycerol from the biodiesel industry in the Republic of Serbia. Glycerol content in cultivation media was 20.00 g/L. This concentration was selected based on the results from the previous study, conducted by the authors [22]. The cultivation medium also contained yeast extract (3.0 g/L), $(\text{NH}_4)_2\text{SO}_4$ (1.5 g/L), K_2HPO_4 (3.0 g/L) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (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).

2.3 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 under aerobic conditions at 25 °C and 150 rpm (laboratory shaker KS 4000i control, Ika® Werke, Germany) for 48 h.

2.4 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 inocu-

lum 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 and 240 h.

2.5 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 [23].

2.6 Determination of xanthan molecular weight

The average molecular weight of the separated xanthan was estimated based on the intrinsic viscosity of its solution in 0.1 M sodium chloride using the Mark-Houwink type equation [24].

2.7 Data analysis

All experiments were carried out in triplicate and the results were averaged. The experimental data were processed by analysis of variance (one-way ANOVA and two-way ANOVA) at the significance level of $\alpha = 0.05$ using Statistica 13.2 software (Dell Inc., USA).

3 Results and discussion

3.1 Statistical analysis

In accordance with the defined aim of this research, xanthan biosynthesis was performed by reference strain *X. campestris* ATCC 13951 and *X. campestris* strains, isolated from infected crucifers, and *X. euvesicatoria* strains, isolated from pepper leaves, on crude glycerol-based medium for different cultivation time (168 h and 240 h). In order to examine the effect of different *Xanthomonas* strains and cultivation time on xanthan production, statistical analysis of experimental data was carried out. The results summary of two-way ANOVA analysis for xanthan concentration in media and xanthan molecular weight are given in Table 1 and Table 2, respectively.

According to the data presented in Table 1, cultivation time and combination of producing strain and cultivation time have a statistically significant effect on xanthan concentration in cultivation media when biosynthesis is performed by *X. campestris* strains isolated from crucifers ($p < 0.05$). This result is in agreement with the results obtained in the research, performed in Brazil where it is confirmed that cultivation time of *X. campestris* strains has significant effect on xanthan productivity [17]. The mean

Table 1 Two-way ANOVA results for the effect of different *Xanthomonas* strains and cultivation time on xanthan concentration in media

<i>Xanthomonas</i> strain	Variability	SS	DF	MS	F-value	p-value
<i>X. campestris</i> (isolates from crucifers)	Strain	19.973	8	2.497	1.768	0.116252
	Cultivation time	32.328	1	32.328	22.891	0.000029
	Strain and cultivation time	29.383	8	3.673	2.601	0.023529
	Error	50.841	36	1.412	-	-
<i>X. euvesicatoria</i> (isolates from pepper leaves)	Strain	11.016	4	2.754	3.668	0.021343
	Cultivation time	2.391	1	2.391	3.185	0.089501
	Strain and cultivation time	13.554	4	3.388	4.513	0.009243
	Error	15.017	20	0.751	-	-

SS – sum of squares; DF – degrees of freedom; MS – mean square

Table 2 Two-way ANOVA results for the effect of different *Xanthomonas* strains and cultivation time on xanthan molecular weight

<i>Xanthomonas</i> strain	Variability	SS	DF	MS	F-value	p-value
<i>X. campestris</i> (isolates from crucifers)	Strain	4.740×10^{10}	8	5.925×10^9	5.501	0.000144
	Cultivation time	1.976×10^{10}	1	1.976×10^{10}	18.343	0.000131
	Strain and cultivation time	4.453×10^{10}	8	5.566×10^9	5.167	0.000246
	Error	3.878×10^{10}	36	1.077×10^9	-	-
<i>X. euvesicatoria</i> (isolates from pepper leaves)	Strain	3.173×10^{10}	4	7.932×10^9	10.672	0.000004
	Cultivation time	4.964×10^{10}	1	4.964×10^{10}	104.393	0.000000
	Strain and cultivation time	1.992×10^{10}	4	4.981×10^9	10.475	0.000096
	Error	9.511×10^9	20	4.755×10^8	-	-

SS – sum of squares; DF – degrees of freedom; MS – mean square

square values presented in Table 1 suggest that the cultivation time has a much greater effect on this group of results for xanthan concentration in media, while the effect of combination of *X. campestris* strains and cultivation time is considerably lower. The obtained results, presented in Table 1, also show that effect of producing strain on xanthan concentration in cultivation media is insignificant when using strains isolated from crucifers ($p > 0.05$).

On the other side, the results presented in Table 1 also show that producing strain and combination of producing strain and cultivation time have a statistically significant effect on xanthan concentration in cultivation media when biosynthesis is performed by *X. euvesicatoria* strains isolated from pepper leaves ($p < 0.05$). This result is in accordance with previous research findings where it is confirmed that selection of *Xanthomonas* strains isolated from pepper leaves have statistically significant effect on xanthan concentration in media [13]. However, if attention is paid to the mean square values presented in the same table, it can be concluded that the combination of producing strain and cultivation time has a greater effect on this group of results for xanthan concentration in media, while the effect of *X. euvesicatoria* strains is lower. Considering the data from Table 1, it can be concluded that the effect of cultivation time on xanthan concentration in cultivation media is insignificant when using *X. euvesicatoria* strains isolated ($p > 0.05$).

The quality of xanthan can be estimated based on several parameters, such as the viscosity of its solutions, the composition of the macromolecules, the molecular weight, etc. [18]. In this study, the quality of the xanthan produced in applied experimental conditions was estimated based on its average molecular weight. The data presented in Table 2 show that the p -values for the analyzed parameters and their interaction are much lower than 0.05, which indicates that all applied *Xanthomonas* strains and cultivation time as well as their combination have a statistically significant effect on the average molecular weight of xanthan. This is in agreement with scientific findings which suggest that producing strain and cultivation time have an important influence on quality of the xanthan [25]. Considering the mean square values presented in Table 2, it can be concluded that the cultivation time has a much greater effect on both groups of results, while the effect of *Xanthomonas* strains is considerably lower. The results presented in Table 2 indicate that the combination of producing strain and cultivation time has the lowest effect on xanthan molecular weight for both groups of isolates.

The obtained results suggest that cultivation time is of great importance for xanthan quality, if the average molecular weight of biopolymer is considered as an indicator of its quality.

The results of the statistical analysis for xanthan concentration in media and xanthan molecular weight are also presented graphically with Box & Whisker Plots in figures in the following sections. Values in the same figure marked with the same letter are not significantly different at $\alpha = 0.05$ (one-way ANOVA).

3.2 Effect of *Xanthomonas* strains and cultivation time on xanthan concentration in media

The results of statistical analysis of the effect of different *X. campestris* strains, isolated from infected crucifers, and *X. euvesicatoria* strains, isolated from pepper leaves, on xanthan concentration in media containing crude glycerol as a carbon source at the end of biosynthesis, regardless of the cultivation time, are presented in Fig. 1.

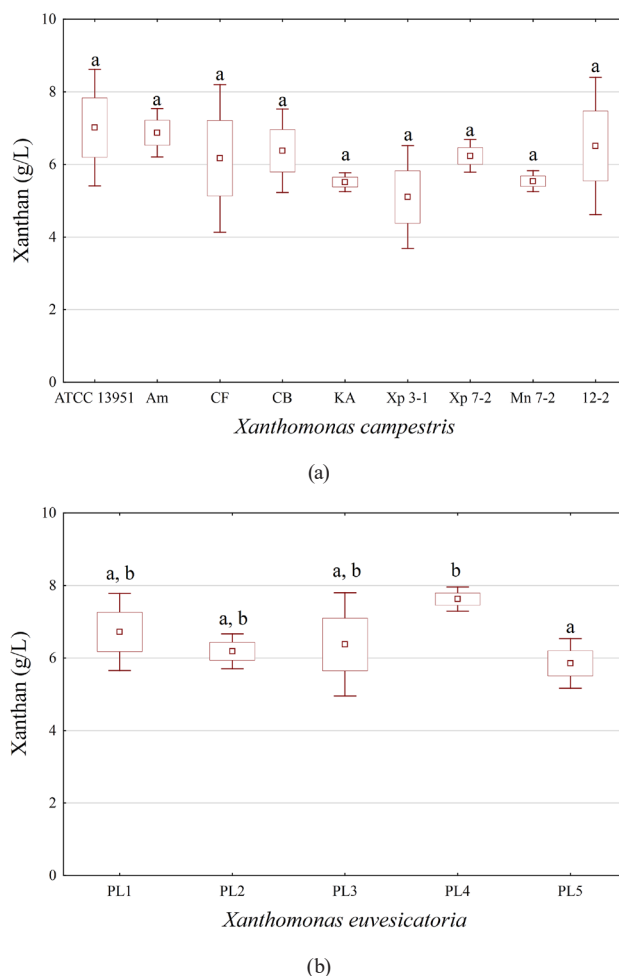


Fig. 1 Effect of different *Xanthomonas* strains isolated from crucifers (a) and pepper leaves, (b) on xanthan concentration in media

Graphically presented results in Fig. 1 (a) indicate that the production of xanthan under the applied experimental conditions is possible by all applied *X. campestris* strains isolated from crucifers, regardless of the cultivation time. Considering the mean value of xanthan concentration in media, the reference strain ATCC 13951 proved to be the best strain for xanthan production. It can also be noticed that other strains from this group showed good productivity. The data presented in Fig. 1 (a) show that xanthan concentration in media varied from around 3.50 g/L to 9.00 g/L. The obtained results are at the same level of statistical significance and are higher comparing to the results obtained in previous research, where the biosynthesis of xanthan was performed by reference strain ATCC 13951 on media containing crude glycerol (15.00 g/L), and where the xanthan concentration in media varied from 6.77 g/L to 7.22 g/L [19].

The results presented in Fig. 1 (b) show that all applied *X. euvesicatoria* strains isolated from pepper leaves have the ability to produce xanthan in applied experimental conditions, regardless of the cultivation time. Graphically represented results (Fig. 1 (b)) indicate that the highest xanthan concentration in media was achieved when cultivation was performed by PL4 strain. It can be noticed that there is no statistically significant difference in the values of xanthan concentration in media when strains PL1, PL2, PL3 and PL4 were used. On the other side, there is also no statistically significant difference in the values of xanthan concentration in media when strains PL1, PL2, PL3 and PL5 were used. The obtained results of xanthan concentration in media varied from around 5.00 g/L to 8.00 g/L and are in agreement with results obtained in the previous study where xanthan production was performed on glycerol-based medium by the same strains, and the xanthan yield was in range from around 5.00 g/L to 10 g/L [26].

Observing the average values of xanthan concentration in media, it can be noticed that *X. euvesicatoria* strains isolated from pepper leaves show slightly better productivity in applied experimental conditions in comparison to *X. campestris* strains, isolated from infected crucifers. The difference in productivity among previously discussed groups of *Xanthomonas* isolates is probably found due to the fact that different *Xanthomonas* species possess different metabolic pathways and cycles [20]. Both *X. campestris* and *X. euvesicatoria* are yellow-pigmented bacteria, generally rod shaped with a single polar flagellum. Bacteria of the species *X. campestris* and *X. euvesicatoria* are catalase positive and oxidase negative and have the ability

to produce acid from glucose, arabinose, galactose, dextrin, mannose etc. [20, 26]. About 90% of glucose is primarily catabolized via the Entner-Doudoroff pathway in *Xanthomonas* sp. [11]. However, *X. campestris* strains have the ability to hydrolyze starch rapidly, while *X. euvesicatoria* strains do not have the ability to hydrolyze starch, but on the other side, have the ability to hydrolyze gelatin and esculin. Strains of *X. campestris* were found causing disease on crucifers while *X. euvesicatoria* strains essentially infects peppers [20, 27]. Results from recent study suggest that *Xanthomonas* strains isolated from crucifers have the ability to metabolize glucose in higher degree than glycerol, while *Xanthomonas* strains isolated from pepper leaves metabolize glycerol in higher degree than glucose [26].

Fig. 2 shows the results of statistical analysis of the effect of different cultivation time on xanthan concentration in media obtained by *X. campestris* strains isolated from crucifers and *X. euvesicatoria* strains isolated from pepper leaves.

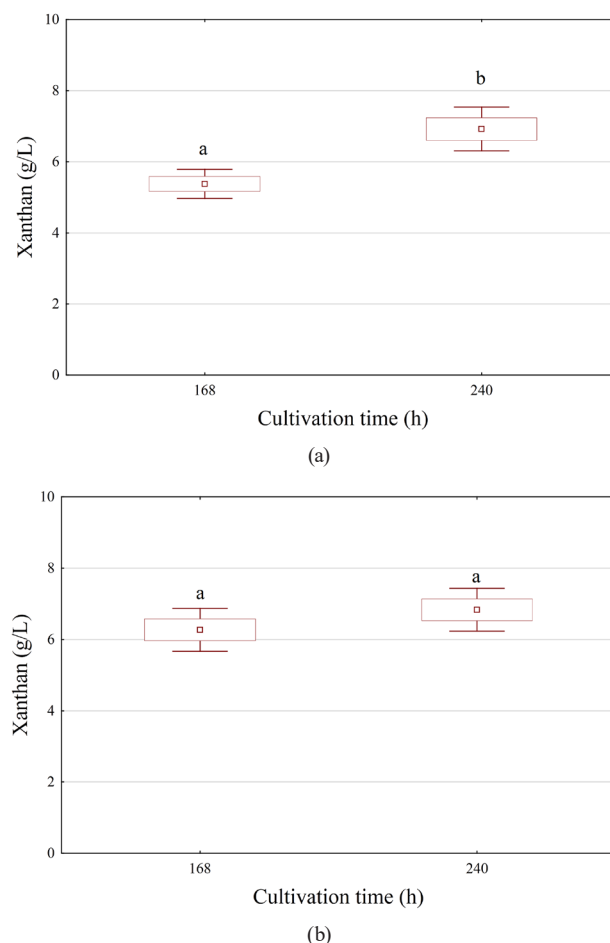


Fig. 2 Effect of different cultivation time on xanthan concentration in media obtained by cultivation of *X. campestris* strains isolated from crucifers (a) and *X. euvesicatoria* strains isolated from pepper leaves (b)

The results presented in Fig. 2 (a) indicate that there is a statistically significant difference in xanthan concentration in media when cultivation of *X. campestris* strains isolated from crucifers on crude glycerol-based medium was conducted for 168 h and 240 h. According to graphically presented results, higher xanthan content was obtained when cultivation of *X. campestris* strains was performed for 240 h. This result is in agreement with findings gained in the previous study where higher xanthan concentration in media was obtained after 240 h of cultivation of *Xanthomonas* strains isolated from crucifers on commercial glycerol containing medium, under similar experimental conditions [28]. This may be due to the greater time needed by the applied strains to adapt to the glycerol as a sole carbon source in cultivation media [6].

Graphically presented results in Fig. 2 (b) indicate that there is no statistically significant difference in xanthan concentration in media when cultivation of *X. euvesicatoria* strains isolated from pepper leaves on crude glycerol-based medium was performed for 168 h and 240 h. The results obtained in this research confirm findings of the previous research, where aforementioned strains were cultivated on commercial glycerol containing media for 168 h and 240 h and there was insignificant difference in xanthan concentration at the end of biosynthesis [28]. Comparing to the previously discussed results presented in Fig. 2 (a), it can be concluded that *X. euvesicatoria* strains isolated from pepper leaves adapt to crude glycerol in a shorter period of time. Taking into account all the above mentioned, it can be concluded that besides the producing strain, cultivation medium and bioprocess parameters, cultivation time is of great importance for bioprocess efficacy too.

3.3 Effect of *Xanthomonas* strains and cultivation time on xanthan molecular weight

The results of statistical analysis of the effect of different *X. campestris* strains, isolated from infected crucifers, and *X. euvesicatoria* strains, isolated from pepper leaves, on average molecular weight of xanthan obtained after different time of cultivation (168 h and 240 h) on crude glycerol-based medium are shown in Fig. 3.

The obtained results presented in Fig. 3 (a) suggest that the most suitable, among all *X. campestris* strains isolated from crucifers, for production of xanthan with the highest average molecular weight under applied experimental conditions is Am strain, regardless of the cultivation time. As it can be seen from the results presented in Fig. 3 (a), Xp 3-1 strain also produces xanthan of high average

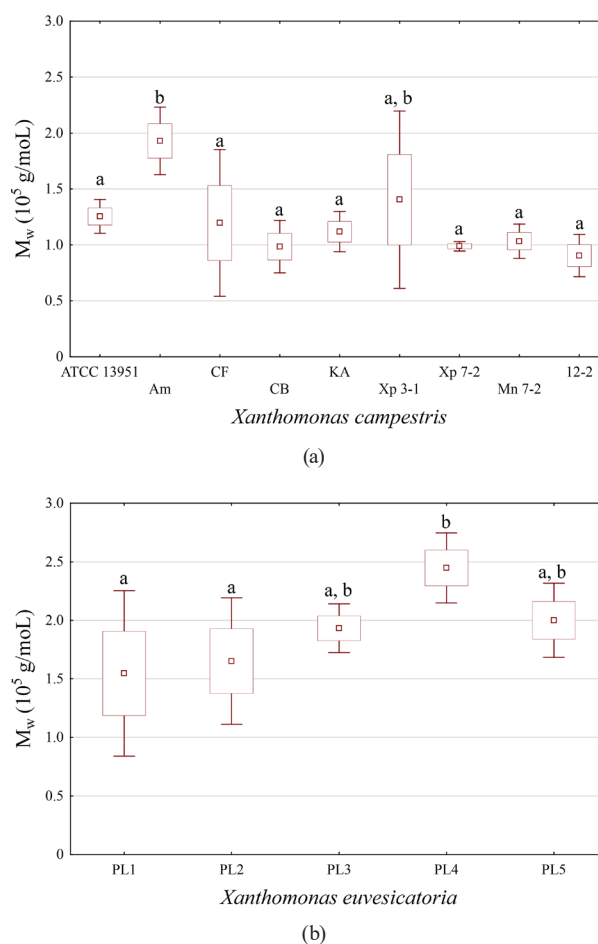


Fig. 3 Effect of different *Xanthomonas* strains isolated from crucifers (a) and pepper leaves, (b) on xanthan molecular weight (M_w)

molecular weight and this value is at the same level of significance as the value of average molecular weight of xanthan produced by Am strain. All the other strains from this group produce xanthan of significantly lower average molecular weight. According to the results shown in Fig. 3 (a), values of average molecular weight of xanthan were in range from 0.5×10^5 g/mol to 2.3×10^5 g/mol and are in agreement with results from previous research (1.0×10^5 g/mol to 6.0×10^5 g/mol) when *Xanthomonas* strains isolated from crucifers were cultivated on media with different carbon sources, including glycerol [26].

Further, the results showed in Fig. 3 (b) suggest that xanthan with the highest average molecular weight was achieved by PL4 strain, among other *X. euvesicatoria* strains isolated from pepper leaves, regardless of the cultivation time. Strains PL3 and PL5 also produced xanthan of the high average molecular weight. In addition, graphically presented results indicate that there is no statistically significant difference in the average molecular weight of xanthan when strains PL1, PL2, PL3 and PL5 were used.

The values of average molecular weight of xanthan obtained in this study were in range from around 0.5×10^5 g/mol to 2.8×10^5 g/mol. The obtained results are in accordance with the results from previous research where *X. euvesicatoria* strains isolated from pepper leaves were cultivated on media with different carbon sources, including glycerol, and average molecular weight of produced xanthan was in range from 1.0×10^5 g/mol to 8.0×10^5 g/mol [26].

Comparing the values of average molecular weights of xanthan presented in Fig. 3 (a) and Fig. 3 (b), it can be seen that *X. euvesicatoria* strains isolated from pepper leaves produce xanthan of higher average molecular weight. This indicate that quality of xanthan produced by *X. euvesicatoria* strains isolated from pepper leaves on medium with crude glycerol is better than quality of biopolymer produced by *X. campestris* strains isolated from crucifers in applied experimental conditions, if the average molecular weight of biopolymer is considered as an indicator of its quality.

In Fig. 4 the results of statistical analysis of the effect of different cultivation time on average molecular weight of xanthan obtained by cultivation of *X. campestris* strains isolated from crucifers and *X. euvesicatoria* strains isolated from pepper leaves are presented.

The results given in Fig. 4 (a) suggest that there is a statistically significant difference in average molecular weight of xanthan when cultivation of *X. campestris* strains isolated from crucifers on crude glycerol-based media was conducted for 168 h and 240 h. As it can be noticed in Fig. 4 (a), *X. campestris* strains isolated from crucifers produce xanthan of higher average molecular weight for 240 h of cultivation. Taking into account the presented results and the results obtained in previous research [28], it can be concluded that *X. campestris* strains isolated from crucifers need more time (240 h) to produce xanthan of higher average molecular weight on crude glycerol-based media comparing to biosynthesis on commercial glycerol containing media. Considering aforementioned results presented in Fig. 2 (a) and Fig. 4 (a), it can be concluded that both quantity and quality of xanthan produced by *X. campestris* strains isolated from crucifers increase over time. The length of macromolecules depends mainly on biosynthesis pathways and genetic variability of the producing strains [20], but the formation of double or triple helices occurs over time and increases molecular weight of biopolymer [29].

The results presented in Fig. 4 (b) indicate that there is statistically significant difference in average molecular weight of xanthan obtained when different *X. euvesicatoria* strains isolated from pepper leaves were cultivated for

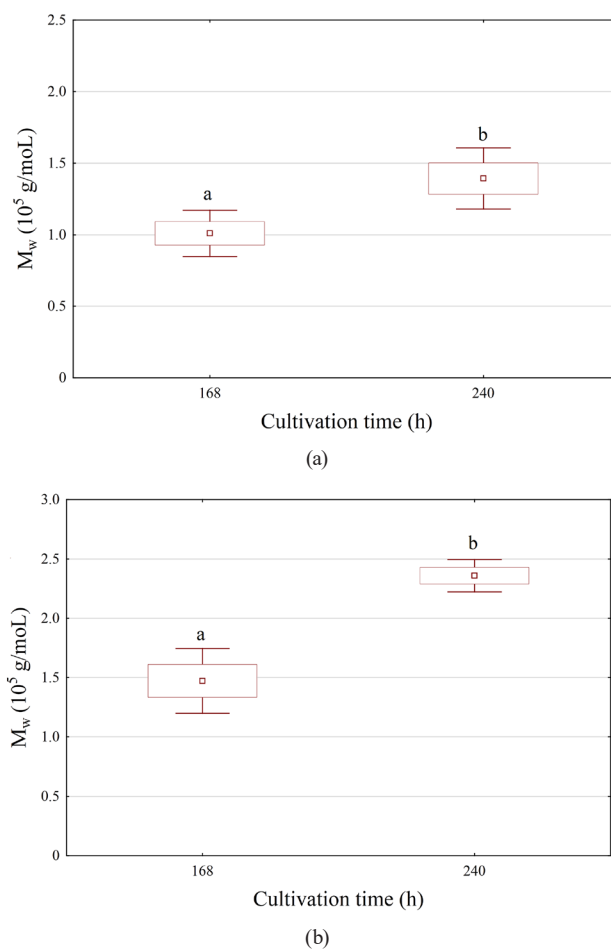


Fig. 4 Effect of different cultivation time on molecular weight (MW) of xanthan obtained by cultivation of *X. campestris* strains isolated from crucifers (a) and *X. euvesicatoria* strains isolated from pepper leaves (b)

different time. Xanthan of higher average molecular weight was produced when cultivation of applied strains was performed for 240 h. Despite the fact that increase in cultivation time does not lead to increase in xanthan concentration, as shown in Fig. 2 (b), it can be assumed that increasing of the average molecular weight of produced biopolymer is the result of the formation of double or triple helices [29].

The presented results of the study suggest that crude glycerol is suitable carbon source for the biosynthesis of xanthan of good quality and in large quantities by cultivation of both groups of *Xanthomonas* isolates under applied experimental conditions. According to the obtained results, the applied strains need different time to adapt to the glycerol and produce xanthan of good quality. This is probably due the fact that impurities present in crude glycerol may have a positive or negative effect on xanthan quantity and quality depending on the used strain and its metabolism [30] and the fact that different *Xanthomonas* species possess different metabolic pathways and cycles [20].

4 Conclusions

The results of this study have confirmed that commonly used carbon sources in cultivation media for xanthan production can be substituted with crude glycerol generated by a biodiesel industry as a cheap alternative substrate. Based on the obtained results, it can be concluded that local *X. euvesicatoria* strains, isolated from pepper leaves, produce xanthan in higher concentration and of better quality for shorter cultivation time (168 h) comparing to local *X. campestris* strains, isolated from crucifers, under applied experimental conditions. This is very important from the economic point of view, considering that reduction in the cultivation time and costs of cultivation medium preparation leads to the reduction in the total production costs. Besides economic, results of this study also have a great importance from an ecological aspect,

since the biotechnological production of xanthan on crude glycerol-based medium represents a promising solution for sustainable valorization of this effluent.

The results obtained in this study represent valuable information that can be used in future research related to the development of biotechnological production of xanthan on crude glycerol-based medium. Further studies should include standardization of inoculum preparation for xanthan production on crude glycerol-based medium, as well as optimization of cultivation medium composition and bioprocess parameters.

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