XANTHAN SYNTHESIZED BY STRAINS OF *Xanthomonas campestris* pv *pruni*: PRODUCTION, VISCOSITY AND CHEMICAL COMPOSITION

XANTANA SINTETIZADA POR CEPAS DE Xanthomonas campestris *pv* pruni*: PRODUÇÃO, VISCOSIDADE E COMPOSIÇÃO QUÍMICA*

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ABSTRACT: The aim of this work was to characterize the xanthan produced by 30 strains of the *Xanthomonas campestris* pv *pruni*, evaluating the production, viscosity and chemical composition. Xanthan production by each strain was evaluated through weight of the dry product per volume of broth fermented ($g.l^{-1}$); the viscosity of 3 % (w/v) aqueous solution of the biopolymers was measured in rheometer rotating mode at 25ºC and the chemical composition was determined by comparative thin-layer chromatography (TLC). Xanthan production was strain dependent and the highest production reached 9.2 $g.l^{-1}$ for strain 83 and the lowest 3.8 $g.l^{-1}$ for strain 51. Strain 107 did not produce xanthan. The aqueous solutions of the xanthans produced showed pseudoplastic behavior. The xanthan synthesized by strain 101 showed the highest viscosity and strain 83 produced the xanthan with the lowest viscosity. All the xanthans synthesized contained glucose, rhamnose, mannose and glucuronic acid in their chemical composition, differently from commercial xanthan, that do not contain rhamnose. There was not relation between yield, viscosity and chemical composition. The yield obtained was not appropriate for industrial production according to the literature, but the viscosity of the aqueous solutions of the xanthans synthesized by sixteen strains was higher than the commercial xanthan, compensating for the low production.

KEYWORDS: Chemical composition. Production. Viscosity. Xanthan. *Xanthomonas campestris* pv *pruni*.

INTRODUCTION

Xanthan is an extracellular polysaccharide produced by several pathovars of *Xanthomonas campestris* and by other species of X*anthomonas* (SUTHERLAND, 1993).

The isolation and screening of *Xanthomonas* strains from natural habitats is still the most efficient method of identifying strains with high capacity of xanthan production and/or high rheological quality (TORRESTIANA; FUCIKOVSKY; GALINDO, 1990; NITSCHKE; THOMAS, 1995; GUPTE; KAMAT ,1997; SÁNCHEZ et al., 1997; MOREIRA et al., 2001; ANTUNES et al., 2003). Other criteria have been questioned for the selection of strains such as colony morphologic characteristics and virulence (GALINDO, 1994a; NITSCHKE; RODRIGUES, 2000). Modern methods of molecular biology as the techniques of Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic (DNA-RAPD) were used without success to correlate the genetic profile with xanthan production, chemical composition and rheological behavior (TESSMANN, 2002; MAYER, 2006).

According to Sánchez et al. (1997), most of the literature regarding xanthan gum production is based on strains obtained from culture collections, mainly *Xanthomonas campestris* NRRL B – 1459 and its derivatives. There are very few reports dealing with the characterization of novel *Xanthomonas* strains.

The results obtained previously have shown that several strains of *X. campestris* pathovar *pruni* have potential for commercial production of xanthan polymer (SOUZA; VENDRUSCOLO, 1999; MOREIRA et al., 2001; ANTUNES et al., 2003), justifying the importance of research with this pathovar.

Xanthan, synthesized by the bacterium *X. campestris* pv *campestris* NRRL B-1459, is constituted by D-glucose, D-mannose, D-glucuronic acid and residues of acetic and pyruvic acid (SLONECKER; JEANES, 1962). However, some species and pathovars produce gums with other sugars in their constitution (ORENTAS; SLONEKER; JEANES, 1963; KONICEK; LASÍK; WURST, 1977; LOWSON; SYMES, 1977; SOUZA; VENDRUSCOLO, 1999).

The commercial interest in the xanthan gum is due to its rheological properties (ROCKS, 1971; GALINDO, 1994a; GARCÍA-OCHOA et al., 2000). These properties are sometimes measured as an indicator of product quality (MARCOTTE; HOSHAHILI; RAMASWAMY, 2001). The species, pathovar and strain influence of *Xanthomonas* in the rheological behavior of polymer produced has been investigated (TORRESTIANA; FUCIKOVSKY;

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GALINDO, 1990; SÁNCHEZ et al., 1997; NITSCHKE; RODRIGUES, 2000; ANTUNES et al., 2003).

The objective of this work was to evaluate the xanthan production, in orbital shaker, for thirty strains of the bacterium *Xanthomonas campestris* pv *pruni*, determining the production, viscosity and chemical composition of the biopolymers obtained.

MATERIAL AND METHODS

Microorganism

Strains 12, 19, 25, 26, 27, 30, 38, 42, 51, 53, 55, 61, 77, 78, 81, 83, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 112, 113, 114 and 115 of *Xanthomonas campestris* pv *pruni* had not been tested for the biopolymer production. These strains were isolated from plum and peach leaves and fruits at the Centro de Pesquisa Agropecuaria de Clima Temperado (EMBRAPA, Pelotas, Brazil). The standard strain NRRL B-1459 of *Xanthomonas campestris* obtained from of Foundation for Research and Technology André Tosello, Campinas, Brazil, was used for comparison of the production results.

Culture media

A) Maintenance of cells in agar SPA, containing ($g.l^{-1}$): sucrose 20.0; peptone 5.0; K_2HPO_4 0.5; $MgSO_4$ $7H_2O$ 0.25; agar 16.0 (HAYWARD, 1964).

B) Production of cells in liquid media YM, containing ($g.l^{-1}$): malt extract 3.0; yeast extract 3.0; peptone 5.0; glucose 10.0 (HAYNES; WICKERHAM; HESSELTINE, 1955).

C) Medium of biopolymers production MPII, containing ($g.l^{-1}$): (1) $NH_4H_2PO_4$ 1.5; K_2HPO_4 2.5; $MgSO_4$ $7H_2O$ 0.2; (2) sucrose 50.0 (CADMUS et al, 1978 modified by VENDRUSCOLO et al., 2000). The media (1) was adjusted to pH 7.0 and the two solutions were sterilized separately at 121ºC for 15 min.

Commercial xanthan

Commercial xanthan was used for comparing of the viscosity results and chemical composition.

Xanthan production

The biopolymer production was made by aerobics fermentation in batch, in orbital shaker (New Brunswick Scientific model Innova 4230), at 28°C and 200 rev. min^{-1} for 72 h (SOUZA; VENDRUSCOLO, 1999). The experiment was carried out in triplicate. The fermented broth was centrifuged for cell removal (Sorvall Instruments model RC-5C) at 4ºC, 16 000 *g* during 30 min. Ethanol 96 GL was added to the supernatant in the proportion of 1:4 (v/v) for recovery of the biopolymer. These were dried at 56°C until reaching constant weight and subsequently ground in a disk mill (Fritsch model Pulverisette), until granulometry of 0.5 μm. The production of the biopolymers of each strain was evaluated by the weight of the dry product per liter of fermented broth and the averages expressed in $g.l^{-1}$.

Viscosity

The viscosity of 3 % (w/v) aqueous solutions of the xanthans synthesized by 29 strains of *X. campestris* pv *pruni* was compared with the viscosity of xanthan synthesized by standard strain *X. campestris* NRRL B-1459 and with dialyzed and not dialyzed samples of a commercial xanthan. The sample was dialyzed to remove its salt contents, against ultra pure water for 48 h, at 4°C. The solutions of the xanthans were agitated for 2 h, heated up to 60ºC during 20 min and kept in rest at room temperature for 24 h (XUEWU et al., 1996). The viscosity was determined in rheometer rotating mode (HAAKE model RS150), at 25ºC. A plate-plate system was used, with a sensor PP35Ti and shear rate of 0.01-100 s^{-1}; the time of each assay was 300 s.

Chemical composition

The polymers synthesized by the 29 strains of *X. campestris* pv *pruni* and the dialyzed and not dialyzed samples of commercial xanthan were hydrolyzed using 2 N HCl [3:100 (w/v)] at 80ºC for 16 h. The chemical composition of the biopolymer was determined through comparative thin-layer chromatography (TLC) to the comparison with authentic samples of glucose, rhamnose, mannose and glucuronic acid. Three μL were applied from the hydrolyzed samples and from the standards in silica gel 60 F_{254} plates (Merck) and eluted with chloromethane: methanol: acetic acid: water, in the proportion of 40:40:10:10 (v/v/v/v). For revelation the reagent used was sulfuric-anisaldehyde (MOREIRA; SOUZA; VENDRUSCOLO, 1998).

RESULTS AND DISCUSSION

Production

The analysis of the results showed that the xanthan production by *X. campestris* pv *pruni* was influenced by the strain. The values in $g.l^{-1}$ varied between 3.8 (strain 51) and 9.2 (strain 83), the strain 107 did not produce xanthan, as shown in Figure 1.

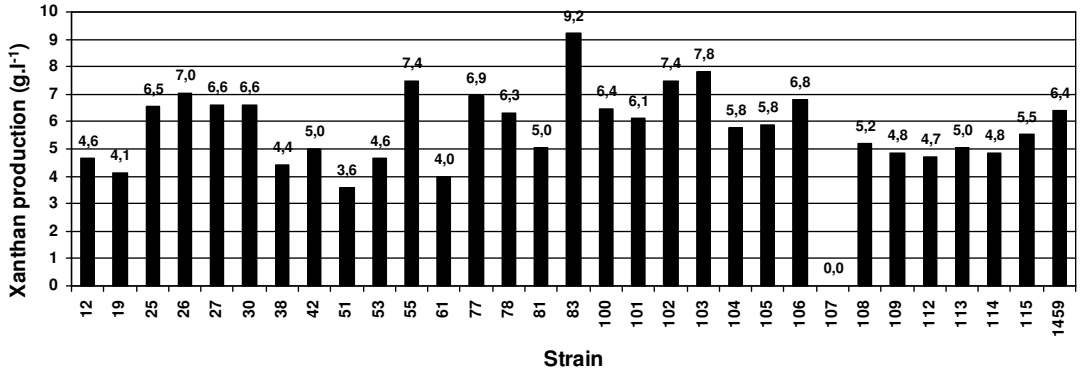

Figure 1. Xanthan production ($g.l^{-1}$) by strains *of X. campestris* pv *pruni* and standard strain *X. campestris* NRRL B-1459.

These results agree with the previous findings of others. Torrestiana et al. (1990) studied xanthan production by six isolated of *X. campestris* pv *campestris* and obtained yields varying from 0.0 to 8.0 $g.l^{-1}$. Sánchez et al. (1997) evaluated xanthan production by several *Xanthomonas campetris* pathovars. For campestris pathovar the production oscillated between 3.4 and 15.3 $g.l^{-1}$, production by juglandis pathovar varied from 0.0 to 7.3 $g.l^{-1}$ and by pruni and manihotis pathovars xanthan production reached 7.4 and 9.0 $g.l^{-1}$, respectively. Moreira et al. (2001) in a study with 18 strain of *X. campestris* pv *pruni* and under the same fermentation conditions used in this work, found similar values. The lowest value 2.3 $g.l^{-1}$ for strain 44 and the highest 8.4 $g.l^{-1}$ for strain 73.

The production of a certain strain can be strongly influenced by the production medium. Souza and Vendruscolo (1999) analyzed the xanthan production by strain 58 of *X. campestris* pv *pruni* in four different production media, and in one of them, also there was not xanthan production.

The influence of the production medium on the xanthan yield by the standard strain NRRL B - 1459 was observed when comparing the value obtained in this experiment, 6.4 $g.l^{-1}$, with the values obtained by other authors. Galindo et al. (1994b) in a the study of xanthan production by *X. campestris* strain B-1459 in a different medium, obtained 9.3 $g.l^{-1}$, while Nitschke and Thomas (1995) in another medium obtained 11.3 $g.l^{-1}$.

The results of previous studies cited show that the production is dependent on the strain and that was also confirmed in this work. It can be stated, therefore, that the selection of strain should be the first stage in the xanthan search with the highest yield and quality.

Viscosity

The aqueous solutions of all xanthans showed pseudoplastic behavior (Table 1). The aqueous solutions of the xanthan synthesized by strain 101 presented the highest viscosity (12.700 mPa.s in 10 s^{-1}), while the xanthan produced by strain 83 presented the lowest value of viscosity (1.370 mPa.s) in the same shear rate. Souza and Vendruscolo (1999) determined the viscosity of the 3 % (w/v) aqueous solutions of xanthan produced by *X. campestris* pv *pruni* strain 24 and 58, obtaining approximately 5.000 mPa.s and 300 mPa.s, respectively in the shear rate 10 s^{-1}.

Among the analyzed strains, sixteen produced xanthan with higher viscosity than the commercial xanthan (Table 1). Antunes et al. (2000) evaluated xanthan viscosity produced by *Xanthomonas campestris* pv *pruni* strains in conventional and alternative medium. The xanthan produced in conventional media (MPI and MPII) showed comparable viscosity with commercial xanthan.

The viscosity of the commercial xanthan after dialyzis decreased of from 3.400 mPa.s to 2.770 mPa.s in shear rate 10 s^{-1}, probably due to the elimination of salts that are added to facilitate the solubilization of the polymer and to increase its viscosity (JEANES; PITTSLEY; SENTI, 1961; MORRIS, 1996). When comparing the viscosity of the xanthan synthesized for *X. campestris* NRRL B-1459 in the shear rate 10 s^{-1}, it reached 2.700 mPa.s, equivalent to the value found for the viscosity of the dialyzed commercial xanthan, 2.770 mPa.s in the same shear rate.

The results of the viscosity of aqueous solutions of the polymers showed that the behavior

was dependent on the used strain. However, no relation was found between viscosity and yield.

Table 1. Viscosity (mPa.s) at 25°C of 3 % (w/v) aqueous solutions of xanthan synthesized by 29 strains of *Xanthomonas campestris* pv *pruni*, standard strain NRRL B-1459 of *X. campestris* and dialyzed and not dialyzed commercial xanthan

Strain	Viscosity (mPa.s) Shear rate			
	10 s^{-1}	30 s^{-1}	60 s^{-1}	100 s^{-1}
12	2.150	0.840	0.500	0.350
19	2.350	0.880	0.530	0.360
25	2.390	0.990	0.580	0.380
26	8.930	2.980	1.620	1.010
27	7.600	2.550	1.340	0.840
30	6.120	2.210	1.210	0.770
38	7.660	2.560	1.410	0.890
42	2.530	0.950	0.550	0.360
51	2.050	0.840	0.490	0.320
53	2.270	0.850	0.500	0.340
55	2.220	0.920	0.540	0.360
61	2.110	0.790	0.460	0.320
77	1.810	0.730	0.440	0.300
78	2.390	0.860	0.490	0.330
81	2.040	0.820	0.490	0.340
83	1.370	0.570	0.340	0.220
100	7.010	2.530	1.400	0.870
101	12.700	4.080	2.080	1.290
102	7.210	2.570	1.440	0.910
103	2.590	1.090	0.650	0.430
104	12.100	3.900	2.050	1.240
105	7.920	2.700	1.480	0.930
106	9.930	3.270	1.750	1.090
108	11.200	3.750	1.970	1.200
109	9.600	3.230	1.770	1.110
112	7.830	2.770	1.490	0.940
113	8.100	2.860	1.570	0.990
114	7.270	2.720	1.520	0.950
115	10.300	3.500	1.870	1.170
X*	3.400	1.190	0.650	0.430
XD**	2.770	0.990	0.560	0.380
1459***	2.700	1.090	0.650	0.440

*Not dialyzed commercial xanthan; ** Dialyzed commercial xanthan; *** Standard strain NRRL B-1459 of *X. campestris*

Chemical composition

In the chromatograms could be observed that all the samples contained rhamnose, mannose, glucose and glucuronic acid, as expected for the pruni pathovar (SOUZA; VENDRUSCOLO, 1999; VENDRUSCOLO et al., 2000; MOREIRA et al., 2001; ANTUNES et al., 2003). The polymer of all the strains differed from the commercial xanthan (dialyzed and not dialyzed) by the presence of rhamnose. There were no differences in the chromatograms from the samples dialyzed and not dialyzed of commercial xanthan.

Differences in xanthan structure have been evaluated by other authors. The polymer produced by *Xanthomonas campestris* pv *vesicatoria* contains galactose instead of mannose (ORENTAS; SLONEKER; JEANES, 1963). Lowson and Symes (1977) reported the rhamnose presence in the xanthan produced by *X. campestris* pv *juglandis* and xylose in the polysaccharide synthesized by the phaseoli pathovar. Konicek et al. (1977) found the galactose and ribose presence in the polysaccharide synthesized by mutant strains of *X. fuscans*.

The difference was visible in the intensity and size of the spots of the components in the chromatograms; however there was no relation between chemical composition with yield and viscosity. According to Antunes et al. (2000) the differences in the intensity and in the size of the spots could explain why some strains produced

biopolymers with higher viscosity than others. According to these authors the polymers with more mannose and glucuronic acid showed greater thickenig properties.

Moreira et al. (2001) found a relation between the chemical composition and the rheological behavior. Biopolymers that contained greater mannose concentration maintained or incresead their viscosity with increasing temperature.

CONCLUSIONS

The yield obtained was not appropriate for industrial production (10 – 20g.l^{-1}), according to the literature, but the viscosity of the aqueous solutions of the xanthan synthesized by sixteen strains was higher than the commercial xanthan, compensating for the low production. These strains should be revaluated in other media and production conditions in an attempt to increase yield.

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RESUMO: O objetivo deste trabalho foi caracterizar a xantana produzida por 30 cepas de *Xanthomonas campestris* pv *pruni*, avaliando a produção, viscosidade e composição química. A produção de xantana por cada cepa foi avaliada pelo peso do produto seco por volume de caldo fermentado (g.l^{-1}); a viscosidade das soluções aquosas dos biopolímeros a 3% (m/v) foi mensurada em reômetro no modo rotativo a 25ºC e a composição química foi determinada por cromatografia comparativa de camada delgada (CCDC). A produção de xantana foi cepa dependente e a maior produção alcançada 9,2 g.l^{-1} para cepa 83 e a menor 3,8 g.l^{-1} para a cepa 51. A cepa 107 não produziu xantana. As soluções aquosas das xantanas produzidas apresentaram comportamento pseudoplástico. A xantana sintetizada pela cepa 101 apresentou a maior viscosidade e a cepa 83 produziu a xantana com a menor viscosidade. Todas as xantanas sintetizadas continham glucose, ramnose, manose e ácido glucurônico em sua composição, diferentemente da xantana comercial, que não contém ramnose. Não houve relação entre rendimento, viscosidade e composição química. O rendimento obtido não foi o apropriado para produção industrial, de acordo com a literatura, mas a viscosidade das soluções aquosas das xantanas sintetizadas por dezesseis cepas foi superior a xantana comercial, compensando a baixa produção.

PALAVRAS-CHAVE: Composição química. Produção. Viscosidade. Xantana. *Xanthomonas campestris* pv *pruni*.

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