Xanthan Pruni, a Singular Biopolymer Produced By Brazilian Bacterium Strains – 25 Years of Discovery

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Keywords: Biopolymer, Xanthomonas arboricola pv pruni, fermentation, application, scale-up

Abstract

Xanthan is a polysaccharide produced by bacteria from Xanthomonas gender. It is widely used by several industries as thickening, stabilizing, suspending agent and emulsifier additive. In 1995 our research group started the inedita xanthan production by *X. arboricola pv pruni*. In 2000 we reported internationally that xanthan produced by the pruni pathovar is chemically different from commercial xanthans; and its polymer has been named xanthan pruni by us. In these 25 years we had studied how this specific bacterium combined with tailored fermentations parameters can result in specials xanthans. Xanthan pruni has been successfully applied in foods, vaccines, biodegradable eatable and pharmaceutics films (including an anesthetic bioadhesive for humidity areas) and in probiotic microcapsules. Now, experiments are being made with support of Some institutions as SENAI and industries as Procelys by Lesaffre to reduce costs of fermentation media in scale transposition from 10 to 100L.

Introduction

Microbial Biopolymer were initially researched because they are related to pathogenicity and virulence [1]. At ends od 1950th they were very researched and produced because they have wide industrial applications as thickening and stabilizers in food, oil, ceramic, paints, pharmacy, agrochemical, etc. After 1990th, new uses were developed as bio packages, biodegradable plastic, scaffolds and other [2]. Scientifically and technologically the extracellular polysaccharide produced by bacteria of the Xanthomonas gender is called xanthan [3,4]. Since 1960th and nowadays, the xanthan is the more relevant Microbial Biopolymer from a commercial and technological point of view [5]. The researches about extracellular polysaccharides (EPS) produced by bacterial phytopathogenic gender *Xanthomonas* beginning in the United States, at middle of 1950th [6]. In 1961 were published some considerations about production and viscosity of the polysaccharide B-1459, produced by *X. campestris* pv campestris strain NRRL B-1459 [7]. And in 1962, its chemical composition was disclosed - D-mannose, D-glucose and D-glucuronic acid at proportion 2.8:3:2, and residues of acetate and pyruvate) [8]. Still in 1962, the kelco Company started its commercial production under the name "Xanthan". Traditionally the NRRL B-1459 strain of Xanthomonas campestris pv campestris has been used for this industrial production of xanthan, but others pathovars and species can produce xanthan efficiently [9]. In 1965, the first release for use of xanthan in food occurred in Brazil [10]. Why? In the USA, FDA allowed the use of xanthan as a stabilizer, emulsifier and thickener in foods. It was previously used in other types of industry, mainly drilling of oil wells [11]. The 1970thand 1980th were the decades of xanthan popularization; various publications about production, rheology and uses were done in these periods. In 1975, Janson et al. [12] published the structure of commercial xanthan. Cadmus et al. [13], Sutherland [14], Morris [5] and Margaritis and Pace [15] are among principals publications. The principles announced by Cadmus et al. [13] about xanthan production until now influence the xanthan production. In the Brazil, studies about xanthan beginning in 1980th and were characterizes by use of other patovars, as manihotis [16,17].

Xanthan pruni time line

1995 – Discovering

Dra. Claire Tondo Vendruscolo [18], lieder of our research group, started an unprecedented study about xanthan production by "*Xanthomonas campestris* pv pruni", pathovar which infect *Prunus* specie as peach and prune; in Erlenmeyer flask (50mL) (Fig 1).

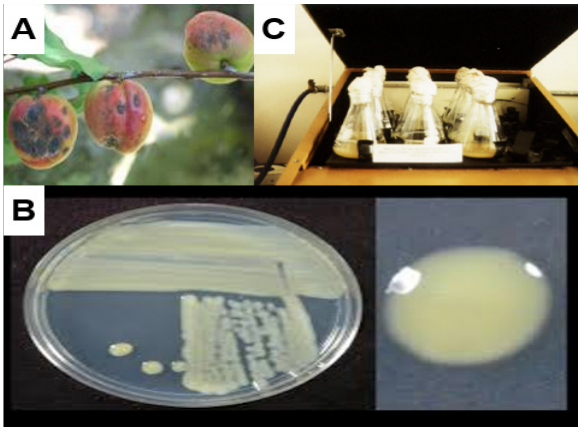

Figure 1: *A) peach infected by Xanthomonas arboricola pv pruni, B) colony of X. arboricola pv pruni, C) first shaker used in xanthan pruni production.*

1998 – Gelling capability and chemical composition

The first analyses of polymers in rheometers revealed that they had characteristics of true gel, differently from commercial xanthans. In order to determine the chemical composition of the xanthan produced by X. campestris pv pruni, we developed a new method for monosaccharide acids and derivatives based in Thin Layer Chromatography (TLC) [19] which became reference to polysaccharide analyse and is used worldwide. The method is based on sample hydrolysis with 2M HCl [3:100 (w/v)] at 80°C for 16 h in closed tubes. The chromatograms are obtaining in silica gel 60 F_{254} plates eluted with chloromethane/ methanol/ acetic acid/ water 40:40:10:10 (v/v/v/v); the disclosed is with sulfuric-anisaldehyde and heating. This cchromatographic system is suitable to all sugar and polysaccharide.

In 2000, we reported internationally [20] that xanthan produced by the pruni pathovar is chemically different due to the presence of sugar rhamnose, absent in commercial xanthan. After, the gel characteristic was related to the presence of rhamnose [9].

For the bacterial natural biopolymers such as xanthan, the parameters utilized in fermentation such as media, time, temperature, aeration and agitation must be extremely well defined and controlled so that the biosynthesized product has the desired properties for each kind of application. To study strain and medium influence in production and viscosity, we growed 13 strain of *X. campestris* pv pruni in two production media and founded two strains that produced polymer with viscosity higher than a commercial xanthan used as control [21,22] (Fig 2).

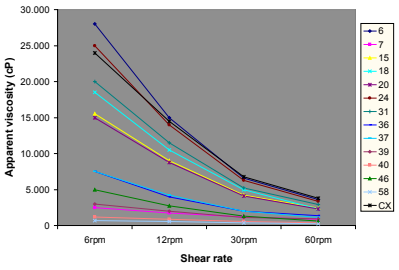

Figure 2: *Apparent viscosity at 25°C of solution at 3% (m/v) of commercial xanthan and synthesized by "Xanthomonas campestris pv pruni ´ s strains in medium MPII.*

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2001 - Different rheology profile and support to reclassification

In 2001 [9], results from an unprecedented study among 18 wild strains was published. Three rheological behaviours until heating were detected– decreasing, maintenance and increasing. Among different strains there was apparent differences among quantity of monosaccharides (Figure 3). Xanthans with high mannose kept or increasing its viscosity with increasing temperature.

Was inferred that xanthans from patovar pruni were chemically near to patovar juglandis by presence of rhamnose. In a previous study Vauterin and Swing [23] found genetics similarities between these pathovars.

Taking account of the bacterial phytopatogenicity and genetic profile and chemical composition of its xanthan, *X. campestris* pv campestris was officially renamed as *Xanthomonas arboricola* pv pruni [24]. Then, timidly we started namely its polymer as xanthan pruni!

2004 - First patent application

The initial application was divided by request of INPI, the Brazilian patent authority, and two patents were granted in 2017, one about culture medium for growth of *Xanthomonas* [25,26] and other about xanthan biopolymer production process.

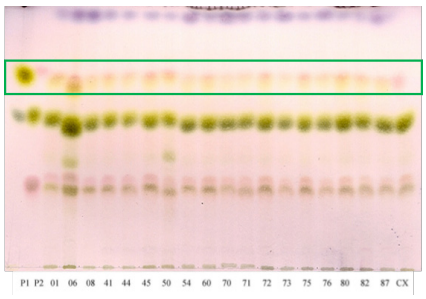

Figure 3: *Chromatographic pattern of xanthans produced by 18 strais of X. campestris pv pruni. P1 - standard of rhamnose ang glucose; P2 – standard of mannose and glucuronic acid. Chromatographic system: silica gel 60 F254 plates (Merck) and eluted with chloromethane/ methanol/ acetic acid/ water 40:40:10:10 (v/v/v/v). Revelation with sulfuric-anisaldehyde and heating.*

2007 – New uses

From that year, food products were developed with xanthan pruni as a stabilizer or thickener (Fig 4). An exceptional stability in the structure of developed products were observed [27]. In pulp fruits, the xanthan pruni use, associated to acid enhance the stability of phenolic compounds during fruit pulp storage, resulting in products with highest final phytochemicals content and the highest antioxidant activities [28].

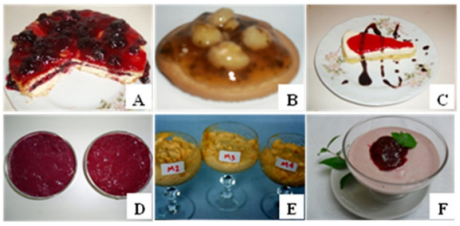

Figure 4: *Food products developed with xanthan pruni as a stabilizer or thickener: A) blueberry topping, B) champignon topping, C) raspberry, D) blueberry mousse, E) passion fruit mousse and F) mousse of raspberry.*

Cakes formulated with xanthan pruni displayed improved quality characteristics such as increased specific volume, enhanced texture in terms of decreased firmness, and delayed staling. The cakes presented desirable quality characteristics that resembled the physical, chemical, and sensory attributes of traditional cakes formulated with wheat flour [29].

We were pioneer in using xanthan as vaccine adjuvant. Bacelo et al [30] compared xanthan pruni (strain 106), aluminium hydroxide (alhydrogel®), and CpG ODN as adjuvants in a LigA subunit vaccine preparation. In a challenge against a virulent strain of *L. interrogans* serovar Copenhageni, significant protection was observed in 100%, 100%, and 67% of hamsters immunized with rLigANI-xanthan, LigA-CpG-xanthan, and rLigANI-alhydrogel, respectively. Furthermore, xanthan did not cause cytotoxicity in Chinese hamster ovary (CHO) cells *in vitro*. The use of xanthan as an adjuvant is a novel alternative for enhancing the immunogenicity of vaccines. A patent application was registered in Brazil [30].

Low-viscosity commercial xanthans have been used as stabilizers for juices and nectars [31], but we studied a new use to xanthan pruni low-viscosity – micro-organism encapsulant. Microencapsulation increasing probiotics preservation against adverse conditions, including probiotic foods storage. Xanthan has been used as an secondary encapsulant in association with other polymers. In the spray drier technique, the high viscosity of the polymers is negative because reduces quality and yield of the powders. Associations of low viscosity xanthan pruni and pyrogenic silica (Aerosil®) added with glycerol were used to obtain microcapsules of *Lactobacillus acidophilus* ATCC 4356 by the spray-drier drying method (Fig 5). Xanthan pruni possess high thermo, osmoprotectant and antioxidant effect and the probiotic microcapsules obtained are able for different applications [32].

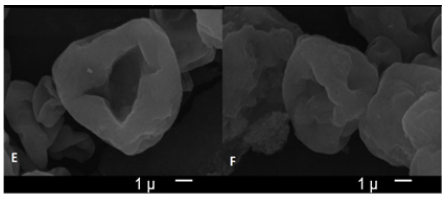

Figure 5: *Scanning Electron Micrographs of probiotic Lactobacillus acidophillus ATCC 4356 preserved in microcapsules of xanthan and pyrogenic silica. E = Xanthan pruni:Pyrogenic silic 2.0/0.5 (w/w); F = Xanthan pruni:Pyrogenic silic 0.19/1.25 (w/w); with 10000x magnification.*

Xanthans pruni was successfully used as filmogenic matrix. Biodegradables, edibles and pharmaceutics films (including an anaesthetic bioadhesive for humidity areas – Fig 6)) were developed with different purposes. A patent application about biodegradable materials was made in Brazil [33] and one patent was granted in United States about topical anaesthetic bioadhesives [34].

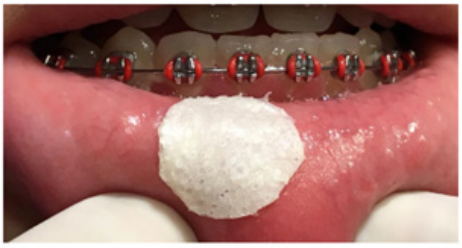

Figure 6: *Topical anesthetic bioadhesive.*

2009 – Chemical modification

Modifying an existing polymer is faster and easier than developing a new one with desired characteristics! Xanthan can be modified during fermentation or after recovery. The modifications can improve viscosity, stability and thermal resistance and increase the compatibility of xanthan with other gums. Ion exchange was the first chemical modification made to the xanthan pruni [35].

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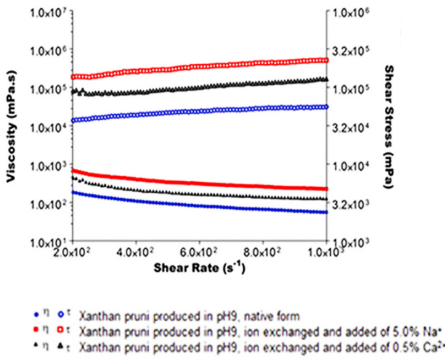

Figure 7: *Viscosity curves (mPas) versus shear stress in different shear rate (0.01-1000s^{-1}) at 25°C of aqueous solutions (1% w/v) of the natural autodeacetylated Xp 106 pH9; Xp 106 pH9 ion exchange and added of 5.0% Na$^+$; Xp 106 pH9 ion exchange and added of 0.5% Ca^{2+}*

Increasing amounts of Na^+ (0.5–15% w/w relative to xanthan content) and Ca^{2+} (0.5–10% w/w) were added to the xanthan salt free solutions. After recovering, improvements to xanthan viscosity were verified (Fig 7). The ion-exchange enhanced the viscosifier capacity of xanthan pruni, which was already excellent, approximately 2–7 fold, and was mainly due to the addition of 5% of Na^+ and 0.5% of Ca^{2+}.

We produced natural xanthans pruni from strain 106 under pH 7 and 9 and performed the comparison and combination of chemical modifications of deacetylation, crosslinking and ion exchange. A patent application was made [36]. Ion exchange (replacement of naturally occurring ions for Na^+) increased the rheological parameters of natural and deacetylated xanthan pruni (Fig 8)

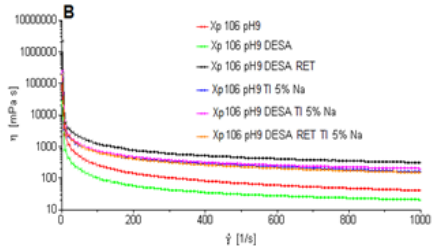

Figure 8: *Viscosity curves (mPas) versus shear rate (0.01-1000s^{-1}) at 25°C of aqueous solutions (1% w/v) of the natural, deacetylated Xp 106 pH9; deacetylated + reticulated; ion exchange; deacetylated + ion exchange; deacetylated + reticulated + ion exchange*

Deacetylation as a single modification reduced viscosity and elastic and viscous modules, but it necessary before cross-linking (Fig 8 and 9).

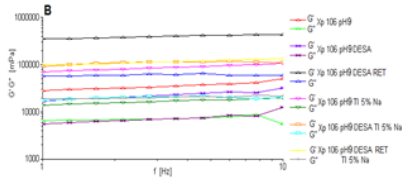

Figure 9: *Viscoelasticity curves at 25°C of aqueous solutions (1% w/v), at the frequency of 1 to 10Hz, of the natural; deacetylated 106 pH9; deacetylated + reticulated; ion exchange; deacetylated + ion exchange; deacetylated + reticulated + ion exchange.*

The greatest increase in thermal resistance was promoted by ion exchange, while deacetylation followed by cross-linking provided highest improvement in rheological parameters. The crosslinked deacetylated xanthans had their rheological parameters decreased after ion exchange. Among the Xp pH7 and derivative xanthans, the greatest thermal stability, determined by TGA and DTG, was observed for xanthans subjected to ion exchange, in this order: natural (onset ~ 273°C), deacetylated (onset ~ 271°C) and reticulated deacetylated (onset ~ 269°C) (Fig 10). Ion exchange increased the thermal resistance of samples, but as the single change was relevant only to the natural xanthan pruni.

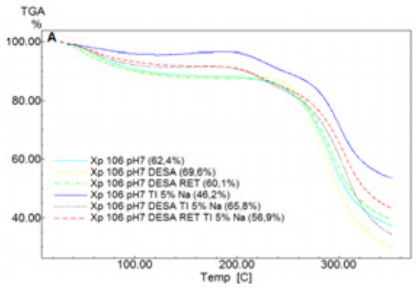

Figure 10: *Curves of thermogravimetric analysis (TGA) of natural xanthanes Xp 106 pH7 natural, deacetylated, deacetylated reticulated, naturals under ion exchange, deacetylated under ionic exchange and deacetylated reticulated under ion exchange with their respective mass loss percentages in the temperature range of 30 to 350°C with a heating rate of 10°C / min.*

2019 – Business

Recently the technology of Topical Anaesthetics Bioadhesive (TAB) was licensed to Biopolix Technological Materials, a Brazilian start up. A partnership has also been established with Procelys by Lesaffre to test new inputs and develop new means of cultivation for the production of xanthan pruni. Now, new project seeks partnerships for the scale up production.

Conclusion

In these 25 years we had studied how the specific bacteria that produce xanthan with different chemical composition combined with fermentations with tailored parameters can result in specials xanthans. Generalizing, xanthan pruni is gelling, very resistant to salt addition, possess high melting and degradation temperature, form strong physical or chemical crosslinking by interactions with cations or chemical crosslinkers and posses higher antioxidant activity than the commercial xanthans.

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