Microbial synthesis of xanthan gum and application

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Summary

This paper deals with microbial synthesis of xanthan gum by *Xanthamonas campestris* strains and its applications in various industrial processes. The proposed biosynthetic pathway of xanthan synthesis by *X. campestris* strains and biotechnological methods of improving xanthan production were discussed. The biotechnological parameters for efficient improvement of xanthan yield and productivity such as agitation rate, pH, temperature and various additives were described. Furthermore, the applications of xanthan gum in the food, pharmaceutical, textile, oil drilling and cosmetic industries were presented.

Key words:
application, biochemistry of xanthan synthesis, xanthan fermentation.

1. Introduction

Xanthan gum is a microbial exopolysaccharide (EPS) produced by members of the *Xanthamonas* genus, a phytopathogenic gram-negative bacteria (*X. campestris pv campestris; X. campestris pv translucens; X. fragariae; X. oryzae pv oryzae)* that has proved to be an attractive and alternative product for the replacement of traditional gums obtained from plants and marine algae by chemical extraction processes (1-7). This gum was first officially recorded by its allocation to the ARS Culture Collections in 1953 and was approved by the FDA in the United States in 1969 as food additive (8-10). It is a polymerised pentasaccharide repeating unit (linear or unlinear), consisting of cellulosic (1,4-β-D-glu-

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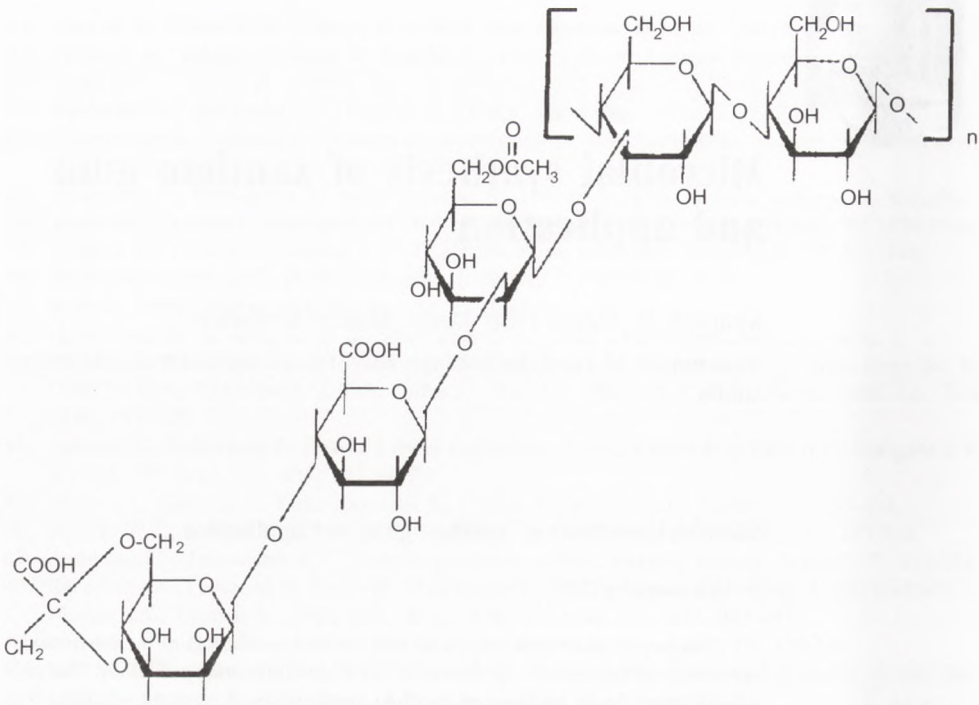

Fig. 1. The structure of xanthan (7,9,14).

cose) backbone, to which a trisaccharide side chain that contains two mannose and a glucouronic acid in the order mannose-glucouronic acid-mannose is linked with α-1,3, β-1,2 and β-1,4-glycosidic bonds, respectively, at every second glucose (11-14). The molecule of xanthan is partially substituted with acetyl and pyruvyl radicals at both internal mannose residues at various degrees [Fig. 1] (9,15,16). It has been postulated that the tertiary structure of the xanthan is a helix. The high molecular weight (2000-15,000 kDa) and the helical structure of this gum give its solution the property of being very viscous. The world-wide annual production of xanthan is about 25,000 tons and the demand for xanthan is estimated to increase continuously also at an annual rate of 5-10% (9,16-19). However, the high demand and the biotechnological problem concerned with its low productivity and yield have increasingly led to high production costs, which needs to be tackled. This problem is associated with certain biotechnological drawbacks such as oxygen transfer, agitation rate due to viscous nature of the fermentation broth, physiology of the strain, growth rate and xanthan production in terms of carbon to nitrogen (C/N) availability (8,16,20,21). These factors to a great extent determine the quality and quantity of produced xanthan. Currently, xanthan has meet all technical parameters required for an EPS like high viscosity at low concentration, low shear stress, good solubility

and unique rheological properties (pseudoplasticity) (22,23). Due to this, it has found increasing applications in food and dairy industries as stabilisers, emulsifiers, thickening agents or food enhancers, cosmetics, pharmaceuticals, paper, paint, textile, adhesive and tertiary oil recovery (2,9,10,23,24). Furthermore, xanthan fermentation is an excellent model for study. Broth viscosity undergoes changes of about four orders of magnitude during cultivation, whereas broth rheology has shear thinning, yield stress and viscoelastic characteristics. This makes mixing a key factor in xanthan fermentation and therefore determines its productivity. Another important aspect in this process is the bacterial stability during cultivation, as well as, strain preservation. Preservation of bacteria is a cause of concern, as good reproductivity is required in any industrial fermentation processes (18,25).

The objective of this paper was to present and discuss the underlying biosynthetic and regulatory mechanisms, as well as biotechnological aspects of xanthan formation that could lead to an increased production.

2. Biochemistry/Genetic of xanthan synthesis

2.1. Biosynthesis of xanthan gum

The biosynthesis of xanthan has been the subject of current study in the field of genetics. Although, several aspects of its synthesis on the molecular level has been revealed, the act of secretion and regulatory mechanism still remain to be elucidated. Xanthan is composed of polymerised pentasaccharide repeating units, synthesized by sequential addition of glucose-1-phosphate, glucose, mannose and glucouronic acid on a polyprenol lipid linked carrier with both pyruvyl and acetyl residues at specific sites (11-14,26-28). Studies on the bacterial chromosome and DNA sequence analysis of *X. campestris* strain revealed a number of important genetic loci involved in xanthan synthesis through the isolation and selection of nonmucoid mutants defective in the production of xanthan gum, developed by chemical, transposon and site-directed mutagenesis (7,13,26,27). A cluster of 12 genes, predicted as operon (ORFS), gum B to M and two additional flanking genes, gum A and gum N located in the region assigned as *xpsI* or gums have been suggested to code proteins responsible for the synthesis and polymerisation of xanthan, whereas no specific roles have been proposed for the two flanking genes (7,15, 26,29,30). Based on these studies, several regions denoted as *xps III*, *xps IV* and *xps VI* (31), as well as 35.3 kb gene cluster contain genes that are responsible for the synthesis of sugar nucleotide acceptors essential for both xanthan and lipo--polysaccharide formation. The 35.3 kb gene cluster contains two separate regions, one of which overlaps the *xps III* region. Analysis of the DNA sequences in the common segment of this region revealed two genes, known as *XanA* and *XanB*

(13,32,33). The *Xan A* gene codes an enzyme with both phospho-glucomutase and phospho-mannomutase activities; whereas XanB encodes a bifunctional enzyme having both phosphomannose isomerase and GDP-mannose pyrophosphorylase activities. Therefore, a part of this region codes two enzymes of which the former is necessary for the biosynthesis of all the sugar nucleotide precursors of xanthan, while the latter is responsible for the synthesis of GDP-mannose (13,34). Thus, the biosynthetic pathway of xanthan comprises of five stage biochemical reactions: 1. conversion of simple sugars to their respective nucleotide derivative precursors; 2. assembly of pentasaccharide subunits or repeating units linked to their inner membrane lipid polyprenol phosphate carriers; 3. addition or incorporation of acetyl and pyruvyl residues; 4. polymerisation of pentasaccharide repeating units (linear or unlinear); 5. secretion of the polymer-xanthan (13,27,35,36). The proposed pathway of xanthan synthesis based on the five stage biochemical reactions is shown in Figure 2. Reactions 1 to 5 represent the synthesis of the pentasaccharide repeating units on a polyprenol lipid acceptor by sequential addition of individual sugar residues from the corresponding sugar nucleotide precursors. Recent studies have demonstrated that these precursors UDP-Glu, UDP-GluA and GDP-man are all synthesised by *X. campestris pv campestris* (7,14,26-28,31,35). The assembly of these pentasaccharide units in sequence [from reactions 1 to 5] is governed by the function of *gum M, H, K* and *I* genes that encode glycosylation precursors *II, III, IV* and *V*, respectively. These genes code respectively for the sequential transfer of glucose-1-phosphate, glucose, mannose, glucouronic acid and, finally, mannose from the nucleotide sugars to C_{55}-isoprenol phosphate carriers. Therefore, the first step of xanthan synthesis involves the incorporation of glucose 1-P in an endogenous lipid linked to isoprenol phosphate (IP) acceptor by the action of UDP-Glu [reaction 1] (7,15,36). The synthesis of cellobiose-PP polyprenol involves the transfer of glucose residue from UDP-Glu by the action of glucose diphosphate polyprenol [reaction 2]. The incorporation of mannose in cellobiose diphosphate polyprenol by GDP-man results in the formation of the trisaccharide diphosphate polyprenol [reaction 3]. Mannose is formed in this reaction from phosphoglucose isomerase acting on glucose 1-phosphate and its conversion to fructose-G-P and subsequently to mannose-G-P by gene XanB (15,24). The next step involves the transfer of glucuronic acid by UDP-GluA to trisaccharide-P-P polypropenol [reaction 4]. Finally, a second mannose residue is attached to tetrasaccharide diphosphate polyprenol from GDP-man to synthesize the polyprenol – bound pentasaccharide [reaction 5], leading to the polymerisation of polysaccharide lipid-linked diphosphate [reaction 6]. The polymerisation of polysaccharide lipid-linked diphosphate (linear or unlinear) involves the addition of pyruvyl and acetyl residues to mannose, catalysed by both acetyl Co-enzyme A and phosphoenolopyruvate irrespectively *via* Entner-Doudoroff (ED) pathway [reaction 7] (28,29,35,37), whereas the act of polymerisation is governed by the gum B to M genes, generally termed as *xps1* gene (34).

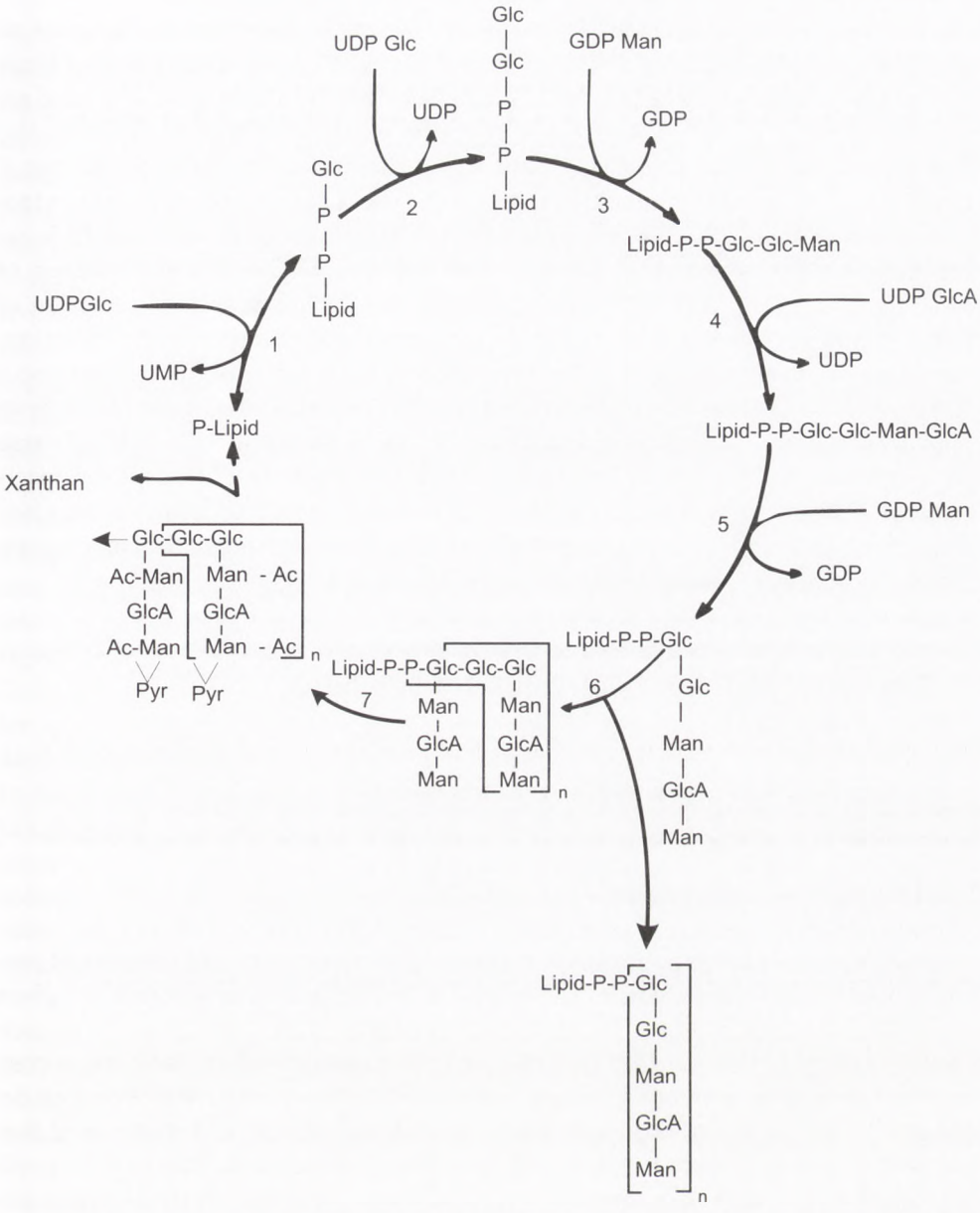

Fig. 2. The proposed pathway of xanthan biosynthesis (adapted and modified from 7 and 14).

The genes responsible for the lipid linked intermediate assembly, polymerisation and secretion have been isolated and sequenced (27). They are clustered in a 16kb region, termed *xps1* or gum. This region is separated from those required for

the synthesis of sugar nucleotide precursors, and its arrangement is dissimilar to other known exopolysaccharide biosynthetic systems. 12 open reading frames (gum B to M) were deduced by nucleotide sequence analysis for this region. The gum region is expressed as a single operon in which the promoter is located upstream the first gene B, while the second promoter was identified at the upstream of *gum K* based on the transcription analysis through *gum-lac Z* fusion and promoter extension assays (7,13,15). In this reaction, a cluster of two genes (gum F and G) is responsible for the acetylation, whereas gum L is responsible for pyruvilation of xanthan. The regeneration of polyprenol phosphate carrier as well as secretion of polymer xanthan has not been fully explained and require further study. However, permease has been suggested to be responsible for the secretion of xanthan and a cluster of at least three permease genes (Gum B, C, E), as well as gum J have been implicated for the secretion of xanthan (34). The evidence for the role of these genes product are lacking due to the apparent lethal effects of transposon insertions in some of these genes, and thus, they are suggested to code unidentified steps in the polymerisation and secretion (7,15,26), while the regulatory mechanism for overproduction of xanthan is yet to be elucidated.

3. Biotechnological aspects of xanthan production

3.1. Conditions affecting xanthan fermentation

3.1.2. Agitation and aeration

The influence of oxygen supply and agitation on the quality and quantity of produced xanthan has been studied (20,38-40). The oxygen mass transfer rate in a given stirred tank bioreactor is a function of many variables such as the physical properties of liquid (viscosity, surface tension, etc.), the geometry of the tank and stirrer, the type of impeller and the operating conditions (3). The distribution of oxygen depends also on the agitation speed and on the type of impeller or turbine used during fermentation. Efforts have been made to retrofit the existing standard large diameter Rushton turbines in the bioreactors, specially for more effective oxygen distribution during the fermentation of xanthan. On the other hand, the power embedded during fermentation represents a significant cost in running a large-scale process and changes the impeller type or relative size should be justified by either a reduction in energy costs in order to achieve the same productivity or an increase in productivity at the same costs (1). However, a linear correlation was found suggesting that different oxygen transfer rates affect the formation or absence of stagnant zones in the bioreactor and these were crucial demands for achieving high produc-

tivity, as well as, high molecular weight of this polysaccharide. The viscous nature of the broths limits the intake of oxygen supplied into the medium during fermentation, therefore, affects the specific production rate, which decreases together with an increased viscosity. In addition, the agitation rate affected not only the xanthan yield but also the pyruvate content and the mean molecular weight (MMW). Funahashi et al. (39) proposed a mechanistic model describing xanthan cultures in stirred vessels and divided them into three regions: 1. the micromixing region around the impeller (high shear stress and high oxygen supply producing highly active *Xanthomonas campestris* cells); 2. a macromixing region characterised by a circulating flow (many cells are not producing xanthan) and 3. a stagnant region. Several reports have been made that the polymer formation rate was lower at 500 rpm than at 800 rpm (20,38-40). It has been hypothesized that the observed product kinetics was inhibited by some feedback mechanisms caused by the formation of the slime layer around the cells that could restrict the rate of nutrients diffusion and oxygen intake by the cells. It was further reported that the increased agitation or shear stress may enhance the removal of this slime layer and improve nutrient transport and oxygen distribution (39,40). However, Peters et al. (41) differed this phenomenon and using transmission electron microscopy, showed that there was no slime layer around the cells when cultured at different agitation speeds (41-44). Serrano-Careón et al. (44), examined the xanthan production as a function of impeller speed in a bioreactor (8 L working vol.; T = 0.2 m, Dt 0.5 with two different Rushston turbines) and found that at an agitation rate of 800 rpm, the power drawn to the broth was largely sufficient to keep the medium completely well-mixed, and in consequence, they obtained an increased xanthan productivity. They further compared the DOT-controlled and model controlled cultures. During the DOT-controlled culture, the agitation rate was feedback-controlled in order to maintain the DOT value above 10% saturation. The main difference between the two types of fermentations was the low growth rate and low xanthan productivity observed in model controlled cultures when compared with the DOT-controlled culture (0.84 g l^{-1} h^{-1} versus 1.18 g l^{-1} h^{-1}), but both cultures had similar biomass and productivity. The DOT controlled experiments consumed about 46% more power than the model controlled cultures. The effect of impeller speeds at 600 and 1000 rev/min on xanthan synthesis was also investigated by Jana and Ghosh, (45). Due to better oxygen transfer rate and mixing in the fermentation broth at 1000 rev/min, the xanthan concentration reached 27 g/dm^3 compared to 19 g/dm^3 at 600 rev/min. The yield of xanthan (g xanthan per g glucose consumed) also increased from 0.49 at 600 rpm to 0.59 at 1000 rev/min. Flores et al. (20), studied also the effect of the dissolved oxygen tension (DOT) in the range of 10-100% of saturation on the formation and the quality of xanthan gum. The DOT level during cultivation markedly affected the molecular weight and size of produced xanthan gum. The molecular weight of xanthan increased together with increased DOT values between 10-40%. A higher DOT between 40-100% of saturation gave higher MMW but the largest MMW was obtained

at 100% DOT, while the rest DOT values were lower (10,40 and 80%). They also observed that the pyruvate contents of xanthan gum depended on the level of DOT. At 10% DOT the level of pyruvilation was lower when compared with that found at 40, 80 and 100% of saturation. Different levels of pyruvate contents have been observed in various cultures. This can be attributed to the difference in strains, culture medium, mode of cultivation and different analytical procedures used. It has been shown that under oxygen – limited conditions, the content of pyruvate was low, and this result was attributed to (be associated with) the function of oxygen supply (20,43). The viscosity of polysaccharide solutions is a function of gum concentration and determines the rheological characteristic of the polymer. One important characteristic which reflects the polymer quality is the viscosifying ability, which can be defined as the viscosity generated per unit of gum concentration. The pyruvic acid content of xanthan has been implicated to be an important factor in the viscosifying ability, thermostability and salt compatibility of the solutions (27). Nevertheless, no correlation was found between η values (values of intrinsic viscosity) and pyruvate contents (46). It rather seems to correlate with the pyruvate/acetate contents and the mean molecular weight of the xanthan or there exists a more complex relationship that, however, needs further studies (38,47). Nitschke and Thomas, (48) studied the relationship between the yield and the viscosifying ability of xanthan produced by isolated strains of *X. campestris* in comparison to the xanthan obtained using the standard strain *X. campestris* NRRL-1489, based on the pyruvic acid contents and yield. Although a higher xanthan yield was obtained by the standard strain *X. campestris* NRRL-1489 under the conditions of study, the viscosifying ability of the solution was very low (500 mPa.s) when compared with isolated strains that had a viscosifying index value of 500-1300 mPa.s.

3.1.3. Effect of carbon/nitrogen availability and mode of cultivations

Generally, cell growth and xanthan production have different nutrient requirements and conditions for achieving a high xanthan yield. Biosynthesis of xanthan is favoured by a high concentration ratio of carbon source to the limiting nutrient that can be nitrogen, phosphorous or sulphur (8,49). When nitrogen is used as the limiting nutrient, a high C/N ratio is required for the stimulation of xanthan formation. A relatively high nitrogen concentration is required for fast cell growth and high cell density, which lead to fast fermentation, but at extreme high nitrogen concentration in the medium the xanthan production will be low or poor. Furthermore, the available amount of nitrogen source at the beginning of the stationary phase determines to a great extent the yield of xanthan gum produced (43,50). The rate and yield parameter estimates seemed to be independent of the initial nitrogen concentration, but the specific xanthan production rate in the stationary phase is dependent on the initial nitrogen content in the medium. This problem can be moderated

by providing a condition that allows both cell growth and xanthan formation. Due to this, a two-stage fermentation concept was described and proposed for the formation of xanthan gum in batch cultivation i.e 1, the trophophase in which there is propagation of cells and 2; the idiophase in which xanthan gum production proceeds (51). It has been reported that the beginning of xanthan formation can be attributed to cell physiological changes caused by the nutrient limitations, and that both cell growth and xanthan production were limited by availability of two different substrates at two different times of cultivation i.e carbon and nitrogen sources (50,52). The cell biomass is limited by the concentration of available N sources, whereas xanthan production is limited by C availability and can be modulated by the addition of the lacking nutrient at a given period of cultivation (43). The attainment of optimal conditions for production is a time-consuming task, though, with many variables under study. However, it is feasible to undertake a rational study by using adequate experimental statistical design procedures, which avoids a lot of tedious works. This two-stage formation concept can be used for continuous, semi-continuous or repeated batch-processes, in order to obtain a high xanthan production rate throughout cultivation (41-43,49,50).

Lo et al. (49) examined the effect of glucose and yeast extract concentrations separately and in combination on the synthesis of xanthan gum using batch, two--stage batch and fed-batch mode of fermentations. They found that a higher glucose concentration yielded a higher xanthan concentration. Both, xanthan yield and the specific xanthan production rate increased together with G/YE in the medium in all examined processes. The effect of different glucose/yeast extract ratios (G/YE) on xanthan gum production was opposite to the effect of glucose because at the same glucose concentration with a higher yeast extract concentration, a higher cell yield and specific growth rate was achieved, then again, at higher G/YE a lower specific xanthan production rate and xanthan yield were obtained. Nevertheless, it was found that this effect can be consolidated by finding an optimal glucose/YE ratio for increased xanthan yield and specific xanthan production rate. Among the examined processes, the two-stage fermentation was the preferred mode of production, since it provided fast cell growth, gave a higher xanthan yield and specific growth rate than the use of other processes. A glucose/YE ratio of 16.667:1 was found to be optimal for the production of xanthan and about 39.72% higher yield was achieved using a *X. campestris* strain NRRRL-B 1459 with the two-stage mode of fermentation.

Organic nitrogen sources in form of corn steep liquor (CSL), peptone, soya flour, distiller's yeast soluble, yeast extract and inorganic nitrogen sources such as NH_4Cl, $NaNO_3$ and $(NH_4)_2SO_4$ have been used for the synthesis of xanthan by *X. campestris* strains. These nitrogen sources in various experiments showed different effects, but yeast extract or peptone have been the most promising nitrogen source at a given C/N ratio (49,50,53). Gupte and Kamat, (53) showed that a combination of yeast extract and peptone at 3 and 5 g/dm^3, respectively, exhibited a synergistic effect with an increased formation of this EPS. Lopez and Ramos-Cormanzane, (54), used an ol-

ive mill waste water (OMW) for the production of xanthan gum by *X. campestris* NRRL B-1459 S4-L11 strain. The olive mill wastewater had a high C:N ratio with about 4.5% concentration of free sugars, which was optimal for polymer production. In this medium, OMW was the sole nutrient source at 30-40% and a maximum of 4g/dm^3 xanthan gum was produced. When ammonium chloride and yeast extract were supplemented at 0.5% and 0.3%, respectively, maximum. yield of 3.1 g/dm^3 and 7.7 g/dm^3 xanthan was achieved at 200 rpm with 10% inoculum and at an initial pH value of 7.0.

The ability of *X. campestris* to utilise glucose, sucrose (molasses or baggasse) and starch is common (2,53), but the utilisation of lactose is rare because most of these strains have low level of β-galactosidase activity. However, the ability to assimilate lactose has been demonstrated by naturally isolated and genetically engineered strains (53-57). Sucrose concentration in the range of 1.5-4.0% (53) and up to the range of 20-30% in form of sugar cane molasses has been used for the production of xanthan (2). The former has been reported as optimal concentration for improved maximum productivity of xanthan by *X. campestris* NRRL-13-1409. Abd El-Salem et al. (2) used sugarcane molasses at sucrose concentration of 2-30% and stated that between 2-3%, the productivity of xanthan was 0.58-0.63 g/g, but at higher sugar concentration the productivity and the sugar consumption declined. Glucose concentration in the range of 1.2-3.0% and dextrose in the range of 2.0-3.0% have been reported for improved synthesis and productivity of xanthan by *X. campestris* (53). The ability to utilise lactose or whey, which is unnatural for this strain and starch for xanthan synthesis, has been demonstrated for wild strains of *X. campestris* ICa-125, isolated from cabbage and modified *X. campestris* strain by encoding a gene responsible for β-galactosidase activity from other bacteria such as *Sphinogomas* and *Lactococcus lactis* (5,26,53,55,56). Generally, the optimal temperature, pH and inoculum size for effective synthesis of xanthan were 30-32°C, 6-7 and 10%, respectively. Among tested inorganic salts, a clear rise in yield of xanthan has been reported for added NaCl, $CaCO_3$, KH_2PO_4 and $MgSO_4.7H_2O$ at concentrations of 0.05-0.1% for various examined *X. campestris* strains. Addition of $CaCO_3$ gave the highest yield of about 16.2-16.4 g/dm^3 of xanthan in a medium containing 5.0% of sucrose for *X. campestris* ICa-125 (53,57), but concentration of different mineral salts at 2-5.0 g/dm^3 showed inhibitory effect for both growth and xanthan production.

Temperature shift method was also used to improve the xanthan synthesis and its quality. A temperature shift from 27 to 32°C after 20 h or 25 h of cultivation, respectively, was applied for the production of xanthan. As expected, the fermentation rate and xanthan yield were improved as compared to fermentation performed either at 27 or 32°C alone. A better yield was obtained for temperature shift after 25 h (stationary phase) of cultivation than after 20 h (exponential phase) of cultivation which showed that the timing for temperature shift process is important. The pyruvate content of xanthan gum produced by this method of fermentation was about 30% higher than the average value of that produced at 27°C (4.5%) and 32°C

(3.3%), respectively. At 22°C, xanthan yield was 54% but at 33°C, about 90% yield was obtained indicating that high temperatures of cultivation favoured xanthan synthesis. The pyruvate contents of xanthan also varied with a shift in fermentation temperature from 1.9 to 4.5%, with a maximum of about 4.5% occurring between 27 and 30°C of cultivation (58). Esgalhado et al. (59) used a response surface methodology (Doehlert distribution) to examine the relationship between pH and temperature for optimal xanthan yield, growth and index consistency by *X. campestris* NRRL-B-1459. A pH between 6.0-7.5 and a temperature between 25-27°C were found to be optimal for growth, but for xanthan production, these values were 7-8 and 25-30°C, respectively. At these optimal ranges, a concentration of 3.2 g/dm^3 of biomass and xanthan production of 17.7 g/dm^3 with an index consistency of 100.33 m.Pa.s were obtained (50,58). The optimum range of pH and temperature for xanthan production with an increased index consistency, generally occurs between 7-8 and 25-30°C, respectively. However, optimal parameters for strains in this respect may be lower and broader depending on the type and quality of xanthan gum produced and, most importantly, the used strain (49,53,57,59).

Xanthamonas campestris pv campestris 17pkMQLT, that posseses a high β-galactosidase activity was constructed by ligation of an *X. campestris* phage ϕLO promoter with pkM005, COIEI replicon containing *Escherchia coli* Lac ZY genes and the lpp ribosome-binding site. It was then inserted into an Lnc Pi broad host-range plasmid, pLT and subsequently transferred by conjugation to *X. campestris* 17, where it was expressed (5,11,12,56,60). This constructed strain was used to produce xanthan gum on lactose or whey containing medium at 0.4% and 10%, respectively. Suprisingly, this strain produced as much xanthan gum on whey as the parent cells did in the medium containing glucose, but differed in xanthan gum production on 0.4% lactose medium, resulting to about 0.25 g/dm^3 and 3.61 g/dm^3 for parent and engineered strains, respectively. On the other hand, the β-galactosidase activity found on whey medium for engineered strain was about 170-fold higher activity than in the parent strain (4290 U/cm^3 versus 25U/cm^3) (60).

Torrestina et al. (61) isolated 7 colonies of *X. campestris* strain from cabbage and characterised them based on their morphology, antibiotic resistant, xanthan yield, index consistency of the formed polymer and pyruvic contents. It was concluded that the colony diameter of the examined strains did not correlate with the viscosifying ability of the polymer or either with the xanthan yield or pyruvic acid contents. Rather the resistant to penicillin was suggested to be a good parameter for screening of potential producers of high quality xanthan, and about 6-8 g/dm^3 with a final apparent viscosity, ranging from 1000-2000 cp was obtained by different penicillin resistsnt isolates. Whereas, Rodriquez et al. (46) made use of this idea and selected spontaneously mutant strains of *X. campestris* NRRL–B1459 based only on their resistance to various antibiotics (streptomycin, tetracycline, ampicillin and penicillin) by plating directly on agar plate media containing different concentrations of these antibiotics. Among selected colonies, mutants resistant to certain concentrations of

streptomycin and ampicillin exhibited an improved xanthan viscosity of 15-19% over the parental strain. A selected mutant showed higher response to the formation of xanthan with an increased synthesis from 12.5-13.5 g/dm^3 in comparison to the wild type (11.7 g/dm^3), and with an improved viscosity of about 2866.0-3061.0 cP (*X. campestris* NRRL-B1459 M.-11) as against 2806.0 cP for the wild strain (*X. campestris* NRRL-B1459). *X. campestris* XLMLA carrying a plasmid pNZ521, containing phospho-β-galactosidase, insertion protein and proteinase P genes that were conjugally transferred for the first time from *Lactococus lactis* was used to produce xanthan gum on whey. The transformed strain of *X. campestris* XLMLA produced higher amounts of xanthan gum with a maximum value of 14 g/dm^3 in the medium containing 10% whey (5,56). Twelve genes (gum B-M) coding for synthesis, polymerisation and secretion of polysaccharides xanthan gum were clustered together on the chromosome of the bacterium *X. campestris* and transferred into *Sphinogomas* that is an alike-genus. About 0.9-4.3 g/dm^3 of xanthan were obtained with different modified strains of *Sphinogomas* under anaerobic conditions in glucose containing medium for 48 h. The polysaccharides produced by these recombinant micro-organisms were almost similar structurally and functionally to the native xanthan gum of *X. campestris* (26). These results demonstrated that a complex pathway for the synthesis of a specific polysaccharide exists and can be achieved by a single intergeneric transfer of genes between bacteria. This creates a novel feasibility of synthesizing xanthan or other polysaccharides in non-native host.

3.1.4. Effect of detergent and organic acid additions

Generally, surfactant or detergent and organic acid additions into the culture medium during fermentation affect both the synthesis of a given metabolite, metabolism of the cells under study, and the production of exopolysaccharide is not an exception. Galindo and Salcedo, (62) studied the effect of different detergents (Tween 40, Tween 80, [3-[(3-cholamidopropyl) dimethyilaminoniol]-2 hydroxypropane--1-sulfonate)- CHAPS and Triton X-100 on the synthesis of xanthan gum by *X. campestris* at 0.01 and 0.05% concentrations, respectively for each examined detergent. All examined detergents resulted in an increased production of xanthan when compared with the control. Overall, Triton X-100 (either at 0.01 or 0.05%) gave the best results and produced about 1.5-fold higher xanthan in comparison to the control. The mechanism by which the detergents affect xanthan production is not clear but this may change or alter oxygen transfer rate and the cell membrane permeability. Surfactants affect mass transfer either by changing the surface film resistance or the hydrodynamics of the fluid. It has been shown that bubble rise velocity was significantly reduced both in Newtonian and non-Newtonian fluids in the presence of surface active materials due to existence of surface tension gradients at the bubble interphase (49,63,64). Surfactants drastically change the rate of coalescence, thus making the

bubble size more uniform. The detergents may also interact with the bacterial cell membrane and therefore, could enhance the polymerisation process of the xanthan molecule (that occurs at the cell membrane) and/or the turnover and secretion of the completed xanthan molecules (63,64). In addition to increased synthesis of xanthan, the use of the detergent such as Triton X-100 resulted in a polymer of higher rheological quality with an increased xanthan viscosity of about 3-fold higher than the control. The addition of IBA (isobutryic acid) into the culture of *X. campestris* increased or stimulated also the xanthan synthesis, but decreased the biomass formation depending on the concentration of the acid in the medium (65,66). About 6.5% higher xanthan production was achieved at 0.16 mmol/dm^3 acid concentration than observed for that of control. Further increase in the acid concentration decreased both the synthesis of xanthan and biomass formation. The decrease in the biomass concentration was attributed to sublethal isobutyric stress response at the biosynthetic pathway (49,65,66). The lethal effect of IBA on cells during fermentation is a function of acid toxicity, a mechanism that is associated with the solubility of the undiassociated form of the acid on the cell membrane. The intense of the acid toxicity, as reported, depended on the culture pH *ie* as the pH values approach the acid pK_a, weak acids are extensively undisassociated, therefore penetrating freely into the cell membrane where the molecules disassociate and act as proton carriers, and thus affects the cell metabolism (49,65,66). Jana and Ghosh (45), studied the effect of citric acid concentration for the improvement of xanthan yield by *X. campestris*. They found that citric acid concentration up to 0.3% with optima at 0.26% added after 24 h of cultivation in the culture medium, improved the synthesis of xanthan up to 1.8-fold higher when compared with the control. The suggested presence of citric acid improved also the cell viability and xanthan synthesis under O_2-limited conditions irrespective of the viscous nature of the fermentation broth.

Esgaldaho et al. (49) also studied the effect of acetic, pentanoic and octanoic acids at various concentrations, separately on xanthan formation by *X. campestris* during a continuous process at pH 6. A close correlation was found between the acid concentration, the length of the lag phase and the value of the specific growth rate of the examined micro-organism. Growth inhibition occurred in the presence of these organic acids. The lethality of these compounds depended on the number of carbon atoms in the acid chain increasing from acetic to octanoic acid. At lower concentration of the acids, the strain produced similar biomass and xanthan yields, when compared to the control. At 2.5 mM acetic acid, about 9.26 g/dm^3 was obtained in comparison with the control (9.67 g/dm^3), and both had an identical specific growth rate of 0.13 h^{-1} and lag phase of 2 h. It is worthy of note that at 2.77 mM of octanoic acid, a non-growth associated synthesis of xanthan was observed with a specific production rate of 11.1 g g^{-1}, xanthan yield of 1.67 g/dm^3 and lag phase of 30 h. As described, acid stress changed the levels of the cell physiology leading to modification of the microbial growth curve prior to a critical toxic concentration that hampered culture progression or lysis of the cells.

4. Application of xanthan

Xanthan has gained wide spread applications in different branches of industry. This is concerned with its physico-chemical properties (viscosity, texture, ability to suspend solids and emulsion stabilisation), which makes it a convenient and stable component for application in the food, oil, pharmaceutical, cosmetic, paper, paint and textile industries (2-4,13,26,59,66-68). The chemical structure of xanthan with respect to its secondary structure has been shown to consist of a fivefold helical shaped matrix, therefore it undergoes a thermally induced order-disorder conformational transition. The disordered form is activated by low salt concentration and high temperature, while high salt concentration stabilises the ordered conformation. Furthermore, xanthan is resistant to the attack of cellulases, although it has cellulose at its backbone (7,10,14,59,66-68). This trisaccharide side chains are barriers to the enzyme attack due to specific linkage. However, microbial attack caused by enzymes has been shown to occur when xanthan is in the disordered form, but no such report has been made for the ordered helix conformation. The convenience nature of xanthan may be attributed to its solubility both in cold and in hot water, but requires intensive agitation. Moreover, there is no fixed daily intake value for xanthan, and thus could be added to different food products at *quantum satis* because of its safeness (9-10,15,66-68).

Xanthan gum is widely used as stabiliser and thickener in the food industry for salad dressing in order to prevent oiling off and dispersion of insoluble particles due to the three dimensional network, formed by the associated xanthan gum chains. The shear thinning flow – behaviour (non-newtonian and high pseudoplasticity) coupled with high shear stability makes it easier to mix and pump industrially produced dressings and sauces. Xanthan gum solutions exhibit high viscosity even at low concentration and have shear thinning and yield stress characteristics. The viscosity of xanthan gum solutions is very stable at wide spectrum of pH, temperature and ionic strength (13,19,22,66-68).

In soups and gravies, xanthan gum prevents thickness and acts as a stabiliser. The addition of xanthan gum in bakery products impacts starch granules an essential cohesion, contributes to the structure improvements and increases shelf-life due to its ability to retain moisture. In cold storage technology, xanthan gum is used to improve frozen food products quality and stability due to its ability to bind free water, which may cause syneresis after one or two freeze-defrosting cycles. Besides, it limits the formation of large ice crystals during freezing and provides the required texture for frozen food products. Xanthan gum is also an effective food additive for suspending fruit pulps in beverages and fruit juices. It gives the drink an enhanced mouthful, contributing to good consistency and texture, full-bodied taste and good aroma or flavour release (9,10,66-70). This is especially important for low caloric drinks that are totally or partly devoid of natural sugars, with artificial sweetener as replacement for the preparation of foods for diabetic patients. A combina-

tion of xanthan gum with galactomannan (derived from locust bean or guar gums at a mixture of 1:1) gives a synergistic viscosity increase for the preparation and stabilisation of cottage cheese dressings and spreadable pasteurised cheese products (2,9,69,70). In the near future, a combination of xanthan gum with new microbial gums, generally termed as "Sphingans" (gellan, wellan, rhamsan, sphingan S-88) at a given ratio may provide a synergistic effect with novel and unique rheological properties upon solubilisation and heating for new dairy product developments (26,30, 36). In the field of cosmetics, xanthan gum is used for the preparation of toothpaste and body cream where it acts as thickener and stabiliser because of its shear-thinning flow behaviour. Due to its special rheological properties (such as high shear ability, stability and pseudoplasticity), xanthan is used for the preparation of printing paste, paint, ceramic glaze and colour textile coating or dyeing (22-23,66-69). In the oil industry, xanthan is a well accepted viscosifying agent in drilling fluids and has great potentials in enchancing oil recovery, but needs to be sulphated or should contain low content of pyruvic acid (38,70). A sulphated xanthan is a diminution in shear thinning of aqueous solution of the material when compared with natural xanthan, and it is useful as flocculant for oil extraction or recovery in oil polluted sea shores. In addition to xanthan use in food for human consumption, this polysaccharide is added to pet foods, as a thickening agent and binder in the preparation of semi-moist food. In the agrochemical and cosmetic industries, this gum is used mainly as a suspending agent to increase the spray applications of a variety of active substances in chemicals and to reduce the drift of chemical used for plant protection, herbicides, pesticides, fertilisers and fungicides (18-19,66-70).

5. Conclusion

Xanthan gum has gained wide spread acceptance as an ideal exopolymer with unique rheological properties in different branches of industry for food and industrial product developments. The biochemical/genetic aspects of xanthan synthesis by *X. campestris pv campestris*, in although elucidated in part, need further studies as far as the mechanism of polymerisation, secretion and regulation are concerned. Furthermore, a new type of impellers or turbines should be constructed or retrofitted specially for xanthan fermentation, taking into consideration the efficiency in terms of productivity, energy costs and oxygen distribution during cultivation due to viscous nature of the broth. Research should be conducted both on genetic modification of strains in order to increase their ability to utilise whey and starch effectively, as well as, improving the operation conditions. Since preservation of *X. campestris* strains is a cause of concern, a search for adequate method for long-term storage is needed, due to their instability as good reproductivity is required in any industrial processes. Moreover, emphasis should be laid on the quality of xanthan produced with respect to the viscosifying ability (index consistency), and not only on yields.

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