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ORIGINAL ARTICLE

Production of Xanthan gum from *Xanthomonas compestris* isolated from Cruciferous Vegetables

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ABSTRACT

Xanthomonas campestris produce the xanthan gum which is the polysaccharide of this bacteria. This xanthan gum has various mainly in bakery products, beverages, dairy, dressings, syrup, topping, sauces, polishes, pigments, textile and pet food etc. Black rot of cruciferous vegetables is caused by plant pathogen called Xanthomonas campestris. In the present study Xanthomonas campestris was isolated from cruciferous vegetables including cabbages, cauliflower, turnip and mustard. Different cultural, morphological and biochemical characteristics were performed that shows that the bacteria isXanthomonas campestris. This isolated bacteria was then used for xanthan production by using production media. Viscosity was also measured. Out of 30 different samples 8 samples showed all colonies of yellow color, circular, entire margin and mucoid colony. These 8 isolates were named as x1, x2, x3, x4, x5, x6, x7, and x8.The Xanthan gum production was achieved ranging from 0.074 – 0.570 g/100ml .Viscosity of the Xanthan gum produced by 8 isolates was ranging from1.250cP 4.270cp. X4 isolate indicated maximum xanthan gum production of 0.570g/100ml and x8 gave less production of xanthan gum of 0.074 g/100ml. Isolate X4 produced xanthan gum with high viscosity of 4.270cP while x1showed viscosity of 1.250cP. Our study conclude that Xanthomonas campestris can be isolated from the cruciferous vegetables in Pakistan. Our study also conclude that xanthan gum can be prepared from the isolated species of Xanthomonas campestris isolated from cruciferous vegetables. Preparation of Xanthan gum was done and method was adjusted to balance for the industrial use. Various studies clarified the different factors such as method of recovery, specified microbes and raw material budget involved in the xanthangum production and principal investment. But still there are many problems that needs to be defined for biological production of xanthan gum in targeted budget like to develop microbes with high xanthan gum producing capacity, to lower the expenses of raw material and other production methods. Improvement of the xanthan gum production from inexpensive raw materials need to be studied to make it cost effective.

Key words: Xanthan gum; crucifers; Xanthomonas campestris; raw material, Biological

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INTRODUCTION

Black rot of cruciferous vegetables is caused by plant pathogen called *Xanthomonas campestris* [1]. This bacteria produce xanthan gum which is exopolysaccharide of this bacteria and this property make it important both for both agricultural and industrial point of view[2-3]. .*Xanthomonas campestris* belong to

the family of Xanthomonadaceae and order Xanthomonadales in the γ-Proteobacteria. Xanthomonads can be transferred to a new host, and principally a period of survival is essential in the lack of the host in such instance. Numerous ways might be used for such survival like with seeds, residues of plants, perennial hosts and existence in soil or on insect both epiphytical and saprophytical[4].In case of plant disease caused by *Xanthomonas campestris,* debris of Plant, seed, and weeds are mostly reported to be main source of black rot disease[5-7]. The infested soil may be the source of infection[8-9]. The major xanthan gum producing species *Xanthomonas campestris* pv. *Campestris* grow well particularly in climatic condition that is warmth and humid. In plant debris and infected seeds it survive from one season to another season and rain splashes transmitted it to nearby plant[10-11]. The bacteria might survive for a survival period of two years in case when it is protected by the host debris. Even though this bacteria is largely seed-borne[12].The production of exopolysaccharide by has an important role for their survival in soil[11]. *Xanthomonas campestris* is aerobic gram negative rods with monotrichous flagellum. Colonial morphology is generally yellow color, smooth and viscous[13]. Xanthan was discovered by Department of Agriculture, United States at National Regional Research Laboratories, which is an important biopolymer[14]. Xanthan gum is an important water soluble exopolysaccharide of the microorganism which is a water-soluble microbial polymer with particular rheological properties. Industrially and agriculturally this gum is very important and it has many application. In agriculture xanthan is used in combination with antifungal agent for the prevention of *Bipolaris sorokiniana* which cause disease barley cultivars[15].On the other hand, xanthan gum is used as thickening and emulsifying agent in food industry for many food product and other products including mainly in bakery products, beverages, dairy, dressings, syrup, topping, sauces, polishes, pigments, gravies, textile, jellies, margarine, yoghurt, chocolates and pet food etc. Production cost can be reduced when Xanthan gum is used together with gums such as locust bean gum or guar [16]. In many food products Xanthan gum now a day's provides the required properties like appearance, properties of water control, texture, viscosity, and flavor. In addition to these properties, pseudoplastic behavior of xanthan gum in solutions too progresses rheological properties of the final product[4]. For the evaluation of the enhancement of the viscosity of the bacterial exopolymer, a study was conducted that expose many strain of the bacteria to the ampicillin repeatedly that includes *Xanthomonas campestris.* Both parent and mutant strains shows no alteration in the monosaccharide composition but high molecular weight product was observed in case of high viscosity mutant strain[17]. Enhancement of oil recovery is another important application of xanthan gum. The important property of this gum is that in small amount it form solution with high viscosity and pseudo plasticity. In order to efficiently extract the oil, it is essential to pump the xanthan gum in the rocks as the oil is detained in the petit pores of the small sand stone[18]. Xanthan gum can be produced by approximately all *Xanthomonas* spp., particularly *Xanthomonas campestris* pv. *Campestris* [19] xanthan production and their properties are dependent on the microbial strain, are shown by many studies [20-21]. Hence it is important to isolate and identify new strains of *Xanthomonas campestris.* That can provide a chance to increase the rheological quality products and enhance xanthan gum yield[22-26].The market capitalization of xanthan gum is estimated at $ 270 million, and the 2015 estimate exceeds $ 400 million[27]. To supply the xanthan gum to various sectors for consumption, 400million dollars are consumed every year to produce 86000 ton of xanthan gum[28]. Out of total xanthan gum produced by the word, 65% is consumed in food industry, 15% is consumed in oil industry and the 20% is consumed by other application while these demands need to be increased by estimated annual growth of5–10% [4]. The main producers for this raw material is china and Austria. The annual xanthan gum consumption of the Brazil is about 30 000 ton as stated by Brazilian Department of Trade and Industry. This whole demand is supplied by international industries as Brazil have no industry for xanthan production. The development and economic empowerment can be developed in countries having local production of xanthan[29].

In Pakistan there is limited research work on the biological production of xanthan gum. So we conduct this study to scale up and optimize the biological production method for the xanthan gum. The aim of our study was the isolation of *Xanthomonas campestris* from the cruciferous vegetables including cabbages, cauliflower, turnip and mustard and then this isolated bacteria was used for the production of xanthan gum.

MATERIAL AND METHODS

30 samples were collected from the fields of cruciferous vegetables in Lahore, Punjab province. Sampling was done from the leaves having yellow lesions and black. The samples collected were washed and the areas of infection were cut into small pieces and the surface was disinfected for one minute with 70% ethyl alcohol. These small pieces were washed three time with sterile water. Then the pieces were

crushed on sterilized glass slides to get bacterial suspension. This suspension was streaked on Potato Sucrose Peptone Agar (PSPA) medium was used for the growth of these bacterial suspension. The composition of the Potato Sucrose Peptone Agar is given in the Table 1.Washed pieces were also inoculated directly on Potato Sucrose Peptone Agar plates. Incubation period for the plates was 48 hours at room temperature. To get single colonies of the specified microorganism the single colonies were selected based on colony color, sliminess and viscosity and these colonies were purified by repeated streaking on Potato Sucrose Peptone Agar plates. In refrigerated condition the pure culture was stored both in sterile water and in slants.

Table 1: Composition of Potato Sucrose Peptone Agar

Serial No	Chemical	Concentration (g/l)
1.	NaHPO4	0.5g
2.	calcium nitrate	0.5g
3.	potato	300g
4.	peptone,	2g;
5.	sucrose,	20g
6.	KH2PO4,	0.2g
7.	KCl,	0.05g
8.	ferrous sulphate	0.05g
9.	agar	20g per litre

Morphological, cultural and biochemical characters were considered for the characterization and identification. For colonial characteristics the colonies were purified on PSPA plates. For gram reaction and morphological characteristics Gram staining was done.For the identification of the microorganism many biochemical test Sugar fermentation tests, Indol production test, Methyl red test, citrate utilization test, urea hydrolysis test, Hydrogen-sulphide production, gelatin liquefaction, catalase test etc, were performed[25-29].The media for all these test was inoculated aseptically. The incubation period for the inoculated media was 24-48 hours at 37°C.The composition of the production media for the production of Xanthan gum from *Xanthomonas campestris* is shown in the table 2. Before sterilization of the media the pH was adjusted to be 7.

Table 2: Composition of the production media

Serial No	Chemical	Concentration (g/l)
1.	Glucose	20g
2.	KH2PO4	5.0g
3.	MgSO4. 7H2O	0.2g
4.	(NH4)2	2.0g
5.	Citric acid	2.0g
6.	H2BO3	0.006g
7.	ZnO	0.006
8.	FeCl3.6H2O	0.0024g
9.	Caco3	0.02g
10.	HCl	0.13ml

Xanthan gum production

500ml Erlenmeyer flask was used in which 90ml of the production media was added and then it was inoculated with 10 ml of the microorganism. For fermentation the flask was kept at 200rpm in rotary shaker at $28C^0$ for four days. The final broth was heated for half an hour at $80C^0$ after 4 days. It was then diluted five times with water. Then for 40 minutes the broth was centrifuged at 8000g.For xanthan gum isolation supernatant was used while suspended cell mass was discarded. To the supernatant solution three volumes of chilled 96% alcohol was supplemented. Xanthan gum precipitation and settling down occur after some time. The precipitated gum was left at $50C^0$ in oven. At the end the weight of the xanthan gum was measured. Rheoviscometer was used for viscosity measurement by using 0.1% (w/v) gum solution with 1% KCl.

RESULTS

Out of 30 different samples collected from the fields of cruciferous vegetables in Lahore, Punjab province, 8 samples showed all colonies of yellow color circular, entire in margin and mucoid. These 8 isolates were named as x1, x2, x3, x4, x5, x6, x7, and x8. These isolates were observed to be aerobic motile Gram negative rods with Glucose, Lactose and Sucrose fermentation. The cultural, morphological and biochemical characteristics were as shown in table 3 and table 4.

Table 3: Cultural and morphological characters of the isolates

Serial No	Cultural and morphological character	observation
1	Gram reaction	Negative
2	Margin	Entire
3	Surface	Small smoot
4	configuration	Rod
5	Colony color	Yellow mucoid

Table 4: Biochemical characters of the isolates

Serial No	Biochemical characteristics	observation
1.	Citrate utilization test	+ve
2.	Starch utilization	+ve
3.	H2s production	+ve
4.	Lipolytic activity	+ve
5.	Mannitol hydrolysis with acid production	-ve
6.	MR test	-ve
7.	Nitrate reduction	-ve
8.	Indole production	-ve
9.	Catalase test	+ve
10.	Growth in 6% NaCl	-ve
11.	Ammonia production	+ve
12.	Urease test	-ve
13.	Arginine dihydrogenase	-ve
14.	Gelatin liqufication	+ve

The obtained production of Xanthan gum was ranging from 0.074 – 0.570 g/100ml as shown in the table 5 by the different isolates. Viscosity of the Xanthan produced by 8 isolates were observed to range from 1.250cP 4.270cp. (Table 6)

Maximum xanthan gum production was shown byX4 isolate of 0.570g/100ml and x8shows less xanthan production of 0.074 g/100ml.(Table 5)high viscosity xanthan gum wasproduced by Isolate X4 (4.270cP) whilex1showed low viscosity (1.250cP) xanthan gum.(table 6).

Table 5: Xanthan gum production by different isolates

Serial No	Isolate	xanthan gm/100ml
1	X1	0.190
2	X2	0.320
3	X3	0.540
4	X4	0.570
5	X5	0.292
6	X6	0.210
7	X7	0.470
8	X8	0.074

Table 6: Viscosity of the Xanthan gum production by different isolates

Serial No	Isolate	Viscosity (cp)
1	X1	1.250
2	X2	2.52
3	X3	3.46
4	X4	4.270
5	X5	3.27
6	X6	2.270
7	X7	1.11
8	X8	1.60

DISCUSSION

Xanthomonas campestris was isolated from the cruciferous vegetables by using Potato Sucrose Peptone Agar (PSPA). For xanthan gum production 8 different isolates were used. These 8 isolates were named as x1, x2, x3, x4, x5, x6, x7, and x8. These isolates were observed to be aerobic motile Gram negative rods with Glucose, Lactose and Sucrose fermentation. These 8 isolates were identical biochemically and morphologically.

Out of 8 isolates X4 isolate showed maximum xanthan gum production of 0.570g/100ml and x8 showslowest xanthan production of 0.074 g/100ml while the other isolate shows intermediate xanthan gum production. The xanthan gum production range of 2.3-8.3 is shown in other study[30]. The productivity report by Moreira *et al* [30] is comparable to our study .The literature shows that xanthan gum production is relate to the substrate used for productivity. Moreno et al. obtained maximum xanthan production of 1.6 g/L by using *xanthomonas compestris* NRRL B-1459 and melon as a substrate[32]. Bilanovic *et al*. reported that highest xanthan gum production of 12 g/L can be obtained by using citrus waste as low cost substrate[32].Lopez *et al*. [33]studied 4 strains of *Xanthomonas compestris* for xanthan gum production by using olive mill wastewaters. The supreme valued strain was *xanthomonas compestris* NRRL B-1459 S4LII asset has capability to produce xanthan gum of 7 g/L byusing 7% of olive mill waste waters as substrate. Earlier study used Whey to get maximum xanthan production of 1.2 g/100 mL of whey with X*anthomonas compestris* XLM 1521. Molina et al. reported maximum xanthan gum of 14g/L by using as a carbon source[35]. Xanthan gum from different agricultural waste products was compared to xanthan gum produced from sucrose and glucose. For optimization of variables for xanthan gum production numerous tries have been done that reported that glucose is still good on the basis of yield and quality of the product[35].Rheoviscometer was used for viscosity measurement of each isolate. Isolate X4 produced gum with high viscosity of 4.270cP while x1showed low viscosity of 1.250cP. X2, x3, x5, x6, x7 and x8 shows viscosity values of 2.52cp, 3.46cp, 3.27cp, 2.70cp, 1.11cp and 1.60cp.The viscosity of xanthan gum solution is dependent upon on the temperature of the viscosity measurement and the temperature of the xanthan dissolution. By increasing temperature the viscosity decreases. Between$10C^0$-$80C^0$ this conduct is completely reversible[36]. The viscosity of the xanthan gum solution decreases by increasing temperature. With the increase of xanthan gum concentration the viscosity increases with statistical significance as projected. Rottova *et al* [37] reported that viscosity has no correlation with the pH value because the viscosity values were not influenced by pH value.

CONCLUSION

Our study conclude that *Xanthomonas campestris* can be isolated from the cruciferous vegetables in Pakistan. Preparation of Xanthan gum was done and method was adjusted to balance for the industrial use. Various studies clarified the different factors such as method of recovery, specified microbes and raw material budget involved in the xanthangum production and principal investment. But still there are many problems that needs to be defined for biological production of xanthan gum in targeted budget like to develop microbes with high xanthan gum producing capacity, to lower the expenses of raw material and other production methods. Improvement of the xanthan gum production from inexpensive raw materials need to be studied to make it cost effective.

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