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Research Article

Production of Xanthan from Agro-Industrial Waste

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ABSTRACT

Xanthan are water soluble Exopolysaccharides produced by Xanthomonas species. These polysaccharides have much common application and normally produced in submerged fermentation by using different carbon sources. Solid state fermentation offers numerous advantages for the production of bulk chemicals and enzymes. An attempt to synthesize Xanthan from agro-industrial wastes by solid state fermentation yielded a dry weight of 2.9 gm/50gm of substrate when fermented by *Xanthomonas citri*, 2.87 gm by standard strain *Xanthomonas campestries* (MTCC) 2286 and 1.5 gm by *Xanthomonas oryzae*. Potato peel was employed as carbon substrate in which *X. Citri* produced high amount of Xanthan. The recovered xanthan was checked for its purity and composition by chemical analysis and structural analysis by FT-IR. TLC and HPLC confirmed the sugars in isolated Xanthan while FTIR ensured the presence of uronic acids. This work emphasizes the possibility of using agricultural wastes as lower cost alternative substrates for Xanthan production which is a widely used food additive.

Keywords: Xanthan, Xanthomonas sps, Exopolysaccharides, Polymers

1. INTRODUCTION

Exopolysaccharides (EPS) are chemical compounds which are synthesized by various microbes using different carbon sources during fermentation process and are secreted outside the cell wall or as slime or into the extra cellular medium as jelly like material. Microbial EPS are mainly linear molecule to which side chains of varying length and complexity are attached at regular intervals [1]. The bio synthesis of EPS is believed to serve many functions like cell attachment to solid surfaces, enhanced bio film formation, tolerance to environmental stress, etc.[2] Xanthan formed by *Xanthomonas* species serve the bacterium in acting as plant pathogen by allowing its penetration and formation of local lesions, soft roots, scabs, cancers. Black rots caused by *X. campestris* pv campestris (XCC) is one of the most damaging diseases of cauliflower and other crueifers *X. campestris* and pv musacearum is the causal agent of banana bacterial wilt disease. Diverting the EPS secretion to beneficial counterpart of this *Xanthomonas campestris* resulted in Xanthan production and now we use Xanthan as food additives. Solid state fermentation has been defined as the fermentation process occurring in the absence or near absence of free water. SSF has emerged as a potential technology for the production of microbial products such as feed, fuel, food, industrial chemicals and pharmaceutical products.

Utilization of agro-industrial residue as substrates in SSF process provides an alternative avenue and value addition to these otherwise under or unutilized resources, which we call as residues. More recently the problem of effluent from processing operation and their disposal has gained public recognition. Human beings produce large quantities of waste as we go about our daily lives. Waste is defined as any material which has not yet been utilized (i.e.) the left overs from production and consumption. This includes agricultural, industrial, municipal wastes and residues. The bulk of the wastes from agricultural and food processing are not suitable for food and fodder as they are too fibrous to be digested by mono gastric animals. These substances carry 3-70% carbohydrates that are consumed by microbes. Thus these microbes can be employed in utilizing wastes where the cell biomass is achieved along with the synthesis of valuable by products. This work attempts to synthesize Xanthan-industrial gum using potato peel which is an unutilized raw material of potato chips industry.

2. MATERIAL AND METHODS

2.1. Screening & isolation

Microorganisms from infected samples were isolated, characterized and used in the fermentation process. Infected

leaves and plant parts were isolation sources and the isolate was characterized by biochemical tests and comparative studies with standard strain of *Xanthomonas campestries* MTCC 2286 from IMTECH Chandigarh.

2.2. Fermentation

Fresh potato peel was collected from local potato processing shops.50g of potato peel was taken for each isolate and was finally trimmed and grounded thoroughly by adding 20ml of water in order to moisturize the substrate. The pH was adjusted to 7.2 throughout the process. The substrate was used after sterilization. 24 hours broth culture of three isolates identified as *Xanthomonas* species along with the standard were inoculated to the autoclaved substrate and incubated for 6 days at 28°C. All the samples were processed in duplicates and totally 8 flasks were incubated.

2.3. Isolation of Gum/Gum recovery

To remove the bacterial cells from viscous broth, the culture broth was diluted. The cells are removed by centrifugation and 1% potassium chloride is added to the broth. Precipitation of gum is achieved by addition of 3 volumes of chilled 95% ethanol. Xanthan gum was recovered by precipitation with two volumes of isopropyl alcohol using 2% (w/w) potassium chloride as electrolyte. The precipitate was dried in an oven at 40°C for 24 hours (approx). Relative purity is defined as the content of polysaccharide (based on total carbohydrate) per gram of the product, was determined according to the sulphuric acid resorcinol method [3]. This method allows the determination of Xanthan gum concentration as a function of total carbohydrates using a standard solution (5-250 ppm).

2.4. Analytical Determinations

The isolated crude Xanthan from four *Xanthomonas* species were used for various analytical determinations. Of a commercial xanthan gum, a reconstituted solution of 1g/l of sample powder was prepared and diluted in the ratio 1:10 to set a standard. A sample of 1ml of the diluted solution was taken and 1ml of conc.H_2SO_4 phenol mixture was added and placed in ice bath for 5-15 minutes and the absorbance of the samples at 494nm was determined in the spectrophotometer. The proteinaceous nitrogen of the four samples was determined by Lowry's method. Pyruvic acid content of the recovered Xanthan sample was determined by Edwin Martin case method .The sugar composition of Xanthan was further confirmed by TLC and HPLC and the relative purity of Xanthan and presence of amino sugar was confirmed by FTIR.

3. RESULTS & DISCUSSION

The source of plant disease includes citrus canker of Lemon Bacterial blight of paddy and Bacterial wilt of banana. The species of *Xanthomonas* isolated are listed in Table I. [4] elaborated the survival of *Xanthomonas* campestris pv. Vesicatoria in pepper seeds [5]. Identified and characterized the bacteria from micropropagated mint plant and nearly 22 bacteria were characterized out of which 8 were identified as *Xanthomonas*. The presence of amino sugar was confirmed by FTIR reports Fig-1&2.

Table 1. List of Xanthomonas isolated from various sources

Sample	*Plant disease*	*Propable isolate*
X_1	Citrus canker	*X.* citri
X_2	Bacterial blight	*X.* oryzae
X_3	Banana wilt	*X.* musacearum

Table 2. Biochemical characterization of isolates

Tests	**X_1**	**X_2**	**X_3**	**Standard**
Indole	Negative	Negative	Negative	Negative
Methyl red	Positive	Positive	Positive	Positive
Vp	Negative	Negative	Negative	Negative
Citrate	Positive	Negative	Positive	Positive
TSI	$K^+/A^+/G^+/H_2S^+$	$K^+/A^+/G^-$	$K^+/A^+/G^-$	$K^+/A^+/G^-/H_2S^+$
Catalase	Positive	Positive	Positive	Positive

The biochemical test done for characterization and the results are listed in Table II. These reports clearly indicate or help in identification of *Xanthomonas* strain when they are compared with standard strain of MTCC strain of *Xanthomonas* campestries 2286. The amount of Xanthan obtained by fermentation on potato peel with various isolated Xanthomonas is tabulated in Table III.

Table 3. Xanthan yield from various isolates

SAMPLE	XANTHAN OBTAINED for 50gm peel
X. citri	2.900g
MTTC2286 *X.*campestries	2.870g
X. oryzae	1.500g
X. musacearum	0.500g

X. citri seems to be the high yielding isolate as they yield 2.900 gm which is 0.03 gm more than standard strain MTCC-2286. *X.*oryzae isolated from paddy blight yielded 1.500 gm of Xanthan [6] compared two conventional media with 13 strains

of *Xanthomonas* campestris pv pruni and reported the highest yield as 26.4g/l in defined medium. The yield here of ***X.***campestries MTCC 2286 is 2.6gm/50gm of potato peel by SSF. Natural substrates are easily available and are cheaper than synthetic substrates. But they generally require pre treatment to make their chemical constituents more accessible and their physical structure more susceptible for microbial penetration. Here the potato peel used as substrate was only autoclaved and the substrate is comprised of a complex starch medium, which is a source of carbon and other nutrients during the fermentation.

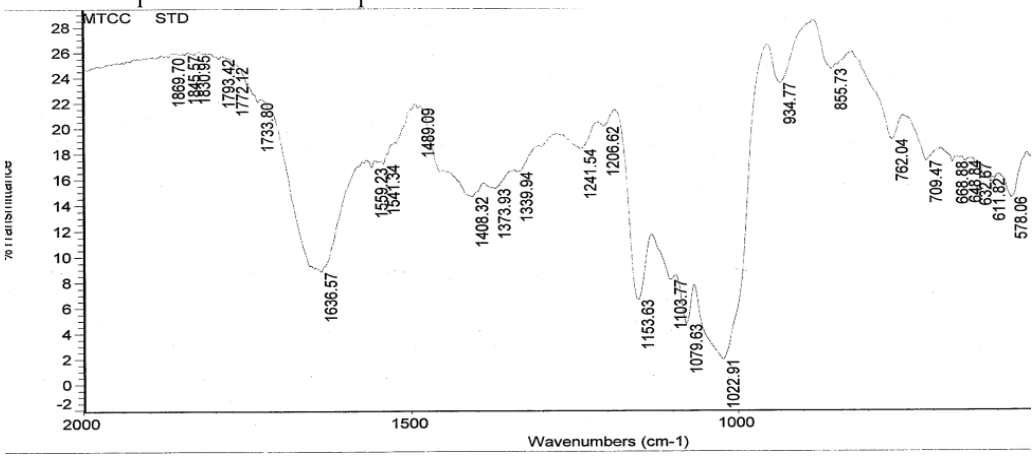

Figure 1. FTIR-X. campestries MTCC STD

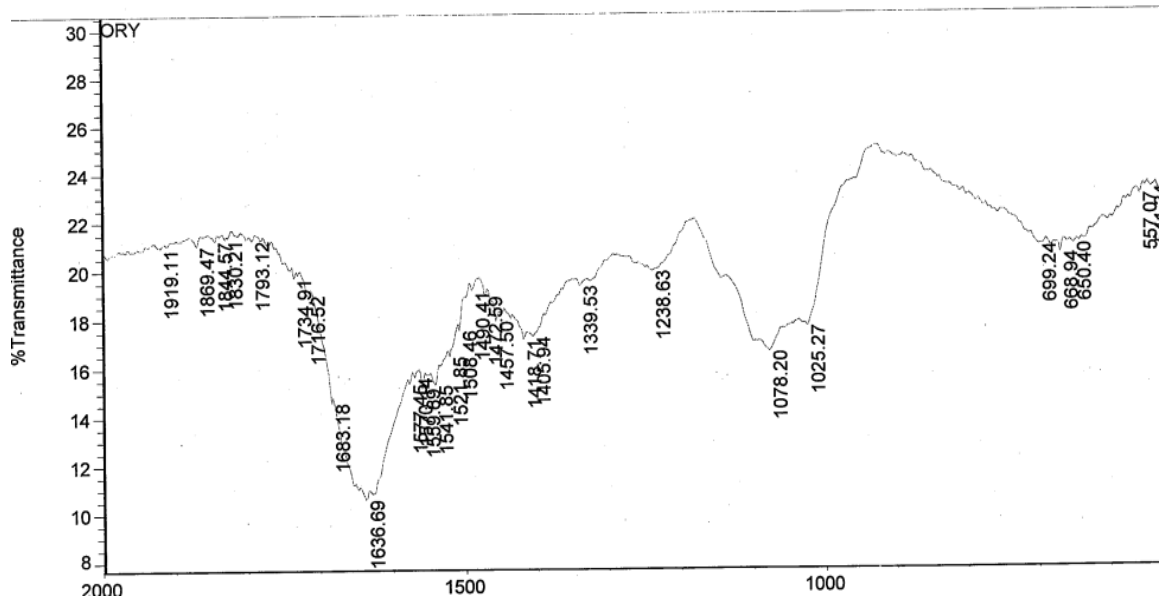

Figure 2 FTIR –X. oryzae

Comparison of four different fractions of citrus waste as substrate for Xanthan formation had been studied [7].The whole waste was proved to be a good substitute for glucose media for Xanthan production. The total sugar content of produced Xanthan was high for X. *citri* and less for *X. oryzae* where the protein content is high. Xanthan from X. *campestris* is composed of high amount of pyruvic acid than that of *X*.citri and *X. oryzae*. Use of orange and potato peel extracts as bacillus substrates for the production of enzymes was reported [8]. These substrates were employed to induce the synthesis of extra cellular hydrolytic enzyme production of *B. subtilis* 11089. The work reports on the performance of potato filtrate that supported the highest levels. Here the extra cellular hydrolytic enzymes are induced due to the presence of extra cellular substances synthesized from carbon substrates like potato peel and thus this serves as an indirect evidence to use potato peel as substrate to synthesize extra cellular substances like Xanthan [9, 10]. Use of agricultural wastes for Xanthan production by *X. campestris* also documented [11]. Melon, Watermelon, Cucumber and Tomato were compared for

Xanthan production and melon acid hydrolysate was proved to be the best source [12]. Investigation of the Xanthan gum production from *X. campestris* using waste sugar beet pulp has been done [13, 14]. Reported the Xanthan gum as an economical substitute for agar in plant tissue culture media. Presence of peaks at the range 1560 to 1540 indicated the presence of Uronic acid. Comparative study of FTIR reports of commercial Xanthan and newly produced Xanthan by SSF on potato peel ensures the purity of recovered product.

4. CONCLUSION

To conclude we would like to suggest the use of agro-industrial waste like fruit or vegetable peels as substrate for synthesis of Xanthan gum that holds a high industrial demand. Using agro-waste in production of commercial products like Xanthan which has high demand ensures a low cost alternative with high productivity. But anyway, the Xanthan obtained needs purification to obtain food grade Xanthan.

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