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STUDIES ON ACCLIMATIZATION OF *XANTHOMONAS CAMPESTRIS* ON WHEY FOR PRODUCTION OF XANTHAN GUM

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ABSTRACT

Xanthomonas campestris is an industrially important microorganism which produces xanthan gum, which is extensively applied in food and other industries. *Xanthomonas campestris* possesses a low level of β-galactosidase activity and therefore is not able to grow and produce significant amounts of xanthan gum, in a medium containing lactose as the sole carbon. β-galactosidase level of *Xanthomonas campestris* was elevated by physical mutagenesis using controlled exposure to ultraviolet radiation. The mutated strain was then acclimatized on medium containing whey. The mutated strain was found to give the 245% more yield of Xanthan gum on medium containing whey.

INTRODUCTION

Xanthomonas campestris is an agriculturally and industrially important bacterium. It causes black rot disease in crucifier's members like cauliflower, cabbage etc. resulting in tremendous economic losses worldwide (Williams, 1980). On the other hand, this bacterium produces xanthan gum, which has many applications in food, cosmetics and oil industries as a stabilizing, emulsifying, suspending and thickening agent (Becker *et al.*, 1998). For xanthan gum production sucrose, glucose or starch is normally used as a carbon source. Xanthan gum is a high molecular weight exopolysaccharide composed of cellulosic backbone with trisaccharide side chains attached with alternate glucose residues in the backbone. The side chains are composed of two mannose and one glucuronic acid molecule (Jansson *et al.*, 1975 and Melton *et al.*, 1976). It displays an extra ordinary combination of physical and chemical properties that make this exopolysaccharide ideal for a wide range of applications (Sandford and Baird, 1983).

Souw and Demain, (1979) showed that a medium with 4% of sucrose or glucose and a suitable nitrogen source gives the highest yield of xanthan gum. Growth of *X. campestris* in lactose based medium produces very less amount of biomass due to low levels of β-galactosidase. As a result on a lactose-based medium significant amount of xanthan gum is not produced (Frank and Somkuti, 1979).

Whey, a byproduct of cheese industry contains 4 to 5% lactose, 0.8 to 1% proteins, and small amount of organic acids, minerals and vitamins (Charles and Radjai, 1977). In this study an attempt has been made to use the whey medium for the production of xanthan gum using mutant *X.campestris* developed by physical mutagenesis using UV- radiations.

MATERIALS AND METHODS

Bacterial strain

The wild type *X.campestris* was isolated by inoculating the plates with crushed leaves of cabbage infected with *X.campestris*.

Media and cultivation

The general purpose media used were Luria Bertani broth and Luria Bertani agar. (Miller, 1972) XOLN medium (Jen-Fen and Tseng, 1992) which was used as a cultivation medium contained (per liter): K_2HPO_4, 0.7g; KH_2PO_4, 0.2 g;$(NH_4)_2SO_4$, 1.0g; $MgCl_2$ - $6H_2O$, 0.1g; $FeSO_4$ - $7H_2O$, 0.01g; $MnCl_2$, 0.001g; 0.0625% yeast extract; 0.0625% tryptone, pH 7.15. The last two components were added to the medium after autoclaving. Ampicillin with concentration 50μg/mL was added to the media.

X.campestris was cultured in 250mL flasks containing liquid media with vigorous shaking at 28ºC at 200rpm for 24 hr.

Extraction and determination of xanthan

Xanthan gum was extracted from the medium using the method described by Yang *et al.*, (2002). The overnight culture of *X.campestris* in XOLN medium was harvested. It was then inoculated into fresh XOLN medium containing 0.4% glucose. An initial O.D. (at 550 nm) of around 0.4 was recorded. After 72 hr the cultures were diluted twofold with sterile distilled water, and centrifuged at 12000g for 10 min to remove the bacterial cells. The xanthan gum in the supernatant was

precipitated in the presence of 40 mM of NaCl and 70% ethanol at -20ºC overnight. The precipitates were collected by centrifuging at 12000g for 30 min, washed once with 70% ethanol and resuspended in distilled water. The amount of the xanthan gum produced was determined by the modified Anthrone method (Lin and Tseng, 1979) and was calculated by plotting the graph of Amount of Xanthan gum produced Vs Time.

Acclimatization of *X. campestris* on whey by physical mutagenesis using U. V light source

Initially *X.campestris* cells were tested for growth on whey and on XOLN medium. But there was no significant growth on whey and less production of xanthan gum on XOLN medium containing 10% whey. A loopful culture of *X.campestris* was taken in saline and 0.1mL suspension from saline tubes was spread on plates containing XOLN medium and were exposed to U.V. for 5, 10, 15 and 20 minutes. The petri plates were immediately kept for incubation in dark to avoid the reversion effect of U.V. exposure. Colonies that were grown on XOLN medium were transferred to medium containing whey with concentrations 10%, 20%, 30% and 50%.

RESULTS

Isolation, characterization and screening of *Xanthomonas campestris* from plant source

Table 1: Characterization

Sr. No.	Criteria	Feature
1	Size of colony	8 mm (diameter)
2	Shape	Circular
3	Margin	Entire
4	Colour	Yellow
5	Opacity	Opaque
6	Elevation	Elevated
7	Consistency	Butyrous

Table 2: Primary Screening

Sr. No.	Test	Result
1	Gram test	Gram negative
2	Motility	Motile
3	Capsule staining	Capsulated

Table 3: Secondary Screening

Sr. No.	Test	Result
1	H_2S production	Positive
2	Milk Proteolysis	Positive
3	Methyl red test	Positive
4	Citrate utilization test	Positive

Table 4: Xanthan gum produced at interval of 12 hrs by isolated wild strain of *X. Campestris* on XOLN medium containing 4% glucose

Sr. No.	TIME (hr)	XANTHAN GUM (g/100mL)
1	0.0	0
2	12	0.088
3	24	0.112
4	36	0.294
5	48	0.460
6	60	0.552
7	72	0.823

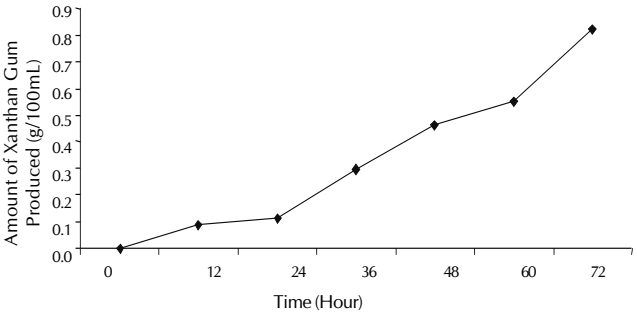

Figure 1: Graph of Amount of Xanthan Gum Produced Vs Time

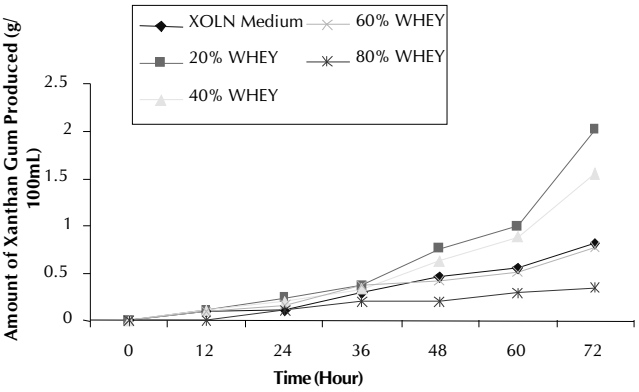

Figure 2: Comparative graph of Amount of xanthan gum produced Vs Time

Table 5: Physical mutagenesis in wild strain of *X .campestris* by U.V. treatment

Sr. No.	Time interval (min)	Growth observed
1	5	Slow growth, Isolated colonies
2	10	Isolated colonies
3	15	Isolated few colonies.
4	20	Isolated colonies

Table 6: The yield of xanthan gum on a medium containing whey

Sr. No.	Medium	Yield (g/100 mL)
1	20% whey	2.015
2	40% whey	1.553
3	60% whey	0.779
4	80% whey	0.355

Wild strain of *X.campestris* was isolated from infected cabbage leaves and conformation was done by colony characterization and secondary screening method as per the Burgey's Manual.

Production of xanthan gum

The strain was successfully tested for the production of Xanthan gum using routine production medium. Xanthan gum was produced. The yield of Xanthan gum (dry weight) was calculated which was about 0.8 g/100 mL.

Detection of xanthan gum

The xanthan gum produced in the medium was then harvested and its amount was determined by the modified Anthrone method. Development of bluish green colour indicated the positive test.

Acclimatization of *Xanthomonas campestris* on whey

Xanthomonas campestris can be acclimatized on the whey.

Table 7: Xanthan gum produced at interval of 12 hr by mutant strain in comparison with wild strain on whey medium

Sr. No	TIME (hr)	XOLN Medium Yield (g/100mL)	20% WHEY Yield (g/100mL)	40% WHEY Yield (g/100mL)	60% WHEY Yield (g/100mL)	80% WHE YYield (g/100mL)
1	0	0.0	0.0	0	0.0	0.0
2	12	0.088	0.11	0.12	0.091	0.008
3	24	0.112	0.245	0.201	0.169	0.11
4	36	0.294	0.369	0.326	0.369	0.195
5	48	0.460	0.752	0.623	0.421	0.21
6	60	0.552	0.996	0.885	0.512	0.29
7	72	0.823	2.015	1.553	0.779	0.352

The wild strain grows slowly on a medium containing whey.

DISCUSSION

It is well known that *X.campestris* because of low levels of β-galactosidase activity cannot grow in a medium contacting lactose as a sole source of carbon. Attempts have been made to develop lactose utilizing *X.campestries* strain for xanthan gum production but, stable strain production was not obtained.

In the present study attempt has been made to induce mutation in *X.campestris* by physical agent like U.V. radiation for better use of lactose from whey. Results indicate that normal strain produces about 0.8g/100mL in routine production medium. When strain was subjected to UV radiations and acclimatized 20% whey medium, it produced 2.015g/100mL yield in 72 hr. This yield is around 245% more as compared to routine production medium.

The mutant strain developed was superior to wild type strain of *X.campestris* in many respects especially, utilization of lactose of from whey and more yield of xanthan gum. By physical mutation of *X.campestris* strain with UV radiation indigenous activity of β-galactosidase is enhanced significantly.

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