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BACTERIAL XANTHAN GUM: SCREENING OF PRODUCER BACTERIA, PRODUCTION USING RICE STRAW AND IT'S BASIC APPLICATION FOR FRUIT PRESERVATION

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ABSTRACT :

For evironment safety, there is need of development of materials from natural polymers for different application. In present study, biogum producing bacteria were isolated from infected cabbage leaves collected from local vegetable market at Kalamboli, Navi Mumbai and screened for xanthan gum producing bacteria using yeast malt extract medium.Total 14 isolates i.e. predominant yellow, convex, mucoid colonies were selected, of which 04 isolates were retained after comparing the similarities between these isolates based on their biochemical identification. The isolates were positive in tests for KOH string assay, H_2S, catalase and showed negative reactions in gram staining, oxidase and nitrate reduction. From these charecteristics, isolates were found alike Xanthomonas spp. The biopolymer producing ability of these isolates was studied using yeast malt extract broth. The fermentation studies were carried out in Erlenmeyer flasks and using ice chilled ethanol, calcium chloride, the product was precipitated, then dried, and weighed. The recovered xanthan gum was confirmed by FTIR. The highest level of xanthan gum was produced by C-2 isolate at 30^{o}C, pH 7 and using starch and peptone as carbon and nitrogen source respectively. Cultivation media composition affects the yield and quality of the desired product as well as production costs. Many studies focus on cheap alternative raw materials, especially carbon sources, to replace commercially used glucose and sucrose. In this study, rice straw was introduced as an inexpensive and readily available carbon source for bacterial xanthan gum production. This study also aimed to evaluate xanthan gum as a carrier of preservative solution in edible coating applied to fresh sliced apples.

KEYWORDS : *Xanthan, Fermentation, FTIR, Rice Straw, Apple preservation.*

INTRODUCTION:

Approximately 140 million tone of synthetic polymers are produced worldwide every year. Because of more stability and much more resistance to biological degradation, these polymers are accumulating in the different ecosystems, at the rate of about 8% by weight and 20% by volume of the landfills. Presently, microorganisms are used for industrial scale production of a wide variety of products such as agrochemicals, biopharmaceuticals and therapeutics, biopolymers or bio-gums and biofuels in the energy and environment sectors.

There are some microbes which causes diseases to plants; these microbes can be used to produce the biogums commercially. *Dowson*, is the most common and destructive disease of the cabbage family worldwide. *Xanthomonas* is a large genus of Gram-negative, yellow-

pigmented bacteria that are associated with plants. This genus can be used to get the xanthan gum.

Xanthan gum is an extracellular heteropolysaccharide that is produced by different *Xanthomonas* spp. such as *Xanthomonas* (X.) *campestris*, *Xanthomonas pelargonii*, *Xanthomonas phaseoli* and *Xanthomonas malvacearum* during aerobic fermentation. The microbial production of xanthan gum at an industrial scale is a non-continuous process.

There are several factors which affects the xanthan gum production includes carbon, nitrogen sources, temperature, pH etc. Bacterial xanthan gum obtained by fermentation can be used in variety of ways in different products. It can be used in food industry, pharmaceutical industry, cosmetic industry etc. One of the basic applications is use as preservative for different types of fruits like apples.

MATERIALS AND METHODS:

i. **Collection of leaf sample:** Leaves of cabbage with V-shaped necrotic lesions on the leaf margin were collected from local vegetable market at *Kalamboli, Navi Mumbai, Maharashtra.*

ii. **Treatment of leaf sample:** Leaves were surface sterilized using 95% ethanol; small pieces of infected leaves were made. Then it was kept in sterile distilled water until it becomes turbid. This sample was then used for further steps.

iii. **Isolation of xanthan gum producing organism:** Sample was then streaked on St. Nutrient agar plate, incubated at 28-30^{o}C for 48 hours.Yellow pigmented colonies were selected for further characterization.

iv. **Screening and maintenance of xanthan gum producing organism:** Xanthan gum producing microorganisms were identified by the presence of microbial colonies with yellow, mucoid, convex on sterile yeast malt extract agar plates. Selective colonies were separated out and streaked on yeast malt extract agar slants for maintenance and further characterization.

v. **Identification by morphological and biochemical tests:** Morphological identification based on Colony characters & Gram nature, while biochemical tests viz. Fermentation of carbohydrate, hydrogen sulphide production, Nitrate reduction, Gelatin liquefaction, Casein, Starch and Esculin hydrolysis, Kovac's oxidase, Catalase and KOH string test were performmed as per standard procedures.

vi. **Production of xanthan gum using synthetic medium:** Loopful of culture was inoculated twice in Sterile Glucose broth, incubated at room temperature under shaking condition for 5 days.

vii. **Qualitative analysis of fermentation broth by Molisch's test:** 2ml of fermentation broth was taken in the tubes**,** then 2 drops of α – naphthol solution was added to the broth, concentrated sulphuric acid was added drop wise using a dropper along the sides of the tube, tubes were observed for the formation of violet ring at the junction of the two liquids.

viii. **Recovery of xanthan gum and determination of its concentration:** The fermentation broth was subjected to centrifugation at 10,000 rpm for 20min at 4^{o}C to remove the cells, Supernatant was collected and debris was discarded, Centrifugation was carried out 1-2 times for complete removal of cells, The collected supernatant was used for the extraction of gum, Gum was precipitated from supernatant using two volumes of 95% ice cold ethanol and 0.1g calcium chloride and it was kept overnight under refrigeration condition, After overnight refrigeration, supernatant was filtered through filter paper, Precipitate was collected through filtration and subjected to drying at 45^{o}C for 2-3 hour.

ix. **Fourier Transform Infrared Spectrophotometer (FTIR) Analysis::** Extracted gum was analysed by FTIR (IR affinity-1 Shimadzu Model no: 03632). Thus, The characteristics peack and functional group was confirmed by by FTIR analysis.

x. **Optimization of Parameters:**

- **Optimization of pH:** Isolates were inoculated in sterile glucose broth of different pH i.e. 6,7 & 8.Then incubated at room temperature for 5 days.
- **Optimization of temperature:** Isolates were inoculated in sterile glucose broth (pH 7) and then incubated at 25^{o}C, 30^{o}C and 40^{o}C for 5 days.
- **Optimization of carbon source:** Isolates were inoculated in sterile glucose broth with different carbon source such as starch, sucrose, lactose and maltose and then incubated at 30^{o}C for 5 days.

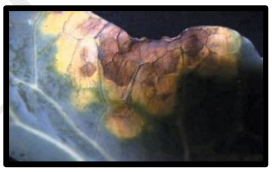
- **Optimization of nitrogen source:** Isolates were inoculated in sterile glucose broth with different nitrogen source such as Potassium nitrate, Ammonium nitrate, Ammonium sulphate and peptone and then incubated at 30°C for 5 days.

xi. **Production of Xanthan gum using rice straw:** Sterile production medium was prepared with separetely autoclaved rice straw powder as carbon source and peptone as nitrogen source including other component such as KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$ $7H_2O$. Highest xanthan yielding organism was then inoculated into the production medium, the flask was incubated at 30°C for 5 days.After incubation period the broth was subjected to product extraction procedure.

xii. **Application of xanthan gum as Preservative :** One slice of freshly cut apple was coated with 1% xanthan gum solution and another slice was coated with 1% salt solution.Both slices were placed on plate with appropriate labelling. It was then kept for 10 days in the refrigerator. After that results were noted down.

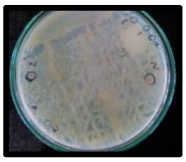
RESULTS AND DISCUSSION:

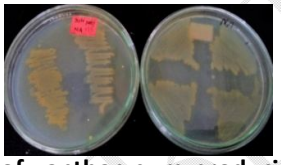
i. **Collection of leaf sample:** Collected Leave of cabbage with V-shaped necrotic lesions from local vegetable market at *Kalamboli, Navi Mumbai.*



ii. **Treatment of leaf sample:** The treated leaves sample kept in sterile distilled water was ready to use in further steps.

iii. **Isolation of xanthan gum producing organism:** After incubation at 28°C for 48 hours different types of colonies were observed on nutrient agar plate. Amongst them yellow coloured colonies were selected for screening purpose.





iv. **Screening and maintenance of xanthan gum producing organism::** Yellow, mucoid, convex colonies on yeast malt extract agar plates were marked as xanthan gum producing microorganisms. Screening revealed the ability of only 14 isolates to produce xanthan gum. Those isolates were labeled as C (2, 7, 11, 11.1, 12, 13, 15, 16, 18, 20, 24, 24.1, 27), and C-32 These selective isolates were maintained on yeast malt extract agar slants for further characterization. After every 15 days isolates were sub-cultured

v. **Identification by morphological and biochemical tests:** On the basis of distinct morphological differences and biochemical characterization only four isolates C-2, C-12, C-18 and C-20 were selected for xathan gum production and further studies.

Table 1 : Biochemical chart showing results of 14 isolates in comparison to the standard *(Xanthomonas)*

Sr.no	Biochemical Test	C-2	C-7	C-11	C-11.1	C-12	C-13	C-15	C-16	C-18	C-20	C-24	C-24.1	C-27	C-32	Standard
1.Sugars																
a	Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b	Xylose	+	+	-	+	+	-	-	+	+	+	-	-	+	-	+
c	Maltose	+	+	+	+	+	-	-	+	+	+	-	-	+	-	+
d	Arabinose	+	+	-	+	+	-	+	+	+	+	-	-	+	+	+
e	Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	Catalase	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+
3.	Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	Gelatin liquefaction	+	-	+	-	+	-	-	-	+	+	-	+	-	-	+
5.	Starch hydrolysis	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+
6.	Casein hydrolysis	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
7.	Esculin hydrolysis	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+
8.	Nitrate reduction test	-	+	+	-	-	+	+	-	+	-	-	-	+	+	-
9.	H_2S production	+	-	-	+	+	-	+	-	+	-	+	-	-	+	+
10.	KOH string test	+	-	+	-	+	-	+	+	-	+	-	-	-	+	+

These propeties matches with the properties of *Xanthomonas spp.*

vi. **Production of xanthan gum using synthetic medium:** After inoculation of culture in sterile Glucose broth, and incubation at room temperature under shaking condition for 5 days**, i**ntensity of colour was increased and it changed to the bright yellow, which was primary indication of xanthan gum formation.

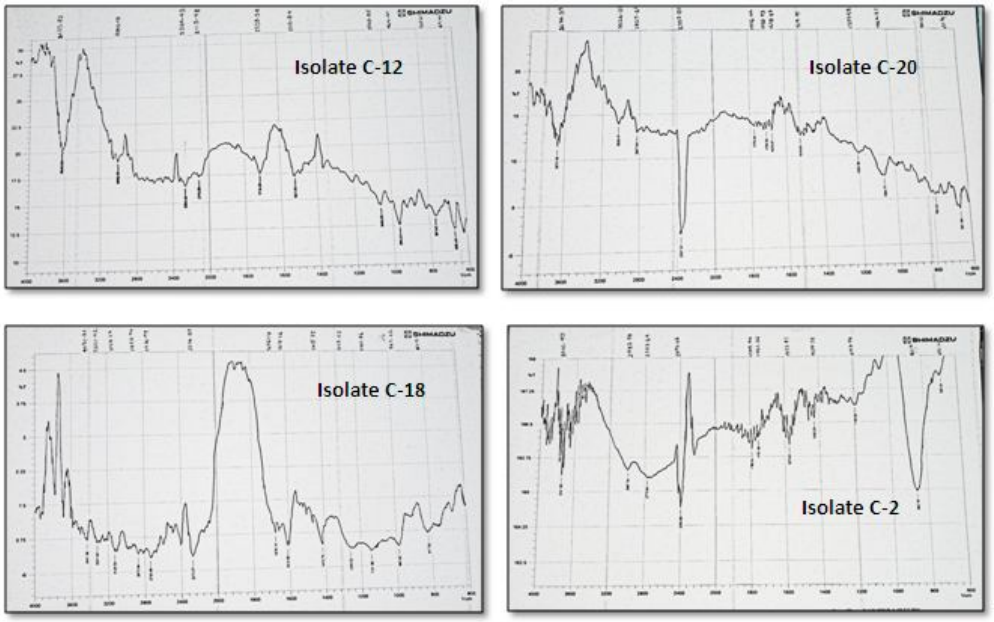
vii. **Qualitative analysis of fermentation broth by Molisch's test:** When 2ml of fermentation broth was taken in the tubes**,** 2 drops of α – naphthol solution and concentrated sulphuric acid was added to the broth, the formation of violet ring at the junction of the two liquids confirmed the presence of general carbohydrates in the broth.

viii. **Recovery of xanthan gum and determination of its concentration:** After subsequent centrifugation and precipitation by using chilled ethanol, extract was kept overnight under refrigeration condition, After overnight refrigeration, supernatant was filtered through filter paper, precipitate obtained was collected subjected to drying at 45°C for 2-3 hour and then flakes of xanthan gum were collected. The dry weight of product by all four isolates was recorded:

Sr. No	Isolates	Xanthan gum (g/100ml)
1.	C-2	**0.65**
2.	C-12	0.26
3.	C-18	0.21
4.	C-20	0.29

Hence, from the above table it was revealed that C-2 is the highest xanthan gum yielding organism. Hence further work was carried out with C-2 isolate and the product obtained from all the above isolates were subjected to FTIR analysis.

ix. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: FTIR spectroscopic analysis was performed to know the information about functional groups and structure using IR Affinity-1, Shimadzu (Model no: 03632.) The details of spectra are as follows: A broad absorption peak at 3650-3200 cm^{-1} indicates the hydrogen bonded OH groups. A band of 3300-2700 cm^{-1} is due to C-H bending from $C-H_2$ and $C-H_3$ vibrations. The peak at 1780-1650 cm^{-1} is due to C=O stretching vibration of carbonyl. The peaks at 1602cm^{-1} (COO^- asymmetric stretching) and 1419cm^{-1} (COO^- symmetric stretching) are due to carboxyl group. A band of 1250-1050 cm^{-1} is due to C-O-C stretching vibration band of ether. The observed IR patterns for the extracted product were similar to the standard xanthan.



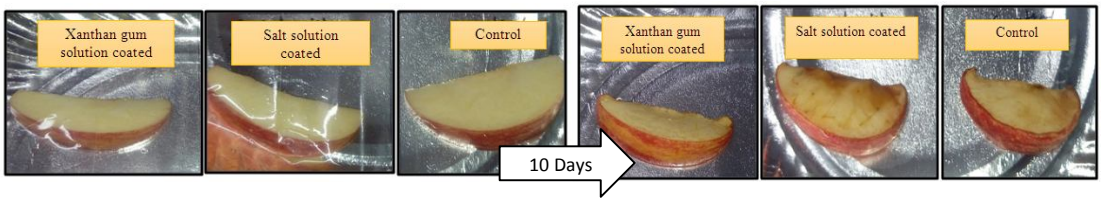
x. Optimization of Parameters:

- It was revealed that, at pH 7 isolate exhibits maximum optical density of culture and even maximum xanthan gum production, while optimal temperature for growth and xanthan gum production was found as 30°C. At 45°C, no growth of isolate was observed. Carbon and nitrogen sources also effectively affect the yeild of xanthan gum. In this study, starch was found as effective source of carbon which gives maximum yeild of xantham as compare to other used carbon sources. Whereas only peptone found as effective nitrogen source for xanthan gum production, but yeild was less i.e.02g/50ml. The maximum yeild at standardized parameters was obtained as 0.82g/100ml of media.

xi. Production of Xanthan gum using rice straw:

As the important objective of this study, production of xanthan gum was carried out using Sterile production medium prepared with separetely autoclaved rice straw powder as carbon source and peptone as nitrogen source including other necessary components. The pH of medium was set at 7. After inoculation of highest xanthan yielding organism into the production medium, the flask were incubated at 30°C for 5 days. After incubation and extraction process, it was revealed that, rice straw support the growth of microorganisms just like synthetic media and exhibited the xanthan yeild up to 0.7g/100ml. which was 85.36% of actual yeild at standardized conditions.

xii. Application of xanthan gum as Preservative: After 10 days of refrigeration, slice coated with salt solution and the one without any coating showed browning of the slice of apple, whereas slice coated with xanthan gum solution showed significantly less browning as compared to others.



So this was revealed that, xanthan gum can be used as a good preservative agent. However, there is need to improve the preservative capacities of gum by studying the different factors involved in it and standardizing the process at higher scale.

CONCLUSION:

From the results obtained in present study, it was concluded that, Xanthan- biogum producing bacteria could be isolated from infected cabbage. Yeast malt extract medium was found as satisfactory medium for screening of xanthan gum producing bacteria, which was promoting growth of isolates as well as gum production. Total 14 isolates were selected, which were found alike *Xanthomonas spp*. based on their morphological and biochemical charecterization. But obviously, there is need to identify the isolates by 16S rRNA sequencing. In the present study, fermentation was carried out in flaks at laboratory level, which proved the ability of isolates to produce the xanthan gum. Potential isolate could give product up to 0.65g/100ml of synthetic media. This fermentation also concluded that, there is need to optimiz the other influencing parameters and modify the method of fermentation for higher scale. To improve the yield of product, we found that there is need to design the media as per the nutritional requirements of organisms by using some other agriculturlal wastes. From the study it was also supported that, we can use agricultural waste i.e. rice straw as major source of carbon and peptone as nitrogen source as well in media for optimal xanthan production. As Xanthan gum can increase the self life of cut or sliced fruits up to 10 days, which can be increased by increasing the concentration of gum for coating. Thus instead of using chemical preservative xanthan can be a good alternative.

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