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Manjunath R
Department of Natural Resources Management, College of Horticulture, Bengaluru, Karnataka, India

Livleen Shukla
Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India

Corresponding Author:
Manjunath R
Department of Natural Resources Management, College of Horticulture, Bengaluru, Karnataka, India

Isolation, screening and characterization of efficient *Xanthomonas* spp. for enhanced xanthan gum production

Manjunath R and Livleen Shukla

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Abstract
Xanthan gum, a microbial polysaccharide of significant industrial importance, is widely utilized in food, pharmaceutical, cosmetic, and petroleum sectors owing to its exceptional rheological and stabilizing properties. Members of the genus *Xanthomonas* are well known for their ability to produce xanthan gum, although their pathogenicity toward agriculturally important crops poses constraints. In the present study, *Xanthomonas* spp. were isolated from diseased tissues of cabbage (*Brassica oleracea*), rice (*Oryza sativa*), French bean (*Phaseolus vulgaris*), and citrus (*Citrus* spp.) collected from different agro-ecological regions of India. Forty-five isolates were obtained and subjected to morphological and functional screening. Based on colony characteristics and gum production potential, ten isolates were selected for secondary screening. Growth kinetics were evaluated through serial dilution and colony-forming unit (CFU) counts. Strains derived from cabbage and French bean demonstrated comparatively higher gum yields, highlighting their potential as candidates for large-scale xanthan production. This study explores the morphological, biochemical, cultural, and molecular characteristics of four xanthan-producing isolates. All were rod-shaped, Gram-negative, non-spore-forming, motile (flagellated), capsule-forming bacteria with raised, yellow, mucoid colonies. 16S rDNA sequencing followed by BLAST analysis, multiple sequence alignment, and phylogenetic reconstruction confirmed them as *Xanthomonas campestris* with minimal genetic divergence. Recent findings on non-pathogenic *Xanthomonas* spp. from healthy rice seeds underline the importance of accurate molecular characterization.

Keywords: Xanthan gum, *Xanthomonas*; biopolymer, screening, growth kinetics, 16S rDNA, molecular taxonomy

1. Introduction
Biopolymers of microbial origin have emerged as sustainable alternatives to petrochemical-based polymers owing to their biodegradability, biocompatibility, and wide industrial applications (Sharma *et al*., 2021) [13]. Xanthan gum is one of the most commercially important microbial exopolysaccharides, with annual production exceeding 30,000 tons and an estimated global market size projected to surpass USD 1.2 billion by 2030 (MarketWatch, 2023) [8].

Structurally, xanthan gum is composed of repeated pentasaccharide units containing glucose, mannose, and glucuronic acid, and its unique rheological properties—including high viscosity at low concentrations, stability across temperature and pH ranges, and salt tolerance—have contributed to its widespread industrial adoption (Jansson *et al*., 2020; Bagewadi *et al*., 2022) [7, 1]. Applications extend from food processing and cosmetics to enhanced oil recovery and drug delivery systems (EFSA, 2017; Patel *et al*., 2022) [4, 9].

Naturally, *Xanthomonas* species are plant pathogens, but they remain the principal producers of xanthan gum, especially *X. campestris* pv. *campestris* (Zhang *et al*., 2021) [15]. Colony morphology, strain genetics, and fermentation conditions are strongly associated with xanthan productivity (da Silva *et al*., 2019) [3]. Large, mucoid, bright-yellow colonies generally correspond to higher gum yields, while smaller colonies are less productive (Cadmus *et al*., 1976; Harding *et al*., 1987) [2, 6].

Despite global progress, there is limited exploration of indigenous Indian *Xanthomonas* strains for efficient xanthan gum production.

This study was therefore undertaken to isolate, characterize, and evaluate *Xanthomonas* spp. from multiple crop hosts for their xanthan productivity and growth kinetics.
Traditional identification methods often struggle to distinguish *Xanthomonas* from Pseudomonas. Molecular tools like 16S rRNA gene sequencing have become essential for accurate taxonomy.

2. Materials and Methods

2.1 Sample collection

Diseased tissues were collected from cabbage, rice, French bean, and citrus plants showing bacterial infection symptoms across Indian agro-climatic zones (2022-2023). Samples were transported under refrigeration and processed within 24 h.

2.2 Isolation of *Xanthomonas* spp.

Isolation was carried out following Schaad (1992) with minor modifications. Tissue sections (5 mm) were surface-sterilized (1% NaOCl, 1 min), rinsed with sterile water, macerated, and streaked on Yeast Dextrose Calcium carbonate agar (YDCA). Plates were incubated at 30±1°C for 48 h. Distinct yellow, mucoid colonies were purified by repeated streaking and maintained on YDCA slants.

2.3 Primary screening

Isolates were screened for gum production on glucose-supplemented nutrient agar (Cadmus *et al*., 1976) [2]. Colony characteristics such as pigmentation, mucoidity, and size were recorded.

2.4 Secondary screening and xanthan production

Selected isolates were subjected to shake-flask fermentation. Ten percent inoculum was added to production broth containing glucose, yeast extract, and mineral salts, incubated at 28 °C, 200 rpm for 72 h. Xanthan was recovered from culture supernatants by ethanol precipitation, dried, and quantified (g/L).

2.5 Growth kinetics

Growth was monitored by serial dilution and CFU counts at 12-h intervals up to 84 h. Growth phases were determined by CFU dynamics, and peak xanthan yields were correlated with growth phases.

2.6 Characterization studies

Four xanthan-producing isolates from cabbage, rice, French bean, and citrus were studied. Morphological and staining tests (Gram, spore, motility, capsule) were performed. Cultural tests included colony morphology on YDC agar. Biochemical assays followed standard protocols including starch, casein, gelatin hydrolysis, catalase, urease, indole, MR-VP, citrate, nitrate, oxidase, and OF tests. Molecular analysis involved 16S rDNA sequencing, BLAST, multiple sequence alignment, and phylogram construction.

3. Results

3.1 Isolation and screening

A total of 45 isolates were obtained: 15 from cabbage, 10 from rice, 10 from French bean, and 10 from citrus. Colonies were typically convex, yellow, and mucoid, consistent with *Xanthomonas* morphology. The details of sample collection from different host plants and locations are summarized in Table 1.

Table 1: Collection of samples from different locations.

Source of isolates	Place of sampling
Black rot of cabbage	Fields of IARI, New Delhi
Bacterial blight of rice	Raichur, Karnataka
Bacterial blight of beans	Yamuna Bank, Delhi
Citrus canker	Bangalore, Karnataka

3.2 Secondary screening for xanthan yield

Significant variability was observed in xanthan yields (Figure 1). Cabbage isolates (BRC1-BRC3) produced the highest yields (14-16 g/L), followed by French bean isolates (12-14 g/L). Rice isolates (9-10 g/L) and citrus isolates (8-9 g/L) were comparatively lower producers.

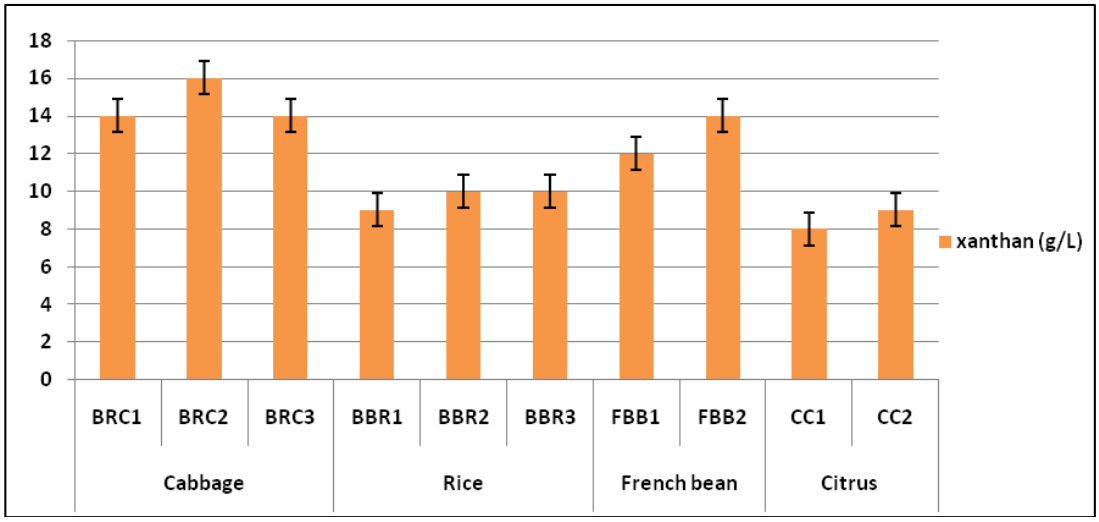

Fig 1: Secondary screening of *Xanthomonas* spp for Xantha gum production

3.3 Growth kinetics

Growth profiles varied among isolates (Figure 2). For example, isolate BBR1 exhibited a lag phase up to 18 h, log phase up to 60 h, stationary phase until 72 h, followed by decline. FBB2 reached the highest CFU (6.3×10^7) at 72 h, while reference strain *X. campestris* 2954 peaked at 7.7×10^7 CFU at 60 h. Maximum gum production coincided with late exponential to early stationary phases.

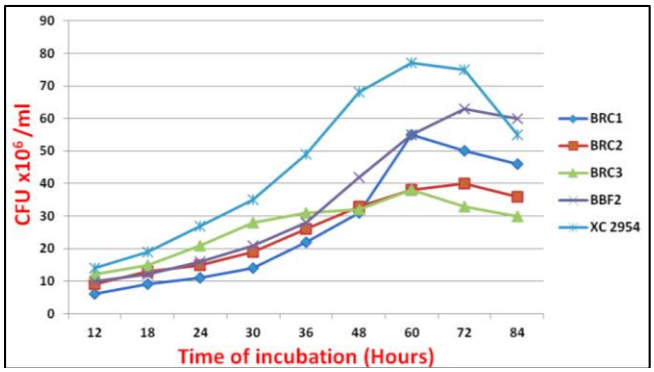

Fig 2: Growth curve of isolated strains of *Xanthomonas* spp.

3.4 Characterization studies

The isolates were rod-shaped, Gram-negative, flagellated, non-spore forming, and capsule-forming. Colonies were yellow, mucoid, and raised. Biochemical profiles were consistent with *X. campestris*. 16S rDNA sequencing showed 100% similarity with *X. campestris*, with highly conserved sequences. Multiple sequence alignment confirmed limited divergence. Phylogram analysis revealed close clustering.

4. Discussion

This study highlights the xanthan-producing potential of indigenous *Xanthomonas* isolates from diverse host plants. Consistent with earlier reports (Garcia-Ochoa *et al*., 2000; Zhang *et al*., 2021) [5, 15], cabbage and French bean isolates exhibited higher gum yields than rice and citrus isolates, underlining host-dependent strain variability.

Morphological traits correlated with xanthan productivity, supporting earlier findings that large, mucoid colonies correspond to high yields (Harding *et al*., 1987; Ramírez *et al*., 1988) [6, 10]. Growth curve analysis revealed that xanthan production was growth-associated, peaking during the late exponential to stationary phase, as reported previously (Sutherland, 1993; Patel *et al*., 2022) [14, 9].

Indigenous high-yielding isolates may serve as promising candidates for industrial exploitation. Further optimization of fermentation parameters, including carbon source selection and aeration control, combined with strain improvement strategies, could enhance yields and reduce production costs.

Morphological and biochemical traits align with *X. campestris*, including mucoid, yellow colonies due to xanthomonadin pigment. Microscopic and biochemical features agree with earlier reports. 16S rDNA analysis confirmed taxonomic identity and genomic homogeneity, supporting classification of all isolates as *X. campestris*.

These four xanthan-producing isolates are unequivocally classified as *X. campestris*. Their morphological, biochemical, and molecular uniformity positions them as robust candidates for industrial and taxonomic studies in India.

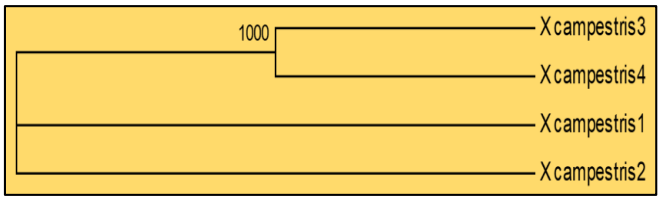

Fig 3: Phylogram showing diversity in *Xanthomonas campestris*

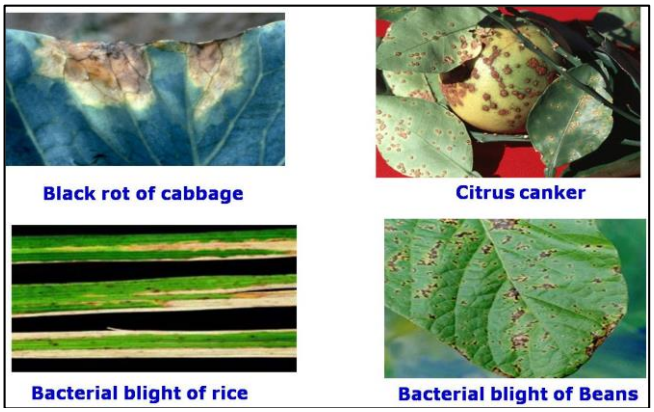

Plate 1: *Xanthomonas* spp. infected samples of agriculturally important crops used for isolation of xanthan gum producing bacteria.

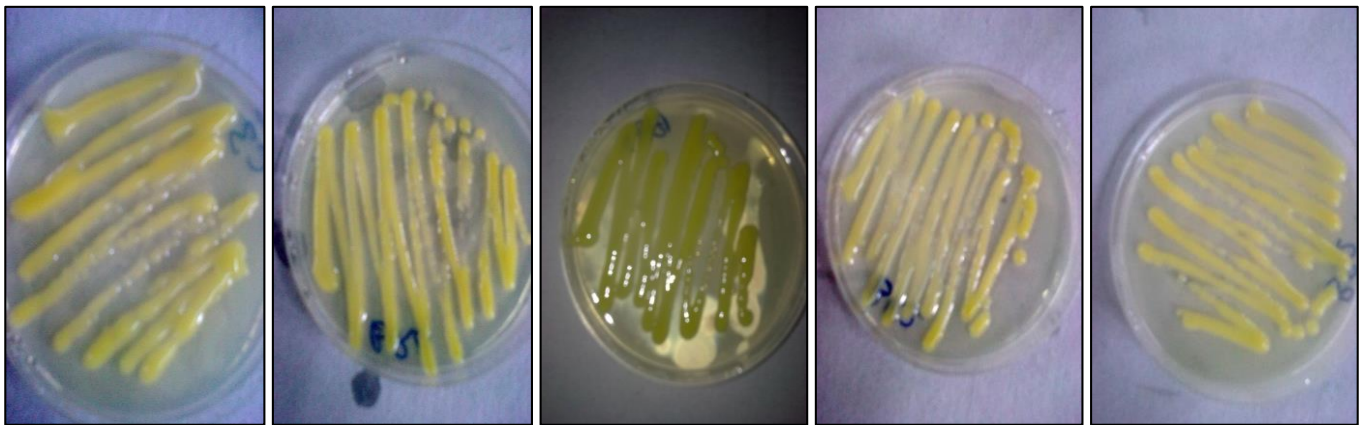

Plate 2: Primary screening of bacterial isolates for xanthan production (multiple colony morphologies).

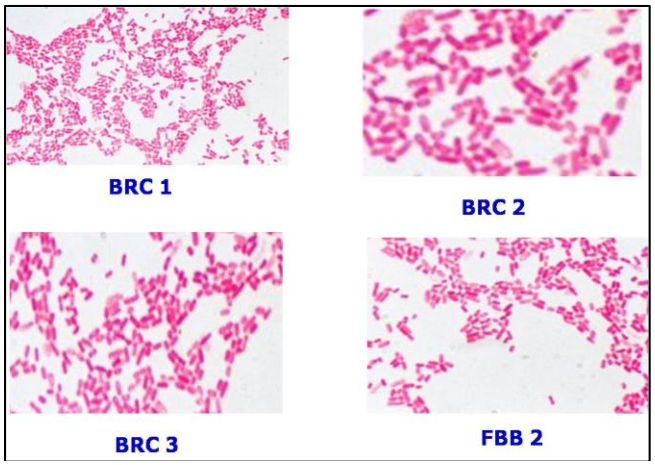

Plate 1: Gram negative staining of *Xanthomonas campestris* (XC) under 100x

5. Conclusion

Indigenous *Xanthomonas* strains isolated from cabbage and French bean demonstrated high xanthan yields and favorable growth kinetics, making them strong candidates for industrial-scale production. These four xanthan-producing isolates are unequivocally classified as *X. campestris*. Their morphological, biochemical, and molecular uniformity positions them as robust candidates for industrial and taxonomic studies in India. These findings provide a foundation for developing cost-effective, locally adapted fermentation processes in India.

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