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MORPHOLOGICAL, MICROSCOPIC, ENZYMATIC AND BIOCHEMICAL CHARACTERISTICS OF XANTHOMONAS ISOLATES ISOLATED FROM BRASSICA L

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Abstract: In this study, bacterial isolates belonging to the genus Xanthomonas were obtained from infected cabbage (Brassica L.) leaves and comparatively evaluated according to their morphological, microscopic, enzymatic and selected biochemical characteristics. YDC medium was used for isolation. The obtained X1, X2 and X3 isolates formed yellowish, convex, smooth-edged and mucoid colonies and showed a Gram-negative reaction. Enzymatic tests included starch and gelatin hydrolysis, proteolytic and lecithinase activities, citrate utilization and lipolytic activity. The results demonstrated differences in the level of enzymatic activity among the isolates and provided a basis for comparative selection of promising variants for subsequent biotechnological studies.

Keywords: Xanthomonas, Brassica L., bacterial isolate, morphology, enzymatic activity, biochemical characteristics, exopolysaccharide.

Introduction

Members of the genus Xanthomonas are Gram-negative bacteria closely associated with plants. In addition to causing diseases in agricultural crops, some representatives are of considerable biotechnological interest because of their ability to synthesize biologically active metabolites and extracellular polysaccharides, particularly xanthan gum. Xanthan produced by X. campestris is widely used in food, pharmaceutical, cosmetic and technical formulations because of its high viscosity, pseudoplastic behavior and stability over a broad range of pH and ionic conditions [1,2]. Therefore, isolation of local Xanthomonas strains, characterization of their phenotypic and enzymatic properties, and selection of promising variants for further biotechnological studies remain relevant. The aim of this work was to comparatively characterize the morphological, microscopic, enzymatic and biochemical properties of Xanthomonas isolates obtained from Brassica L. leaves.

Materials and methods

The objects of the study were bacterial isolates X1, X2 and X3 obtained from cabbage (Brassica L.) leaves showing signs of infection. YDC nutrient medium was used for isolation and colony characterization and contained, g/L: yeast extract, 10.0; sucrose, 20.0; $CaCO_3$, 20.0; and agar, 20.0. Colony color, elevation, margin, growth intensity and mucoid consistency were recorded. Microscopic characterization included Gram staining. Enzymatic and biochemical properties were assessed by tests for starch hydrolysis, gelatin hydrolysis, proteolytic activity, lecithinase activity, lipase activity and citrate utilization. Activity was evaluated according to the diameter of the reaction zones. Numerical values reported in the source experimental data are presented as mean ± deviation.

Results

The isolated X1, X2 and X3 cultures formed yellowish, convex, smooth-edged, profusely growing and mucoid colonies on YDC medium. Colony diameter was approximately 1–2 mm, and

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all isolates showed a Gram-negative reaction. The mucoid phenotype may be associated with extracellular polysaccharide production and is an important diagnostic feature of representatives of the genus Xanthomonas [1]. According to the source table, all three isolates were assigned to the phylum Pseudomonadota.

In enzymatic tests, all three isolates demonstrated the ability to hydrolyze starch, with an average hydrolysis zone of 6±1.0 mm. Gelatin hydrolysis produced an average zone of 5±1.0 mm in all isolates. In tests associated with proteolytic and lecithinase activities, isolate X2 produced a zone of up to 8±1.0 mm, whereas X1 and X3 formed relatively smaller zones. In the citrate-utilization test, values of 1 mm for X1, 7±1.0 mm for X2 and 11±1.0 mm for X3 were recorded. No detectable activity zone was observed for any of the three isolates in the lipase test.

Table 1. Morphological characteristics of the isolated bacterial strains

Strains	Colour	Texture	Margins of colony	Degree of growth	Nature	Gram staining	Size
X1	yellow	raised	smooth	profuse	viscous	-ve	Pseudomonadota
X2	yellow	raised	smooth	profuse	viscous	-ve	Pseudomonadota
X3	yellow	raised	smooth	profuse	viscous	-ve	Pseudomonadota

Discussion

The obtained results show that isolates X1, X2 and X3 were similar in their principal morphological characteristics but differed in several enzymatic properties. In particular, the relatively larger zone observed for X2 in the proteolytic/lecithinase tests and the stronger citrate-associated reaction of X3 indicate differences in the metabolic potential of the isolates. Such phenotypic variation may reflect strain-level physiological diversity. Enzymatic systems of Xanthomonas species are important both for interactions with plant tissues and for utilization of available nutrient sources [3]. At the same time, morphological and biochemical tests alone are insufficient for definitive species-level identification; therefore, promising isolates should subsequently be confirmed by molecular or proteomic methods such as MALDI-TOF MS or 16S rRNA gene sequencing. The present preliminary screening provides a basis for selecting isolates with promising potential for subsequent studies of xanthan production.

Conclusion

The bacterial isolates X1, X2 and X3 obtained from Brassica L. leaves were similar in forming yellowish, mucoid, convex colonies with smooth margins and in showing a Gram-negative reaction. Starch- and gelatin-degrading activities were detected in all isolates, whereas lipase activity was not observed. Differences among the isolates were recorded for selected enzymatic and biochemical parameters. The obtained data make it possible to comparatively evaluate Xanthomonas isolates and to select promising strains for further biotechnological investigation.

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