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RESEARCH ARTICLE OPEN ACCESS

SELECTION AND ISOLATION OF PLANT-ASSOCIATED METHANOTROPHIC BACTERIA BY A RAPID SCREENING PROCEDURE

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ABSTRACT

A rapid screening procedure was used for the selection and isolation of plant-associated bacteria. The results indicated that methanol medium containing crystal violet might have potential application for selective isolation of methanotrophic bacteria from rhizosphere.

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INTRODUCTION

Most plants produce and excrete methanol, probably as a waste product (Fall and Benson, 1996). In nature, methanotrophic bacteria can use as sole carbon and energy sources one-carbon organic compounds, such as methanol and methane (Hanson and Hanson, 1996). The role of methanol in the interaction between plant and rhizosphere microorganisms is still unknown. In this short-communication paper, a rapid screening procedure was used for the selection and isolation of methanotrophic bacteria in the rhizosphere of Zoysia grass. Zoysia grass (*Zoysia japonica*) is the main grass plant cultivated in Brazil and other countries, being used in public and residential gardens and sports areas, mainly in soccer and golf fields. This plant prevents wind and rain erosion, conserving the productive potential of the soil (Brede, 2000). Despite its importance, very little is known about the microbial associated with this plant that may benefits on plant physiology including positive effects on nitrogen and methanol metabolism and stimulation of plant growth.

RESULTS AND DISCUSSION

The isolation of methanotrophic bacteria is very fastidious and difficult task as the use of methane and anaerobic conditions are expensive and time-consuming. It has been suggested the use of alternative growth media such as AMS (ammonium mineral salts), and MET (with methanol as sole carbon and energy source) and the use of crystal violet to avoid contamination and circumvents these limitations (Bouwman et al., 1993; Fung and Miller, 1973).

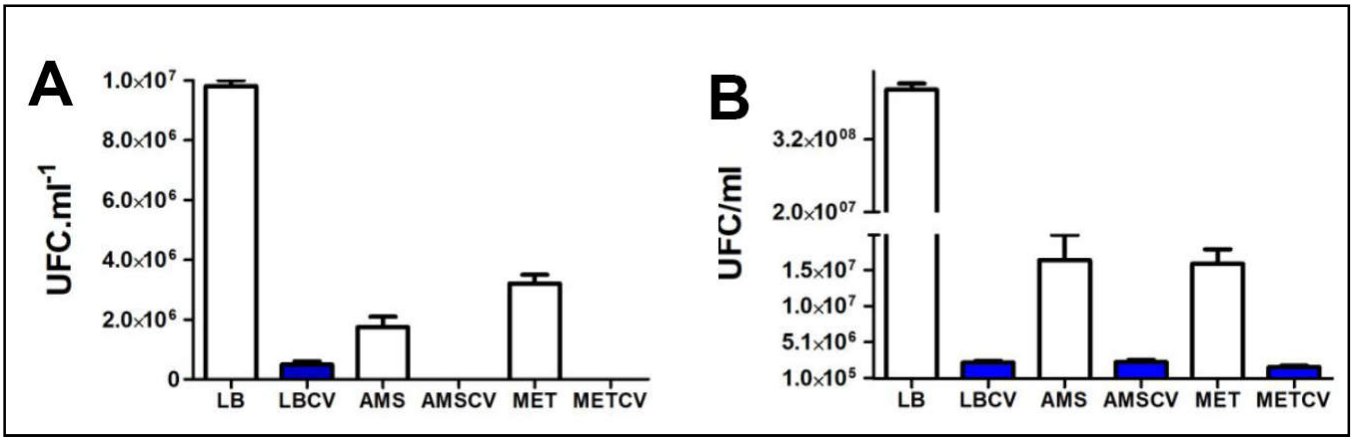

Figure 1. Experiments were conducted on the LB (Luria Bertani), AMS (ammonium mineral salts), and MET (with methanol as sole carbon and energy source) growth media with or without crystal violet – CV (colored in blue). Bacterial growth was monitored by counting the CFU (colony-forming units) at 24 (A) and 168 (B) hours.

The effect of dyes on bacterial growth has been studied for many years (Fung and Miller, 1973). The crystal violet inhibits growth of Gram-positive and some Gram-negative bacteria, besides its antibacterial properties this compound has antifungal, antihelminthic and antitrypanosomal activities (Docampo and Moreno, 1990). In this paper, the crystal violet was used in three media composition: a rich medium (LB), a medium containing ammonium mineral salts (AMS), and MET (with methanol as sole carbon and energy source) to enhance the methanotrophic bacteria isolation. Using LB medium, microbial growth (Figure 1) was $1.0x10^7$ CFU.ml-1 (colony-forming units per ml) in 24 hours (1 day) and after 168 hours (7 days) approximately $5.5x10^8$ CFU.ml^{-1}. In the sample medium with addition of the violet crystal (50 ppm), the bacterial growth was $6.0x10^5$ CFU.ml^{-1} in 24 hours and in 7 days the growth was $2.5x10^6$ CFU.ml^{-1}. For the AMS medium, it was observed for 24 hours $2.1x10^6$ CFU.ml^{-1} and in 7 days (168 hours) $1.3x10^7$ CFU.ml^{-1}. In the AMS medium with violet crystal, no visible growth was recorded during the 24 hours, only within 7 days a growth of $2.6x10^6$ CFU.ml^{-1} was obtained. Using the MET medium, bacterial growth in 24 hours was $3.5x10^6$ and in 7 days (168 hours) $1.4x10^7$ CFU.ml^{-1}. In the MET medium with violet crystal, no growth was observed in 24 hours, only after 7 days $1.4x10^6$ CFU.ml^{-1} were obtained. Isolates were considered to be pure if colonies were similar in appearance morphologically. Methanol-utilizing colonies with crystal violet appeared generally after about 5-7 days.

Conclusion

In this study, different bacteria media containing crystal violet were used. This compound inhibited the growth of Gram-positive organisms and favor the growth of Gram-negative bacteria, which allows for the rapid selection and isolation of methanotrophic bacteria from rhizosphere of the Zoysia grass.

Fifteen bacterial isolates that can grow in methanol as sole carbon and energy source were obtained and preserved for further studies.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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