PRODUCTION POTENTIAL OF METABOLITES OF ISOLATE BRM63426 (*Bacillus* sp.) AT DIFFERENT EVALUATION TIMES

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ABSTRACT

The Bacillus genus has received much attention due to its ability to act as a biocontrol agent for plant diseases. Bacillus uses several mechanisms to suppress plant pathogens, including the production of lipopeptides of antimicrobial action such as iturins, fengycins and surfactins. This study aimed to verify the production capacity of lipopeptides from the iturin and surfactin group by an isolate with known activity against root pathogens. The isolate was inoculated in nutrient broth medium and shaken for 24, 48 and 72 hours. After these periods, the samples were dried, resuspended and subjected to HPLC for quantification of metabolites compared with commercial standards of iturin and surfactin. The isolate showed the capacity to produce these metabolites at all times tested.

Keywords: HPLC. Antagonistic bacteria. Antibiosis. Biological control.

1 INTRODUCTION

One of the characteristics that contributed to the high efficiency of *Bacillus* sp. as a biocontrol agent against plant pathogens is its ability to produce a plethora of antimicrobial metabolites, most notably lipopeptides. Among the main lipopeptides produced by *Bacillus* sp. are iturins, fengicyns and surfactins[3, 7]. Because of this ability, this group of bacteria has been successfully used to control a diverse selection of plant pathogenic fungi and bacteria like *Fusarium graminearum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Xanthomonas oryzae* and *Pseudomonas solanacearum* [5]. Therefore, the objective of this study was to evaluate the ability of the isolate BRM63426 (*Bacillus* sp.) to produce metabolites from the iturin and surfactin group at 24, 48 and 72 hours after inoculation.

2 MATERIAL & METHODS

The BRM 63426 isolate originated from a common bean field from Distrito Federal, Brazil, and was preserved in glycerol 15% at -20 ºC. This isolate has nematocid activity and is a good producer of endospores. The culture medium, nutrient broth (NB), was prepared in 150 mL Erlenmeyer flasks and autoclaved at 120ºC for 20 min. The bacterial inoculum was prepared with 50 µL of the isolate BRM 63426 and shaken in NB at 150 rpm for 24 hours. After this period, aliquots of 50 mL were removed from this flask at 24, 48 and 72 hours after inoculation to estimate the bacterial concentration as UFC/mL (1,05 x10^{11}).

For metabolite analysis, extraction was performed using ethyl acetate as an organic solvent, followed by solvent evaporation and resuspension of metabolites in an acetonitrile and water solution. The solutions containing metabolites were analyzed using high-performance liquid chromatography (HPLC). Standard solutions of iturin and surfactin (Sigma-Aldrich®) were used at concentrations of 0.1 and 1.0 mg/mL as a control for possible metabolites, respectively. The HPLC equipment used was the PerkinElmer Flexar model, with the following chromatographic parameters: an isocratic mobile phase consisting of acetonitrile and 10 mM ammonium acetate (40:60, v/v) (Rao et al., 2008), using a C18 column, 5 µm particle size, 4.0 mm internal diameter, 250 mm length, a flow rate of 1.0 mL/min and a wavelength of 260 nm [8].

3 RESULTS & DISCUSSION

Compared to standards, the isolate BRM63426 synthesized metabolites from both the iturin and surfactin groups in the three time periods evaluated, as shown in Figure 1. At 24 hours, the chromatogram showed a peak of probable lipopeptides at 8-10 min. This observation was repeated more clearly at 72 hours with surfactin production much above the concentration of the standard reagent. HPLC revealed the production of other unknown metabolites by the BRM63426 isolate. By comparing the chromatography of the standard solutions, both surfactin and iturin were confirmed to be present in the fermentation broth. Selectivity was determined by analyzing blank samples and samples amended with standard solutions of surfactin and iturin, and the results were satisfactory. No interferences were observed at the surfactin and iturin retention times in the samples.

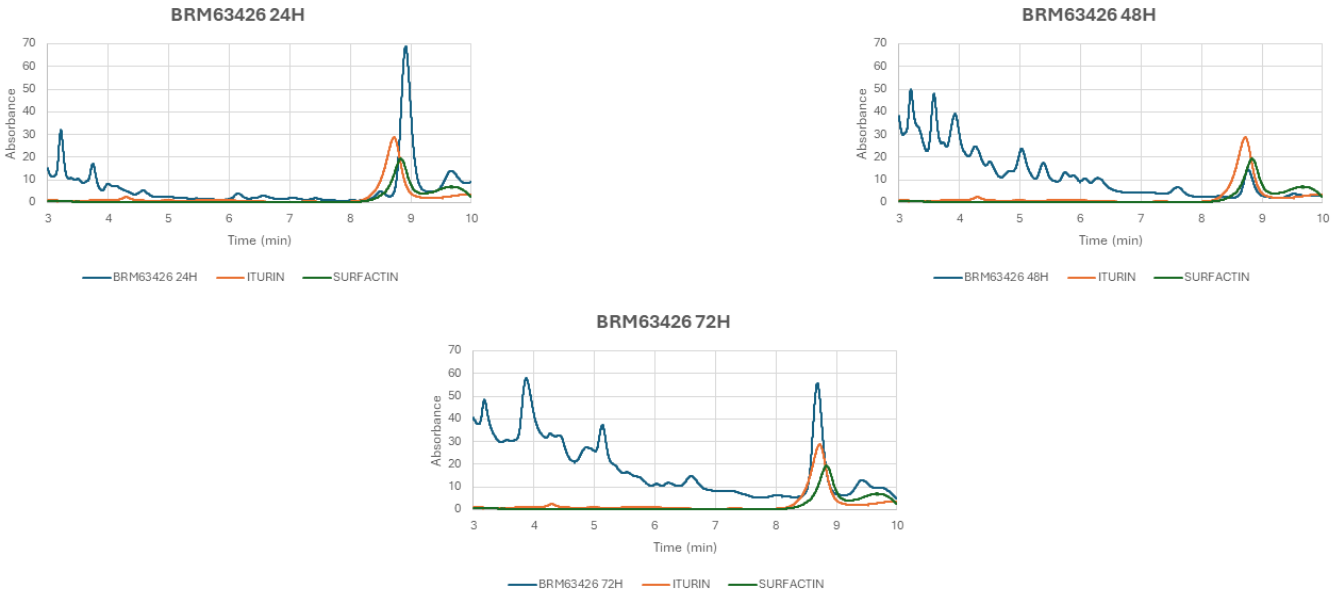

Figure 1 Production of iturin and surfactin by isolate BRM63426 at 24, 48 and 72 hours after inoculation in nutrient broth medium.

Many reports of antimicrobial activity indicated that the extracts of iturin and surfactin by *Bacillus* spp. exhibited powerful inhibitory effects against different pathogenic bacteria[4]. Kim et al. (2020) studied the production of iturin, surfactin, and fengycin after UV irradiation and concluded that a mutant *B. velezensis* strain yielded over 2X of iturin production than its wild-type against *Fusarium oxysporum.* The increased antibacterial surfactin lipopeptide production could be used in various food and biomedical applications as a consequence of good antimicrobial activity against drug-resistant food-born *B. cereus* and the human pathogen *Staphylococcus aureus*[1].

Bouchard-Rochette et al. (2020) worked with *Bacillus pumilus* strain PTB180 and *B. subtilis* strain for their strong in vitro antagonistic activity against several plant pathogens including *Fusarium oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum, Phytophthora capsici,* and *Pythium ultimum* that were investigated for the production of lipopetides. Although both have different LP signatures, they showed comparable in vitro antagonistic activity against *B. cinerea.*

4 CONCLUSION

This study showed that the isolate BRM63426 has the potential to synthesize metabolites from the iturin and surfactin groups at all the different times tested. This result possible explains the nematocid activity of this isolate. Therefore, more trials will be carried out to test the ability of this isolate to control plant diseases of great importance in agriculture through these metabolites.

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