Research Article

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Antimicrobial peptides (AMP)-producing Bacillus spp. for the management of *Fusarium* infection and alfalfa growth promotion

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Abstract

BACKGROUND: Alfalfa is the most extensively cultivated forage legume worldwide. Its yield and quality may be affected by various pests and pathogens. Among them, fungal pathogen *Fusarium oxysporum*, the causal agent of Fusarium wilt, is considered as the main threat. The aim of the present study was to investigate the potential of the Bacillus spp. isolated from alfalfa rhizosphere to be used as biocontrol agents against *F. oxysporum,* as well as plant-growth promoting agents.

RESULTS: A total of six isolates were identified as *B. halotolerans* LA1K3 and LA1NK3, *B. toyonensis* LA1K2, *B. thuringiensis* LA1K4, *B. megaterium* LA2K1, and *B. safensis* LA1NK1. Suppression of *F. oxysporum* was recorded in a range from 2.86% (LA2K1) to 31.43% (LA1NK3), except for LA1K2. The presence of antimicrobial peptide biosynthetic genes was detected: bacyllomicin and fengycin (LA1NK1); subtilin and fengycin (LA1K2); subtilin (LA1K4 and LA2K1), and bacyllomicin (LA1NK3 and LA1K3) by PCR method. *Bacillus halotolerans* LA1NK3 showed six PGP traits (production of indole-3-acetic acid, siderophores, HCN, protease, cellulase and amylase). Bacterial inoculation increased the germination percentage of infected seeds from 42.85% (LA2K1) to 85.71% (LA1NK1, LA1NK3), as well as the yield of infected alfalfa plants of 186.07% (LANK1), compared to the infected control.

CONCLUSION: The results of this study highlight the potential of rhizosphere soil to harbor beneficial bacterial strains that could be exploited for disease control and plant growth promoting. *Bacillus safensis* LANK1 stood out as the most effective strain in promoting the growth of alfalfa infected by *F. oxysporum* under controlled conditions.

Supporting information may be found in the online version of this article.

Keywords: antibiotics; *Bacillus spp*; biocontrol; *Fusarium oxysporum*; PGP bacteria; seed germination

INTRODUCTION

In contemporary agriculture, crop production should be carried out using safe and sustainable methodologies.[1] The utilization of synthetic fertilizers and pesticides has become unsustainable, due to their negative effects on human health and the environment.[2] Further, this practice contributes to the degradation of soil quality and fertility, resulting in the impairment of its physical, chemical, and biological properties.[3,4] Consequently, the quest for alternative agricultural solutions has led researchers to direct their attention towards the development of bio-inoculants, a product that adheres to the environment-friendly principles.[2,5–7] Forage grasslands are essential for livestock feed and are economically significant, covering about 70% of the world's agricultural area and 26% of the total land area.[8] In 2016, forage plants, along with wine and fruit, made up roughly one-third of the European Union's total crop production.[9] Commonly cultivated herbaceous legumes include trefoil (*Lotus corniculatus* L.), clover (Trifolium spp.), vetches (Vicia spp.) and (Medicago spp.). Among these, alfalfa (*Medicago sativa* L.) is the most widely grown perennial forage crop, adaptable to both temperate and tropical grasses or as a standalone crop.[9]

Microbial bio-inoculants are most commonly formulated with beneficial soil microorganisms as their primary constituents.[10,11] This approach offers an environmentally sustainable approach for enhancing soil fertility, crop productivity, and crop quality.[12,13] Moreover, the use of bio-inoculants provides a cost-effective alternative and has the potential to substitute expensive and harmful agrochemicals, including synthetic fungicides.[13] Although the bacteria belonging to *Rhizobium* genus are most commonly used for the promotion of alfalfa growth, it has been shown that these bacteria have low potential for fungal pathogen suppression.[14] On the other hand, the bacteria which are most commonly effective in suppression of plant diseases caused by

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