ECOLETTER

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Lysobacter enzymogenes B25, a novel PGPR strain with nematicidal activity

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Introduction

Lysobacter enzymogenes is a gram negative bacterium commonly isolated from soil and water. It has been widely reported as biocontrol agent of fungal and bacterial pathogens. It produces extracellular enzymes and secondary metabolites that may be responsible of its antimicrobial activity. Moreover, some *L. enzymogenes* strains are able to induce the systemic resistance in plants. However, little is known about its antagonist activity on nematodes or ability to promote plant growth. In this study, *L. enzymogenes* strain B25 (*Le*-B25, patent in progress) was evaluated *in vitro* and *in vivo* for growth-promoting and nematicidal abilities.

Materials and Methods

The *Le*-B25 strain was isolated from pepper roots infested with nematodes from a commercial field under organic management. Following our standard protocol for microbial isolation, the strain was identified as *L. enzymogenes* by 16S rDNA sequencing. The technical-grade active ingredient (TGAI) of *Le*-B25 was produced by liquid fermentation. Firstly, the plant growth-promoting (PGP) activity of *Le*-B25 on tomato plants cv. Marmande was evaluated in seedlings grown under depleted substrate (low organic matter level) with 10^7 CFU/mL. Secondly, seedlings were transplanted into pots filled with standard sterile substrate (loamy sand soil; 3.55% organic matter; pH=7.7 and 39.3mmol/Kg cation-exchange capacity) and treated twice with 10^7 CFU/mL of *Le*-B25 (immediately after transplant and 15 days later). Additional experiments were performed to evaluate the effects of *Le*-B25 on different life cycle stages of the phytonematode *Meloidogyne javanica* at 10^{10} CFU/mL. To confirm nematicidal activity, *in vivo* assays were conducted on 4 week-old tomato plants cv. Marmande under growth chamber conditions. *Le*-B25 (10^8 UFC's/mL) was applied at 3 times: 4 days before inoculation with a suspension of 1000 J_2 *M. javanica*/plant, and 1 week and 2 weeks after nematode inoculation. In addition of the chemical control (a.i. Fenamiphos), a commercial product based on the entomopathogenic fungus *Paecilomyces lilacinus* was included to compare biocontrol activity.

Results

Figure 1. Dry weight (DW) of plants grown in depleted substrate at 35 days after treating with *Le*-B25 (B25) at 10^7 CFU/mL. Data correspond to the mean ± SE of *n*=10 plants. Statistics are shown in the inlet (ANOVA, *P*<0.05).

Fig.1

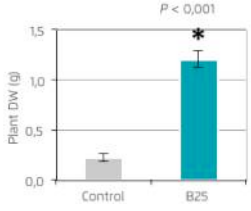


Fig.2

Figure 2. Dry weight (DW) of potted-plants at 30 days after the first treatment (at transplanting) with 10mL/plant of *Le*-B25 (B25) at 10^7CFU/mL. Data correspond to the mean of *n*=10 plants. Results of statistics are shown in the inlet (ANOVA, *P*<0.05).

Figure 3. Plant Growth-Promoting (PGP) activity of *Le*-B25 after third treatment.

Control

Le - B25

Figure 4. Percentage of efficacy of the nematicidal activity of *Le*-B25 vs *Meloidogyne javanica in vitro* assays.

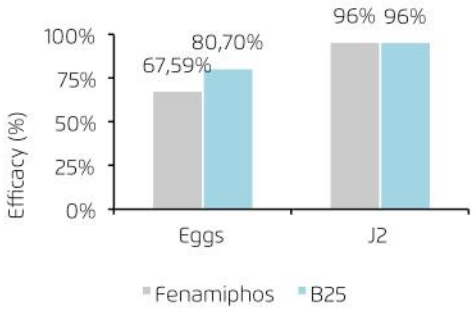
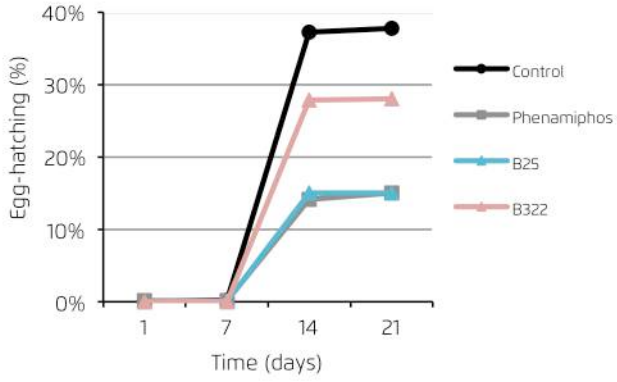
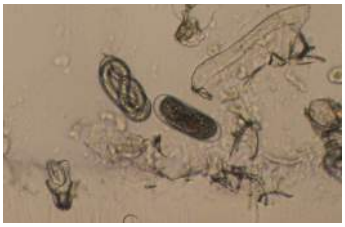


Figure 5. Percentage of egg hatching of the nematicidal activity of *Le*-B25 vs *Meloidogyne javanica in vitro* assays.





Images: Infection of roots by a Meloidogyne javanica female (upper image); eggs of M. javanica in different stages (middle image), and a male adult of M. javanica (lower image).

These trials were performed in the facilities of Futureco Bioscience.
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Figure 6. Effect of *Le*-25 on the final population of *M. javanica* expressed as eggs/gr of fresh root compared to a control and two reference products.

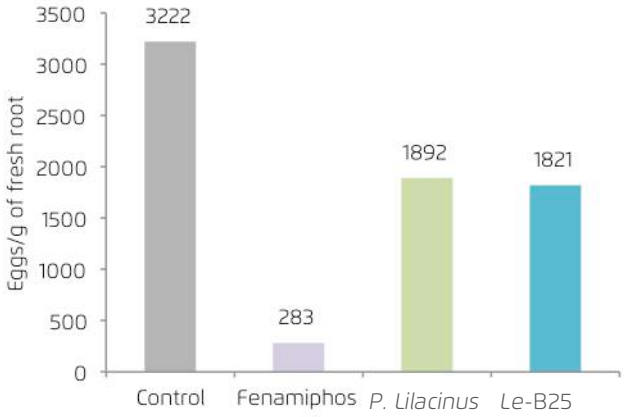
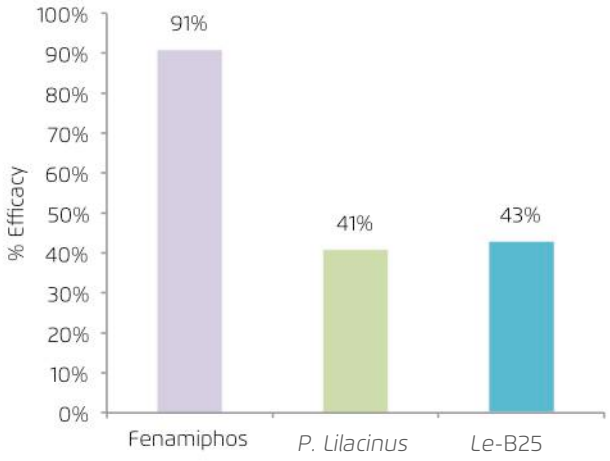


Figure 7. Percentage of efficacy of *Le*-25 on the reduction of the final population of *M. javanica* in tomato roots.



After the treatment with 10^7 CFU/mL, an increase on plant growth was observed (5.79-fold increase in dry weigh respect to control Figure 1). When seedlings were transplanted and treated twice with 10^7 CFU/mL of *Le*-B25 after transplant and 15 days later, results showed strong PGP activity of *Le*-B25 on tomato plants (4.5-fold increase dry weight than control Figures 2 and 3).

Evaluation of the effects of *Le*-B25 on different life cycle stages of the phytonematode *Meloidogyne javanica* showed that *Le*-B25 strain at 10^{10} CFU/mL had high *in vitro* activity in reducing egg-hatching and inducing juveniles (J_2) mortality (81% and 96% respectively) as seen in figures 4 and 5. No significant differences were observed with the efficacy of the chemical control based on the active ingredient Fenamiphos.

A comparative test showed better antagonism of *Le*-B25 than another *L. enzymogenes* strain (*Le*-B322).

Conclusions

Given its effects in both plant growth enhancement and nematode control, *Le*-B25 seems to be a good potential active ingredient for a future Bionematicide with an additional biostimulant effect.