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Lactic acid bacteria as biocontrol agents of soil-borne pathogens

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Abstract: Lactic acid bacteria (LAB) might be a novel promising bacterial group in the biological control of soil-borne pathogens. The potential of this bacterial group to suppress certain fungi has been shown in the control of post-harvest diseases of fruits and vegetables, but not much is known about their ability to protect plant roots against soil-borne pathogens. From soil and the rhizosphere of maize, rye, carrots, garden soils and compost from two origins 294 isolates of LAB have been obtained and tested in a confrontation assay against *Pythium ultimum*. In total, about 75% of the isolates showed an inhibitory effect. The most promising isolates were further tested in the pathosystem *Pythium*/tomato in pot trials. The LAB were able to protect the plants against the pathogen. Additionally the germination of tomato was significantly enhanced when the seeds were treated with LAB. Therefore we conclude that LAB are a promising bacterial group for use in the biological control of soil-borne pathogens.

Key words: Lactic acid bacteria (LAB), *Pythium*, biocontrol, growth promotion, seed germination

Introduction

The ability of lactic acid bacteria (LAB) to suppress certain fungi has been shown in the control of post-harvest diseases of fruits and vegetables. The main mechanism responsible for control in these systems is the production of organic acids such as propionic or lactic acid, a decrease in pH, the accumulation of hydrogen peroxide and some strains were able to produce antimicrobial compounds (Caplice & Fitzgerald, 1999). Beside this antifungal activity LAB have also other properties making them to interesting candidates for the biological control of soil-borne pathogens. From the food industry the technology for mass production for LAB is already available. Furthermore as they are already used in the food technology, LAB are mentioned to be safe for human and animal health. Therefore, we evaluated their potential to control *Pythium* on cucumber and to promote germination of tomato seeds.

Material and methods

Isolation and screening

Soil and root samples of maize, rye, carrots, garden soils and compost were obtained from two origins, Wädenswil and Brugg in Switzerland. From these samples 1g was transferred in a 50ml Falcon-tube containing 30ml of a LAB specific media and shaken for 2 days at room temperature. After serial dilution plating on MRS-agar containing 1% of calcium carbonate single colonies were picked and multiplied. These isolates were then tested in a confrontation assay against *Pythium ultimum* on 1.5 % malt agar plates.

Biocontrol activity in the pathosystem* Pythium*/cucumber
Pythium was grown for one week on malt agar plates. To each pot (10cm diameter) filled with a non-sterilised substrate five *Pythium* covered plugs were added. Two days old cultures of LAB isolates grown in MRS-broth at room temperature were centrifuged. Afterwards, the bacterial pellet was resolved in water and diluted to a optical density of OD600 = 0.250. Seeds of cucumber (Chinesische Schlange) were incubated in this bacterial suspension for 15 min and planted. Root and shoot weight were evaluated after three weeks of cucumber growth.

Germination and seedling emergence of tomato
Bacterial suspensions and seed inoculation were performed as described above. Per treatment 40 seeds were inoculated with LAB and sown in a commercial substrate with no artificial inoculation with *Pythium*. Germination and seedling emergence was followed during three weeks.

Results and discussion

Isolation and screening
In total, 294 isolates were obtained from all samples. Of these around 75% of the isolates showed an inhibitory effect and 50% suppressed *Pythium* growth by more than 60%. Most of the promising strains were isolated from maize roots, compost, or garden soil.

A high number of isolates were easily obtained. Therefore, LAB are present in soil and in the rhizosphere of different plant species and are relatively easy to isolate. Furthermore, a high proportion of the isolates was able to suppress *Pythium* growth in a confrontation assay. These results indicate that soil is a novel source for LAB strains for the control of soilborne pathogens and post-harvest diseases. Currently, the best performing isolates were characterised at the Culture Collection of Switzerland CCOS in Wädenswil.

Biocontrol activity in the pathosystem* Pythium*/cucumber
Plant growth was not significant influenced by the addition of LAB when no pathogen was added to the substrate. In presence of *Pythium* plant weight was strongly reduced and was only 15% of the untreated control (Fig. 1). When additionally the seeds were treated with LAB plant fresh weight was more than 4 times higher and reached up to 65% of the untreated control plants. Furthermore, it was possible to re-isolate the LAB from cucumber roots (data not shown).

LAB were able to protect cucumber roots against *Pythium*. Furthermore, preliminary experiments showed the potential of LAB against *Pythium* on tomato and strawberry. This indicates that LAB are a novel interesting candidate group for the biological control of soilborne pathogens. In the near future, our isolates will be tested also in other pathosystem.

Germination and seedling emergence
Only 34 % of the non-inoculated seeds germinated and emerged (Fig. 2) in a commercial available substrate with no added pathogens. In contrast when the seeds were treated with LAB the germination rate and seedling emergence rate raised up to 54-57 % and therefore differed significantly from the untreated control.

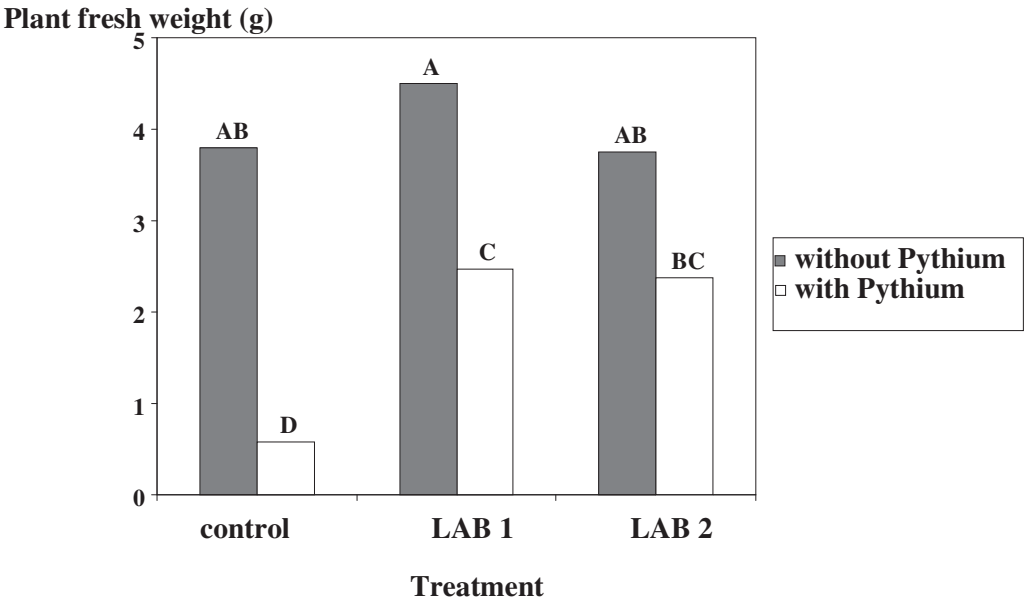

Figure 1. Plant fresh weight of five cucumber plants inoculated with LAB or not in presence or absence of *Pythium* after 3 weeks of growth. Values are the means of one experiment with five replicates each. Columns marked with the same letter are not significantly different according to Fishers protected LSD ($P \leq 0.05$).

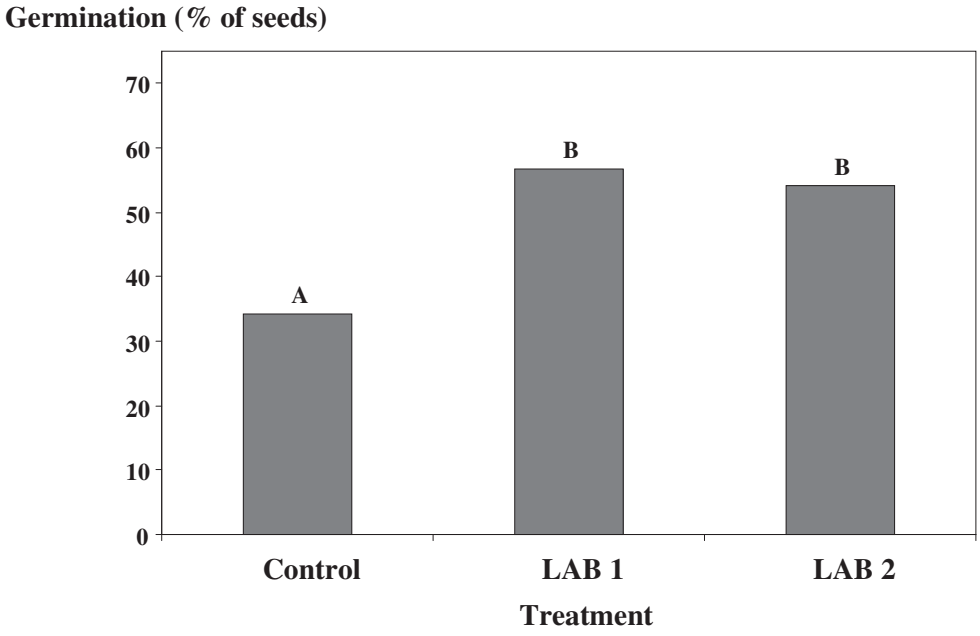

Figure 2. Germination rate of tomato seed inoculated or not with LAB. Values are the means of one experiments with three replicates each containing 40 tomato seeds. Columns marked with the same letter are not significantly different according to Fishers protected LSD ($P \leq 0.05$).

The LAB strains used in this study enhanced germination rate and seedling emergence to a very high extent without negative affecting plant growth. This could be of great interest for the seedling production industry.

In general, we could show that LAB are present in soil and in the rhizosphere of different plant species. A high proportion of the LAB isolates showed an inhibitory effect on *Pythium* growth in a confrontation assay. Furthermore, some isolates were able to protect cucumber against *Pythium* in a pot experiment and to enhance seed germination rates of tomato. Therefore, we conclude that LAB are a promising bacterial group to suppress soil-borne diseases and to promote plant growth.

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References

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