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Challenges in the development of a microbial fungicide based on a strain of *Lysobacter capsici*

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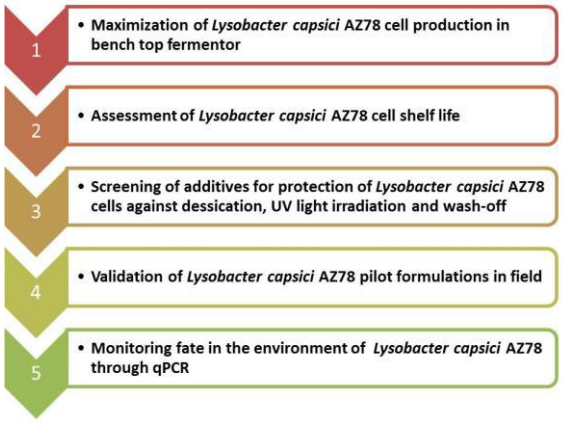
Introduction: *Lysobacter capsici* AZ78 (AZ78) is a Gram-negative bacterium that effectively controls oomycetes, in particular *Plasmopara viticola.* Non-spore forming bacteria populations quickly decline when exposed to harsh enviroments as phyllosphere and formulation can play an important role to achieve consistent efficacy in field applications. Limited information is available in literature on formulation of Gram-negative bacteria. Survival and fate in the environment are also important traits to be assessed for the registration as a biopesticide.
Objectives: The main aim of this work was to set up and apply a stepwise flow diagram to design performing formulations for AZ78 and follow its fate in the environment (Figure 1).
Materials and methods: Cell mass production was maximized in a benchtop fermenter and shelf life of the harvested cells was assessed. We screened compounds capable to protect the bacterial cells against desiccation, UV irradiation and wash-off. We tested the ability of combinations of selected compounds to preserve the efficacy of AZ78 against *P. viticola* and to establish populations on grapevine. A specific primer pair was developed starting from REP-PCR fingerprinting and subsequently used in a qPCR procedure for monitoring the fate of AZ78 in vineyards.
Results: The optimised fermentation protocol gave a harvest of at least 10^{10} AZ78 cell/ml. Viability of cells decreased only one order of magnitude after one year of storage at 4°C. The use of a combination of polyethyleneglycol, corn steep liquor and lignosulfonate in the formulation improved the survival of AZ78 cells in response to environmental stresses and the efficacy against *P. viticola* on grapevine in field conditions. Moreover, the qPCR procedure showed that the AZ78 population reached 10^{6} cell/gram of leaf after its application and revealed that the use of additives in the tank mix enhanced the persistence of AZ78 cells in vineyards.
Conclusion: The stepwise flow diagram allowed us to achieve high biocontrol efficacy of AZ78 in field conditions and fulfill some of the requirement for the registration of AZ78. The same approach could be extended to other members of the genus *Lysobacter* for the development of biofungicides.

Figure 1



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Suitability of an electrolytic disinfector to sanitize irrigation water contaminated with plant pathogens

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Introduction: The transmission of plant pathogens through irrigation water and nutrient solutions is a serious problem in agricultural production. Different physical and chemical techniques such as pasteurization, UV-light, filtration and water treatment by chlorination, ionization and surfactants have been described to decontaminate irrigation water and nutrient solution. Beside cost effectiveness and ecological concerns none of the methods is suitable to inactivate the multitude of relevant viral, bacterial and fungal plant pathogens.

Objective: Evaluation of the efficacy of a disinfectant produced by an electrolytic disinfector processing a salt solution to inactivate plant pathogens *in vitro*.
Materials and methods: The efficacy of the disinfectant, a low concentrated potassium chlorid solution, to inactivate plant pathogens was tested in vitro according to the standard for disinfection in plant protection (OEPP/EPPO, 2008). Eight pathogens were selected: *Fusarium oxysporum*, *F. verticillioides*, *Pythium aphanidermatum*, *Botrytis cinerea*, *Verticillium dahliae*, *Rhizoctonia solani*, *Xanthomonas campestris* pv. *Campestris and Pseudomonas syringae* pv. *syringae*. Dose-effect relations were calculated.
Results: Applying the disinfectant with a concentration of 6 mg KClO/l and a contact time of 60 min, achieved total inactivation of all tested pathogens with exception of *Rhizoctonia solani.* This fungal pathogen even presented activity with 10 mg KClO/l and a contact time of 120 min.
Conclusion: The efficacy of Potassium Hypochlorite (KClO) to inactivate plant pathogens is confirmed in vitro.

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Electron treatment of sprouting seed
An efficient, economical and environmental-friendly process for pathogen reduction
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Providing the world´s growing population with nutritious food is an enormous challenge, that solution starts very early in food production. Beside the known chemical seed dressing there is another way for killing pathogens. This environmental friendly, purely physical disinfection of seed, bases on the biocidal effect of accelerated electrons.
Electrons are a versatile tool for numerous applications in all fields of industry. Beside the known and well established processes in medicine and pharma the electron treatment of seed became more and more important. This technology is well established for treatment of cereal seed. Hence the treatment of sprouting seed is a challenging topic as well. Systemic problems with Bacteria, such as *E.Coli* cannot be solved completely with the common technics.
Due to the current demands, after EHEC crises in 2011, FEP investigated the behavior of electron treated sprouting seeds. When treating seeds, the applied dose, which can be determined by regulating the current strength, and the electron energy, which can be adjusted with the acceleration voltage, are important. When electrons penetrate matter, they lose their energy through collision processes. Once the energy is spent, they do not penetrate further into the material. This fact is used to precisely control the sphere of action during electron treatment.
Infected seeds are treated with electrons and there germination force, germination rate and load of pathogens are investigated.
More than 90 % of the fenugreek and clover samples and more than 80 % of the mung bean samples are sterile, proved with fluid turbidity tests, after electron treatment. Not to influence the embryo, can be proved by testing germination rate and germination force. Both are kept unchanged.
Tests show that the treatment of sprouting seed (Mung bean, clover and fenugreek) to reduce bacteria load is possible, without influencing the embryo.

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Effect of Essential Oil Against Bacterial Cancer Disease Caused by Clavibacter michiganensis subsp. michiganensis in in vitro Conditions
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Bacterial wilt and bacterial canker on tomato caused by *Clavibacter michiganensis* subsp. *Michiganensis* is gram-possitive bacteria. In this study, the antibacterial effects of essential oils of sage (*Salvia* spp), anise (*Pinpinella anisum*), juniperus (*Juniperus* sp.), aloevera (*Aloe vera*), safflower (*Carthamus tinctorius*), bergamot (*Citrus bergamia*), rosemary (*Rosmarinus officinalis*), laurus (*Laurus nabilis*), senien urticae piluliferae (*Urtica diolca*), clove (*Caryophyllus aromaticum*), centaury (*Hypericum perforatum*), priest (*Lavandula stoechas*), thyme (*Tymus vulgaris*), cumin (*Carum carvi*), lavender (*Lavandula officinalis*), melissa (*Melissa officinalis*,), myrtle (*Myrtle* sp.), mint (*Mentha piperita*,), eucalyptus (*Eucolyptus globus*,), fennel (*Foeniculum vulgare*,), daisy (*Matricoria chamomilla)* on *Clavibacter michiganensis* subsp. *michiganensis* were investigated. 100 µl of the pathogen bacterial suspension (10^8 cfu/ml) was spread on 9 cm diameter petri dishes containing King medium B. Sterile discs (Watman No.1 diameter 5mm) were put on the medium and 10 µl of each essential oils was dropped on discs and cover petri with parafilm. Sterile distilled water was used for negative control. All plates were incubated at 25^{o}C for three days. The diameter of clear zone around the disc was measured as millimeters. All treatments were three times replicated and for each