Development of a Rapid Bio-Test to Study the Activity Potential of Biofertilizers

Entwicklung eines schnellen Bio-Tests zur Untersuchung des Wirkungs-potentials von mikrobiellen Pflanzenstärkungsmitteln

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Abstract:

Plant-growth-promoting soil microorganisms are increasingly distributed on the world market. Nutrient mobilization, stimulation of root growth, enhanced resistance to environmental stress factors are discussed as possible mechanisms. These assumptions are based only on scarce scientific evidence due to limited reproducibility of pot and field experiments, limited information concerning the conditions for successful application, limited standardization of inoculum preparation and quality. Thus, the development of rapid screening tests is to demonstrate the principle effectiveness of biofertilizers prior to set-up of labourous pot or field experiments is urgently required.

In this study, a rapid bio-test with cucumber (\textit{Cucumis sativa} L.) as an indicator plant was developed to evaluate the effectiveness of five commercial biofertilizers based on \textit{Trichoderma} spp. and \textit{Bacillus} spp. (Biohealth-G, Biohealth-WSG, Biomex, Vitalin T50 and SP11) using germination rate, root and shoot biomass, maximum root length, and leaf area as test parameters. The experiment was repeated twice with 6 replicates in hydroponic culture under controlled conditions (pH 5.5, 22° C; Light: 230 mmol cm\textsuperscript{-2} sec\textsuperscript{-1}). Biofertilizers were applied at the rate of 3 g per 2.5 l mineral nutrient solution. Germination rate was increased by 20 - 25% in all biofertilizer treatments compared to the control. After 2 weeks culture period, root dry weight and leaf area of Biohealth-G, Vitalin T50, SP-11 and Biomex-treated cucumber seedlings were significantly increased. Biohealth-G and Vitalin T50 showed significantly higher main root length and Biohealth-G higher shoot dry weight than the remaining treatments, while Biohealth-WSG did not cause differences compared to untreated control plants. The pathogen-antagonistic potential of \textit{Trichoderma} strains can be easily tested by co-inoculation with the pathogenic fungus \textit{Gaeumannomyces graminis} on malt extract peptone agar plates. The results suggest that the activity potential of different \textit{Trichoderma}-based biofertilizers could be easily screened by using the described bio-test with cucumber seedlings.

Introduction and Objectives:

Bio-effectors based on plant-growth-promoting soil microorganisms are increasingly distributed on the world market. Mobilization of sparingly available plant mineral nutrients, stimulation of root growth, enhanced resistance to environmental stress factors and direct or indirect suppression of plant pathogens and induced resistance are discussed as possible mechanisms for the effectiveness of these products. However, these assumptions are based only on scarce scientific evidence which is further abused by a lack of standards for production and quality control. Therefore, rapid

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screening tests to evaluate the potential effectiveness of a given product, prior to more
detailed and labourous investigations are urgently needed.
In this study test plants with bioindicator potential for toxins and plant growth regula-
tors (Lepidium and Cucumber) were investigated for responses to biofertil-
izer treatments under controlled condi-
tions.

**Methods:**
(1) Germination test:
Seed surface sterilization: 3 min in 30 %
H$_2$O$_2$, 2 min in 70% ethanol, Two times
washing with sterile distilled H$_2$O. Seeds
of Cucumis sativa cv. Vorgbirgstrauben
and Lepidium were sown in rolls with 4
layers of moist filter paper (MN 710,
Machery and Nagel, Dueren, Germany).
The filter rolls were soaked with 2.5 mM
CaSO$_4$ containing 1% (w/v) of different
commercial bio-fertilizers. Different
biofertilizers, such as T-50 and Sp-11
were collected from Vitalin Pflanzenge-
sundheit GmbH, Ramstadt, Germany;
BioHealth-WSG and BioHealth-G from
Humintech GmbH, Düsseldorf, Germany
and Biomex from Omex Agriculture Inc.,
Manitoba, Canada. Rolls with each 15
seeds were placed in upright position
into a closed plastic box and incubated in the dark for 4 d at 22°C with 6 replicates per
treatment (Fig. 1).
(2) Seedling establishment test:
Seedlings, germinated for 4 days in filter rolls (see (1) were subsequently grown for 7
days under controlled conditions (22°C; 230 mmol light cm$^{-2}$ sec$^{-1}$) in 2.5 L aerated
nutrient solution (mM: 2 Ca(NO$_3$)$_2$, 0.7 K$_2$SO$_4$, 0.5 MgSO$_4$, 0.25 KH$_2$PO$_4$, 0.1 KCl; µM:
10 H$_3$BO$_4$, 0.5 MnSO$_4$, 0.5 ZnSO$_4$, 0.2 CuSO$_4$, 0.01 (NH$_4$)$_6$Mo$_7$O$_{24}$, 20 Fe-EDTA; pH
5.5) supplied with biofertilizers 1.5 - 3g pot$^{-1}$. Each pot contained 10 seedlings in 6
replications (Fig. 2).

**Results and Discussion:**
Most consistent responses were obtained with cucumber (cv. Vorgebirgstrauben).

**Germination test:** biofertilizers increased germination rate by 20-25 % at 4 days after
sowing (DAS) (Fig. 4A). Seedling emergence and plant development was accelerated
by approximately 2 days after inoculation with the biofertilizers.
Similar responses have been reported by other authors (HARMAN et al. 2004, OZBAY
et al. 2004, ARORA et al. 1992) and enhanced seed germination induced by seed
inoculation with Trichoderma spp. has been related to the production of growth factors
such as auxin, cytokinin or gibberellic acid (GA3) and even ethylene.

**Seedling establishment test in hydroponics:** Biofertilizers increased dry matter of roots
(50-90%) and shoots (30-80%) and particularly leaf area (70-100%) during early
seedling growth (7d culture period in nutrient solution, Fig. 4B and C). A differential
effect on root morphology was observed in hydroponic culture. Maximum root length
increased by 30% in T50 and BioHealth-G (Fig. 4D), while T50 stimulated particularly lateral root formation (Fig. 3). The increase in shoot growth and leaf area in *Trichoderma* treated seedlings suggests a common beneficial role of *Trichoderma harzianum* in improving plant growth (Yedidia et al., 2001). The mechanisms involved in increasing growth responses induced by *Trichoderma* sp might be the production of growth-stimulating compounds (GRAVEL et al. 2006, HARMAN et al. 2004, YEDIDIA et al. 2001, ALTOMARE et al. 1999, CHANG et al. 1986).

![Fig. 3: Shoot and root development of cucumber in hydroponics with and without Trichoderma treatments](image)

![Fig. 4: (A) Germination rate at 4 DAS, and dry matter production (B), leaf area (C) and maximum root length (D) of cucumber grown for 7 d in hydroponic culture (B-WSG = BioHealth WSG, B-G = BioHealth G)](image)
Conclusions:
Cucumber (cv. Vorgebirgstrauben) is a suitable test plant to demonstrate effects of various commercial Trichoderma-based biofertilizers on germination, plant development, and root growth within culture periods of 4 – 12 days in simple culture systems (filter paper germination test and hydroponic culture).

Best results were obtained by using seeds with sub-optimal germination rates (approx. 50%). For high quality seeds, this is easily achieved by artificial seed aging treatments: e.g. 1-2 days incubation enclosed in plastic bags at 40-45°C in a water bath (data not shown).

References:


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