About the use of antagonistic bacteria and fungi

Untersuchungen zur Anwendung von antagonistischen Bakterien und Pilzen

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Abstract

Microorganisms isolated from the phylloplane of vine and cereal plants inhibiting different phytopathogenic fungi were tested as biological control agents against *Plasmopara viticola* (downy mildew of grapevine). Based on screening in vitro against *Phytophthora infestans, P. parasitica, Pythium ultimum, Botrytis cinerea* 62 bacterial isolates were selected for tests with *Plasmopara viticola*.. Antifungal bacterial strains were assayed for antagonistic activity towards the grapevine dieback fungus *Eutypa lata in vitro* and on grape wood.

Keywords

Plasmopara viticola, downy mildew of grapevine, *Eutypa lata*, grapevine dieback, antagonistic microorganisms

Plasmopara viticola ...

A suspension of organisms in liquid medium, diluted cultural filtrate and a suspension of washed bacterial cells were sprayed prophylactically onto the lower surface of leaf discs (variety Müller-Thurgau). A susperision of *P. viticola* sporangia were sprayed 24 hours after application. The relevance of the investigations performed with the help of leaf discs was confirmed by tests with detached leaves and potted vines.
Infestation with downy mildew was reduced from 80-100 % by 42 isolates. Application of organisms in liquid medium and suspensions of washed cells were always more effective than spraying the diluted cultural filtrate only.
Curative spraying of six antagonists (inoculation with the pathogen 24 hours before the application of antagonists) resulted in smaller effects compared with prophylactic treatments. Combined application of 2 antagonists could increase reduction rates compared with the organisms separately applied.
42 bacterial isolates were characterised and could be classified taxonomically (69 %). Most of the identified bacteria, both soil-borne organisms (77 %) and epiphytes (75 %), were assigned to the family Pseudomonadaceae. Within this family the species *Stenotrophomonas (Xanthomonas) maltophilia* and *Pseudomonas fluorescens* were identified most often. 31 % of the isolates were identified ambigousiy because of conflicting classifications.

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In further experiments *Pantoea* (*Erwinia) herbicola* and isolate B 2 were used to investigate the influence of formulation additives (cellulose, alginate, xanthane, detergens) on antagonists applied to potted vines. Mixing two or three additives caused an increased amount of antagonist suspension sticked to the leaf surface (up to 200 %). The survival rate of the bacteria was increased by mixing alginate, xanthane or a combination of alginate, xanthane and cellulose with the sprayed antagonist.
When the leaf surface remained wet for 24 hours the survival rate of the antagonists was higher than when the leaves were dried up after application.
Field experiments (two vineyards in the Rheingau region) were performed in order to test six of the most effective organisms (bacteria: *Pantoea herbicola*, two isolates of *Stenotrophomonas maltophilia*, isolate B 2, actinomycetes: isolate A 76, A 132) when prophylactically coated onto grape vine.
Isolates applied without formulation additives, reduced leaf infestation significantly in all cases. The highest reduction rates (> 90 %) were obtained with *Pantoea herbicola and* actinomycete A 76. By mixing additives (cellulose, alginate, xanthane) reduction rates in the field were even higher (up to 95 %).
In order to determine whether the combined application of antagonists and chemical pesticides holds promise, the influence of 34 pesticides used in viticulture on the growth of three antagonists (two bacteria, one actinomycete) was investigated in vitro. The growth of all tested organisms was clearly inhibited by the fungicides Dithane Ultra (active substance Mancozeb), Wacker 83v (active substances copper and sulfur) and Ulmasud (releases aluminium ions). Also some of the insecticides tested (eg Thiodan 35 fl., active substance Endosulfan) had an inhibitory effect.
Microvinification was carried out to determine the effects of four antagonists applied to grapes on the process of wine making. Fermentation course of grapes sprayed 21 days before vintage with original liquid culture of the antagonists was negatively influenced (premature cessation). With a waiting period of 28 days there were no or only some minor irregularities, whereas fermentation course of grapes sprayed 35 days before vintage showed no deviation from the control at all. Parallel to this, wine analysis showed the wines of the shortest waiting period to have an increased sugar content and a decreased alcohol content compared with the control. Both the wines of the other experimental variants (with longer waiting periods) showed no differences.
Investigations using scanning electron microscopy (SEM) indicated that application of the antagonist *Pantoea h*erbicola radically changed the morphology of the vine leaf phylloplane. Bacterial rods occupied internal parts of the stomata. The components of the liquid medium were adsorbed onto the formulation additives.

Eutypa lata.

Antifungal bacterial strains were assayed for antagonistic activity towards the grapevine dieback fungus *Eutypa lata in vitro* and on grape wood. Hundred-twenty-one of 188 isolates (64%) exhibited antagonistic activity towards *Eutypa lata in vitro* (≥ 83% of the tested actinomycetes, *Bacillus* and *Pantoea herbicola* strains

and 32% of the *Pseudomonas* strains). On autoclaved grape wood disks, liquid cultures from 24 of 76 selected bacterial strains (32%) caused at least 50% suppression of mycelial growth over a period of two weeks. Among these were two *Bacillus subtilis*, four *Pantoea herbicola*, two *Serratia plymuthica* and 16 actinomycete isolates. One *Bacillus subtilis* strain (B1α), two *Pantoea herbicola* strains (JII/E2 and JII/E4) and one actinomycete (A123) showed the highest degree of antagonism (70-100% control over a period of four weeks). In *Pantoea herbicola* and the actinomycete A123 the cell free supernatant was the active fraction of the applied liquid culture (100% control); suspended cells showed no or limited effect. Conversely, in *Bacillus subtilis* the cells were the decisive component (50-70% control). *Pantoea herbicola* JII/E2 and *Bacillus subtilis* B1a inhibited growth of 6 different *Eutypa lata* isolates on wood significantly.

The strong antagonistic effect of the four bacterial strains was confirmed by the measurement of fungal hydrolase activity (chitinase, protease, cellulase). Activities of fungal hydrolases were highly correlated with mycelial growth on wood ($r \geq 0.88$). In *Bacillus subtilis* B1a antagonistic activity and ability to colonize grape wood was further demonstrated by cryo-scanning electron microscopy.

The effect of different supplements on the antagonistic activity of *Bacillus subtilis* and *Pantoea herbicola* towards *Eutypa lata in vitro* and on autoclaved grape wood was investigated. Inhibition zones on grape wood agar were enlarged maximally in *Bacillus subtilis* by adding peptone (0.25%) and in *Pantoea herbicola* by adding glucose (0.5%) or high concentrations of peptone (1–2%) to the medium. Nitrate and phosphate salts reduced the antagonistic activity of *Pantoea herbicola* on glucose supplemented medium. Glucose as well as manganese ions did not enlarge inhibition zones of *Bacillus subtilis*. On grape wood, peptone and methylcellulose supplements increased antagonistic performance and cell densities of *Bacillus subtilis* B1a, whereas a negative effect was exerted by calcium alginate and carob (88% galactomannane). A combination of 1% peptone and 4% methylcellulose resulted in 100% inhibition of *Eutypa lata* (70% inhibition without supplements). In *Pantoea herbicola* JII/E2, cells applied without supplements showed no antagonistic effect. Carob was the only supplement which led to a significant antagonistic effect of suspended cells of *Pantoea herbicola* (70%–100% inhibition).

One spontaneous mutant and two transposon mutants of *Pantoea herbicola* JII/E4 did not inhibit *Eutypa lata* on tryptic soy czapek dox agar but showed unreduced antagonistic activity on grape wood extract agar amended with glucose or peptone. on grape wood, the transposon mutants inhibited growth of *Eutypa lata* completely whereas the antagonistic activity of the spontaneous mutant was significantly reduced.

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