Strategies for administration of biosurfactant-producing pseudomonads for biocontrol in closed hydroponic systems

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Abstract

Zoospore-producing oomycetes are major plant pathogens of particular concern in hydroponic systems. Compared with pesticides, biocontrol using antagonistic microorganisms is a sustainable approach to control oomycetes. Previous research has demonstrated that biosurfactants and biosurfactant-producing microorganisms are potentially useful components of a sustainable biocontrol strategy.

In this study three ways of supplying a biosurfactant-producing strain to a recirculating hydroponic cultivation system infected with a zoospore-producing plant pathogen were evaluated. The strain *P. koreensis 2.74* was added as washed cells, in its spent KB broth or in a minimal medium adapted from the nutrient solution, and compared with control treatments. A significant reduction in disease with up to 50% was achieved when a high concentration of washed cells was added weekly to the plant cultivation system. The disease suppression obtained through addition of washed cells equalled the effect achieved when the purified biosurfactant was used. Phytotoxicity was observed when the spent broth was included in the treatment.

Keywords

Biological control, biosurfactants, greenhouse, lettuce, zoospores

1. Introduction

Modern greenhouses employ non-soil culture methods in which the nutrient solution is recirculated in order to prevent the release of excess nutrients to the environment and to save resources. In these systems there is a potential risk of proliferation of zoospore-producing plant pathogens (oomycetes), which are particularly well-adapted to aquatic life (Stanghellini and Rasmussen, 1994). Compared with using pesticide-based managements, biocontrol using antagonistic microorganisms is a sustainable approach to deal with oomycetes in hydroponic systems.

Fluorescent pseudomonads, a group of microorganisms that has been intensively researched for the purposes of biological control (Haas and Defago, 2005), are indigenous in the rhizosphere and are commonly found in non-soil cultivation systems (Hultberg, 1999). The efficacy of biosurfactants produced by fluorescent pseudomonads against zoospore-propagated plant pests was first demonstrated by Stanghellini and Miller (1997) and more recent research has confirmed their observations in many recent studies (De Souza *et al.*, 2003; De Jonghe *et al.*, 2005; Nielsen *et al.*, 2006; Tran, 2007; Hultberg *et al.*, 2010a,b). Zoospores are especially sensitive to surfactants since they lack cell wall (Stanghellini and Miller, 1997; Judelson and Blanco, 2005). *In vitro* studies have shown that zoospores become immotile within 30 seconds of exposure to a particular biosurfactant and subsequently lyse (Stanghellini and Miller, 1997; De Souza *et al.*, 2003).

The natural roles of biosurfactants are not clear but probably relate to increased nutrient uptake of hydrophobic substrates, increased growth on a hydrophobic surface, motility and biofilm formation (Van Hamme *et al.*, 2006; D'aes *et al.*, 2010). These traits are important for

survival of the microorganisms in the rhizosphere, with Nielsen *et al.* (2002) reporting that the ability to produce biosurfactants is a common trait among root-associated fluorescent pseudomonads. This has also been shown in closed hydroponic systems, where approximately 10% of the indigenous fluorescent pseudomonads tested had biosurfactant production capability (Hultberg *et al*., 2008a,b).

Biosurfactants make up a chemically heterogenic group including glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids and polymeric and particulate compounds (Mulligan, 2005). Fluorescent pseudomonads have been reported to produce various types of biosurfactants, including glycolipids and cyclic lipopeptides (Van Hamme *et al.*, 2006; D'aes *et al.*, 2010). Studies to date have mainly focused on the rhamnolipids, glycolipid-type biosurfactants, which have been shown to be effective in hydroponics (Stanghellini and Miller 1997; De Jonghe *et al*., 2005; Nielsen *et al.*, 2006). However, rhamnolipids are mainly produced by *Pseudomonas aeruginosa*, an opportunistic human pathogen. In recent years the zoosporicidal properties of cyclic lipopeptides have been demonstrated (De Souza *et al*., 2003; Tran, 2007). Fortunately these compounds can be produced by other species of fluorescent pseudomonads, which eliminates the need to work with *P. aeruginosa*.

A key element for pathogen control in hydroponic systems is the inactivation of zoospores (Calvo-Bado *et al.,* 2003). While the possible benefits of biosurfactants in non-soil systems and the potential for their production *in situ* using non-pathogenic strains of fluorescent pseudomonads have been discussed, there is a lack of knowledge concerning how biosurfactant-producing strains could be supplied to the cultivation system. The aim of the present study was therefore to evaluate ways of supplying a biosurfactant-producing strain to

a recirculating hydroponic cultivation system infected with a zoospore-producing plant pathogen.

2. Materials and methods

2.1. Microorganisms

The biosurfactant-producing fluorescent pseudomonad, Pseudomonas koreensis 2.75 (CBS 125413), was used in the present study (Hultberg *et al.*, 2008b). This strain produces a cyclic lipopeptide-type of biosurfactant with a molecular mass identical to lokisin (Hultberg *et al.*, 2010a). The strain was stored in glycerol (20% v/v) at -80°C.

The plant pathogenic oomycete *Pythium ultimum*, provided by Dr. W. Wohanka, Geisenheim, Germany, was used for production of zoospores. The abundant production of zoospores suggests that the isolate was a *Pythium ultimum* var. *sporangiferum* (Francis and St Clair, 1997).

2.2. Production of bacterial inoculum

For inoculum preparation *P. koreensis* 2.74 was pre-cultured on nutrient agar for 48 hours at room temperature. A single colony from the nutrient agar was transferred to 50 mL nutrient broth (Difco 234000) and incubated on a rotary shaker (180 rpm) for 18 hours at room temperature. One ml of the seed culture was used as an inoculum and added to a 1-L Erlenmeyer flask with 250 mL of KB broth (King *et al.*, 1954). The flasks were incubated for 48 hours at room temperature on a rotary shaker (180 rpm). Three different types of inocula were tested in the present study: I) The cells were harvested by centrifugation (17 000 g, 35 min: Avanti J-10, Beckman Coulter, CA, USA), washed once with 0.85% NaCl and dissolved in nutrient solution, and added to the plant system. The washed cells were added in two concentrations, log 7.5 cfu mL^{-1} and log 5.2 cfu mL^{-1}. II) KB culture medium including cells

(0.5 L broth added to 20 L nutrient solution) was directly added to the system to achieve a cell concentration of log 7.5 cfu mL^{-1}. III) Pseudomonas koreensis 2.74 were cultivated in oil minimal medium based on the nutrient solution used in the plant system (NH_4Cl 0.02 M, KCl 0.02 M, $MgSO_4$ 0.0016 M, K_2HPO_4, KH_2PO_4 amended with 2% of rapeseed oil, pH 5.9). The minimal oil broth was then incubated for 7 days on a rotary shaker (180 rpm) at room temperature and added directly to the system (0.5 L broth was added to 20 L nutrient solution). In this treatment the concentration of *P. koreensis* 2.74 was log 6.3 cfu mL^{-1} in the plant cultivation system.

2.3. Production of biosurfactant

For biosurfactant production *P. koreensis* 2.74 was cultivated in KB broth under the conditions described in section 2.2, the cells were centrifuged down, and the cell-free broth containing the biosurfactant was autoclaved. The sterile broth was amended with 1% (w/v) Amberlite XAD-16 (Rohm and Haas Company, Philadelphia, USA) and the slurry was shaken (150 rpm) for 12 hours. After this the resin was collected and washed with six volumes of distilled water. The resin was then washed in 25% methanol, after which it was washed once again with 50% methanol. The resin was finally transferred to 100% methanol. After this step, the methanol was collected and evaporated at 50°C and the dry weight of the recovered biosurfactant was recorded. The critical micelle concentration of the biosurfactant was 220 μg ml^{-1}, as determined with SITA DynoTester (SITA, Messtechnik, GmbH) at 22°C, pH 7.0, and the lowest surface tension value obtained was 23.5 mN m^{-1}. The biosurfactant treatment was applied in the nutrient solution to make a final concentration of 50 μg per mL.

2.4. Production of zoospores

Mycelium from *P. ultimum* growing on potato dextrose agar (PDA, Difco 213400) was transferred to water agar plates. After two days small pieces (1 cm x 1 cm) were cut from the periphery of the colony and each piece was transferred to a Petri dish with 15 mL of cleared V8 broth. The broth was prepared by boiling 11.5 g of rolled oats, 125 mL of V8 fruit juice (Friggs, Sweden) and 500 ml water. The suspension was centrifuged for 15 min at 500 g (Avanti J-20, Beckman Coulter, CA, USA). The supernatant was diluted with water to 625 mL, 0.94 g of $CaCO_2$ was added, and the broth was autoclaved. The Petri dishes were incubated in darkness for three days at 25°C. The mycelium was washed twice in an autoclaved mineral solution (0.145 g $Ca(NO_3)_2$, 0.012 g $MgSO_4$, 0.099 KH_2PO_4 and 0.016 $FeCl_3$ per litre) and once in autoclaved distilled water. The mycelium was then transferred to Petri dishes with 15 ml of autoclaved distilled water, incubated at 4°C for 30 minutes and then transferred to room temperature. The zoospores were released after about one hour. The zoospore suspension was collected in sterile vessels and zoospores were counted in a haemocytometer. The zoospores were added in a concentration 10^3 zoospores per mL of nutrient solution.

2.5. Plant material and growth conditions

The plant material used was oakleaf lettuce, *Lactuca sativa* L var. Ashbrook (Olssons frö AB, Sweden). Seeds were sown in rockwool cubes and at two weeks of age the seedlings were transferred to a NFT system. Each treatment consisted of three gutters, each containing 12 plants, and 20 L of recirculating nutrient solution. The flow was set to 800 mL min^{-1}. The nutrient solution used in the experiments was Sonneveld lettuce nutrient solution. It was composed of the macronutrients: (mmol L^{-1}) 10 KNO_3, 4.5 $Ca(NO_3)_2$, 1 $MgSO_4$, 1 KH_2PO_4, 1 Na_2HPO_4, 1.25 NH_4Cl and the micronutrients: (µmol L^{-1}) 40 Fe-EDTA, 5 $MnSO_4$, 4 $ZnSO_4$,

30 H_3BO_3, 0.75 $CuCl_2$, 0.5 Na_2MoO_4. The pH was set to 5.9 using NaOH and H_2SO_4. During the experiment the pH was continuously adjusted to 5.9 and the EC to 2.8 mS cm^{-1}. The experiment was conducted in a greenhouse with a temperature of 18°C, a photoperiod of 17 hours and 80% humidity. Additional artificial lightning (SON-T; 200 W m^{-2}) was provided during the experiment.

2.6. Experimental set-up and analysis

The different treatments (Table 1) were added once a week, starting when the plants were transferred to the gutters. The biocontrol treatments were added two hours before the zoospore suspension was added. The length of the plants was measured every week. At an age of six weeks the plants were harvested. Fresh and dry weight of leaf and roots were determined and leaf area was measured. The total numbers of fluorescent pseudomonads in the nutrient solution of the different treatments were monitored by viable counts on KB agar one hour after addition of the strain, after 24 hours and after six days. The experiment was repeated twice and data were analysed by analysis of variance with fixed effects followed by Tukey's multiple comparison test with differences considered significant at $P<0.05$ (Minitab, version 15).

3. Results

Significant effects on the weight of the seedlings ($F=69.59$; $df_1=11$, $df_2=24$; $p\leq0.000$) and of the roots ($F=45.50$; $df_1=11$, $df_2=24$; $p\leq0.000$) were observed in this study and the results are shown in Table 1. Addition of a zoospore suspension of *P. ultimum* severely impaired growth of lettuce and the untreated, infected treatment had significantly lower weight than the uninfected control. The leaf area of a lettuce head, expressed as mean ± SD, of the uninfected

control was 1383±141 cm^2. In comparison, pathogen addition reduced leaf area by 64%. Addition of the purified biosurfactant at a concentration of 50 µg mL^{-1} of nutrient solution protected the plants and no significant differences in plant weight were observed compared with the uninfected control.

Washed bacterial cells were added to the system in two different concentrations, either log 7.5 cells or log 5.2 cells mL^{-1} nutrient solution. A control treatment, with addition of log 7.5 cells mL^{-1} nutrient solution and no pathogen addition, was included and confirmed that addition of high concentration of *P. koreensis* 2.74 did not affect the growth of the plant. For the infected treatment receiving the higher concentration of cells no significant differences in plant weight was observed compared with the uninfected control. In the treatment receiving the lower concentration of cells the root weight was affected and not significantly higher than for the infected control. However, no significant difference was found when comparing the weight or leaf area of the lettuce with the uninfected control for this treatment.

When the cells were cultured in KB medium and directly added to the system, the growth of the plants was significantly lower than in the control irrespective of pathogen presence in the system. Lettuce weight was similar to that of the infected control and the weight of the roots was significantly lower than in the infected treatments. Furthermore, when KB broth in which no cells had been cultured was added to the system a significant reduction in plant growth was observed. Using *P. koreensis* 2.74 cultured in minimal medium with oil as a carbon source also resulted in significantly decreased growth of the plants.

The amount of fluorescent pseudomonads in the plant cultivation system is shown in Figure 1 and after six days there was significant difference in the amount of fluorescent pseudomonads

compared with the control treatment ($F=10.62$; $df1=4$, $df2=10$; $p=0.001$). In the control treatment the average amount of fluorescent pseudomonads over time, expressed as mean ± SD, was log 2.8±0.2 cfu mL^{-1} of nutrient solution. When a high concentration of washed cells of *P. koreensis* 2.74 was added to the system a rapid decrease in fluorescent pseudomonads was observed, from log 7.5±0.1 cfu mL^{-1} one hour after addition of the cells to log 5.1±0.1 cfu mL^{-1} after 24 hours. After six days there was no significant difference in the amount of fluorescent pseudomonads compared with the control treatment. A very similar pattern was observed for the treatment receiving a high amount of cells directly in KB broth. A comparable trend was found when a lower concentration of washed cells was added to the system, namely a rapid decrease from log 5.2±0.2 cfu mL^{-1} to log 3.7±0.1 cfu mL^{-1} during the first 24 hours, while after six days no significant difference could be found compared with the control. Applying the cells and crude broth, when a minimal medium with rapeseed oil as a carbon source was used for cultivation, resulted in a rapid decrease in the amount of fluorescent pseudomonads during the first 24 hours, from log 6.3±0.2 cfu mL^{-1} to log 4.4±0.1 cfu mL^{-1}. However, after six days the amount of fluorescent pseudomonads in this treatment (log 3.7±0.1 cfu mL^{-1}) was significantly higher than in the control treatment (2.7±0.1 cfu mL^{-1}).

4. Discussion

The present study demonstrated that addition of a biosurfactant-producing strain could protect the plant from disease and that the protective effect was equal to that obtained when the biosurfactant only was added. A biocontrol system based on living microorganisms in the cultivation system or on the plant would obviously require less labour, energy and raw material than *ex situ* production and addition of the biosurfactant as an extract and is therefore

a more sustainable approach. In addition, there is less foaming when living strains are used. The strain used in this study, *P. koreensis* 2.74, was originally isolated from a hydroponic system (Hultberg *et al.*, 2008b) and could be expected to be capable of surviving and growing in the system. However, a rapid decrease in viable cells of fluorescent pseudomonads in the nutrient solution was observed after addition. After six days the amount of fluorescent pseudomonads in treatments with addition of washed cells did not differ significantly from that in the control treatment. Repeated addition of the biocontrol strain is most probably needed to ensure a consistent effect.

Direct addition of *P. koreensis* 2.74 in its spent broth, reducing the need for centrifugation and washing of the cells, was observed to be less favourable, causing a significant reduction in plant weight. Potentially this could be due to the presence of phytotoxic secondary metabolites produced by *P. koreensis* 2.74 in the broth. However, also the treatment which received broth in which no cells had been cultured showed reduced plant weight. There may be several reasons for this effect such as a negative impact on the composition of the nutrient solution when the media was added, or that the increased content of organic carbon allowed proliferation of a deleterious microflora.

A total disease-protective effect was observed when washed cells were added in a concentration of log 7.5 cfu mL^{-1}. Addition of a lower concentration of washed cells (log 5.2 cfu mL^{-1}) reduced disease, but resulted in decreased root weight. Since lettuce is only cultivated for a short period of time, the lower concentration of cells could probably be used in practice. For other greenhouse crops with a longer cultivation time, e.g. tomato and cucumber, the use of a higher concentration of cells is probably more favourable in order to protect the roots.

Addition of the biosurfactant-producing strain in its spent broth will result in a higher concentration of organic carbon in the nutrient solution. This could possibly result in increased survival of the added bacterial strain. In the treatment receiving KB cell culture this was not the case and there was no increase in the amount of fluorescent pseudomonads after six days compared with the control. However, for the treatment receiving the biosurfactant-producing strain cultivated in a broth based on nutrient solution and with rapeseed oil as a carbon source, an increased amount of fluorescent pseudomonads was observed compared with the control. As no selective method was used to trace the added strain, this could have been due to either an increase in the added strain or a general increase in fluorescent pseudomonads. However, the potential of using this in practise is less since even this minor addition of oil (less than 0.2% w/w) was observed to be toxic to the plants, causing a large reduction in plant weight.

Biosurfactant-producing strains could be cultivated using very cheap carbon sources such as waste oils or whey, which would decrease the costs of production. Recent research has shown that agroindustrial waste have a potential to be used for the production of biosurfactants and for cultivation of biosurfactant-producing strains (Sobrinho *et al.*, 2008; Barros *et al.*, 2008). The biosurfactant-producing strains could either be cultivated in this recycling based medium, harvested and added to the system as washed cells, or the concept of a direct addition of cells and spent broth could be further refined, with less phytotoxic carbon sources. Again, this would have the advantage of avoiding expensive downstream processing or extraction with organic solvents.

In the pioneering work on biosurfactants and interactions with zoospore-producing plant pathogens performed by Stanghellini and Miller (1997), a rhamnolipid-producing strain of *Pseudomonas aeruginosa* was used. The fluorescent pseudomonas species used in the present study, *P. koreensis*, is an environmental strain not capable of growth at 37ºC (Kwon *et al.*, 2003). This species is therefore preferable to *P. aeruginosa*, which is an opportunistic human pathogen and therefore not suitable for large-scale use. In the study by Stanghellini and Miller (1997), the biosurfactant-producing *P. aeruginosa* strain was inoculated directly into the cultivation system and a disease control effect was reported, although this was inconsistent. To overcome this problem of inconsistency, a procedure with repeated and dense inoculations of the biosurfactant-producing strains into the cultivation system should be devised. The present study had the advantage of implementation in a greenhouse in the immediate vicinity of a laboratory. However, in practice this is not possible and development of suitable measures to formulate the biosurfactant-producing strains with a high viability and a long shelf-life are key issues for further research.

The present study demonstrates that reduction in plant disease is possible with biosurfactants produced by non-pathogenic strains of fluorescent pseudomonads. The biosurfactant does not have to be added as an extract, but can be supplied through amending the plant cultivation system with biosurfactant-producing bacteria. This reduces the need for labour and downstream processing and increases the sustainability of biological control with biosurfactants in both environmental and economic terms. It can be concluded that biosurfactants are important for biological control in closed hydroponic systems where oomycetes are major plant pathogens. Further research should be directed towards formulation of the biosurfactant-producing strains.

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References

Barros, F.F.C., Ponezi, A.N., Pastore, G.M., 2008. Production of biosurfactant by *Bacillus subtilis* LB5a on a pilot scale using cassava wastewater as substrate. J. Ind. Microbiol. Biotechnol. 35,1071-1078.

Calvo-Bado, L.A., Pettitt, T.R., Parson, N., Petch, G.M., Morgan, A.W., Whipps, J.M., 2003. Spatial and temporal analysis of the microbial community in slow sand filters used for treating horticultural irrigation water. Appl. Environ. Microbiol. 69, 2116-2125.

D´aes, J., De Maeyer, K., Pauwelyn, E., Höfte, M., 2010. Biosurfactants in plant-pseudomonas interactions and their importance to biocontrol. Environ. Microbiol. Reports 2, 359-372.

De Jonghe, K., De Dobbelaere, I., Sarrazyn, R., Höfte, M., 2005. Control of *Phytophthora* in the hydroponic forcing of witloof chicory with the rhamnolipid-based biosurfactant formulation PRO1. Plant Pathol. 54, 219-226.

De Souza, J.T., De Boer, M., De Waard, P., van Beek, T.A., Raaijmakers, J.M., 2003. Biochemical, genetic, and zoosporicidal properties of cyclic lipopeptide surfactants produced by *Pseudomonas fluorescens*. Appl. Environ. Microbiol. 69, 7161-7172.

Francis, D.M., St Clair, D.A.,1997. Population genetics of *Pythium ultimum*. Phytopathol. 87, 454-461.

Haas, D., Defago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Reviews Microbiol. 3, 307-319.

Hultberg, M., Alsberg, T., Khalil, S., Alsanius, B., 2010a. Suppression of disease in tomato infected by *Pythium ultimum* with a biosurfactant produced by *Pseudomonas koreensis.* BioControl 55, 435-444.

Hultberg, M., Bengtsson, T., Liljeroth, E., 2010b. Late blight on potato is suppressed by the biosurfactant-producing strain *Pseudomonas koreensis* 2.74 and its biosurfactant. BioControl 55, 543-550.

Hultberg, M., Bergstrand, K.J., Khalil, S., Alsanius, B., 2008a. Production of biosurfactants and antibiotics by fluorescent pseudomonads isolated from a closed hydroponic system equipped with a slow filter. Antonie van Leeuwenhoek 93, 373-380.

Hultberg, M., Bergstrand, K.J., Khalil, S., Alsanius, B., 2008b. Characterization of biosurfactant-producing strains of fluorescent pseudomonads in a soilless cultivation system. Antonie van Leeuwenhoek 94, 329-334.

Hultberg, M., 1999. Seed bacterization with *Pseudomonas fluorescens* and interactions with *Pythium ultimum* on tomato in soilless systems. Dissertation, Swedish University of Agricultural Sciences. Agria 159.

Judelson, H.S., Blanco, F.A., 2005. The spores of *Phytophthora*: weapons of a plant destroyer. Nat. reveiws 3, 47-58.

King, E.O., Ward, M.K., Raney, D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44, 301-307.

Kwon, S.W., Kim, J.S., Park, I.C., Yoon, S.H., Park, D.H., Lim, C.K., Go, S.J., 2003. *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. Int. J. Syst. Evol. Microbiol. 53, 21-27.

Mulligan, C.N., 2005. Environmental applications for biosurfactants. Environ. Poll. 133, 183-198.

Nielsen, C.J., Ferrin, D.M., Stanghellini, M.E., 2006. Efficacy of biosurfactants in the management of *Phytophthora capsici* on pepper in recirculating hydroponic systems. Can. J. Plant Pathol. 28, 450-460.

Nielsen, T.H., Sørensen, D., Tobiasen, C., Andersen, J.B., Christophersen C., Givskov, M., Sørensen, J., 2002. Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. Appl. Environ. Microbiol. 68, 3416-3423.

Perneel, M., D´hondt, L., De Maeyer, K., Adiobo, A., Rabaey, K., Höfte, M., 2008. Phenazines and biosurfactants interact in the biological control of soil-borne diseases caused by *Pythium* spp. Environ. Microbiol. 10**,** 778-788.

Sobrinho, H.B.S., Rufino, R.D., Luna, J.M., Salgueiro, A.A., Campos-Takaki, G.M., Leite, L.F.C., Sarubbo, L.A., 2008. Utilization of two agroindustrial by-products for the production of a surfactant by *Candida sphaerica* UCP0995. Process Biochem. 43, 912-917.

Stanghellini, M.E., Rasmussen, S.L., 1994. Hydroponics- a solution for zoosporic pathogens. Plant Dis. 78, 1129-1138.

Stanghellini, M.E., Miller, R.M., 1997. Biosurfactants - their identity and potential efficacy in the biological control of zoosporic plant pathogens. Plant Dis. 81, 4-12.

Tran, T.T.H., 2007. Interactions between biosurfactant-producing *Pseudomonas* and *Phytophthora species*. Dissertation. Wageningen University. The Netherlands.

Van Hamme, J.D., Singh, A., Ward, O.P., 2006. Physiological Aspects, Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotech. Advances 24, 604-620.

Van West, P., Appiah, A.A., Gow, N.A.R., 2003. Advances in research on oomycete root pathogens. Physiol. Mol. Plant Pathol. 62, 99-113.

Tables

Table 1 Addition of bacterial cells in a hydroponic-lettuce cultivation system and its effect on lettuce weight, expressed as a percentage ± SD of that in the uninfected control, in the presence of *Pythium ultimum*.

Biocontrol treatment	**Log CFU mL^{-1}**	**Pathogen**	**Seedling FW[a]**	**Root FW**
Control	-	-	100±8.3a[b]	100±11.0a
	-	+[c]	48.1±9.1b	62.0±12.3b
Biosurfactant (50 μg ml^{-1})	-	+	98.5±3.8a	101.5±14.9a
Washed cells	7.5	-	101.3±4.6a	93.9±13.7a
	7.5	+	109.0±4.2a	108.0±9.0a
	5.2	+	93.5±5.9a	81.4±7.9ab
KB culture	7.5	-	57.5±6.2b	38.3±10.9bc
	7.5	+	52.7±7.8b	30.7±13.3c
KB broth	-	-	56.5±7.5b	22.8±16.5c
Minimal oil culture	6.3	-	61.2±7.8b	37.3±8.7c
	6.3	+	58.3±4.1b	37.0±13.6c
Minimal oil broth	-	-	62.3±7.9b	43.4±7.2bc

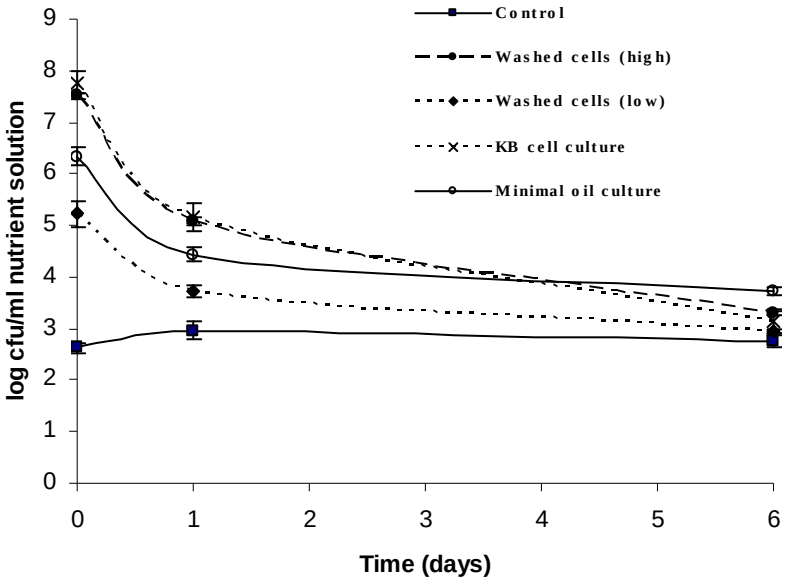
[a] FW, Fresh weight, expressed as a percentage of that in the uninfected control

[b] Values within the same column followed by different letters are significantly different ($P<0.05$, Tukey's test).

[c] A zoospore suspension of *P. ultimum* added every week to a density of 10^3 zoospores mL^{-1}

Legends

Figure 1 Numbers of fluorescent pseudomonads, expressed as log cfu mL^{-1} nutrient solution, over time in the hydroponic systems. Standard deviations are represented by vertical bars for each point.


Control
Washed cells (high)
Washed cells (low)
KB cell culture
Minimal oil culture
log cfu/ml nutrient solution
Time (days)
0
1
2
3
4
5
6
7
8
9

1