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Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis*

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Abstract

The epiphytic bacterium *Rahnella aquatilis*, isolated from fruit and leaves of apples, was tested for antagonistic properties against *Penicillium expansum* and *Botrytis cinerea* on Red Delicious apple fruit. In "in vitro" assays, this bacterium inhibited completely the germination of *P. expansum* and *B. cinerea* spores, but it needed direct contact with the spores to do it. However the putative mechanism seemed be different for the two pathogens. The bacterium did not produce extracellular antibiotic substances and when the acute toxicity test was performed no mortality, toxicity symptoms or organ alterations of the test animals (Wistar rats) were observed.

Assays of biological control of *P. expansum* and *B. cinerea* on apple fruit were carried out at different temperatures. At 15 °C and 90% RH, the incidence of disease caused by *P. expansum* on apples stored for 20 days, was reduced by nearly 100% by *R. aquatilis* (10^6 cells/ml), while in the case of *B. cinerea*, the reduction of decay severity was nearly 64% but there was no reduction in the incidence of disease. At 4 °C and 90% RH the treatment with the bacterium significantly inhibited the development of *B. cinerea* on apples stored for 40 days and the incidence of disease was reduced by nearly 100%, while the incidence of disease caused by *P. expansum* at 4 °C was 60%. The results obtained show that *R. aquatilis* would be an interesting microorganism to be used as a biocontrol agent.

Keywords: *Rahnella aquatilis*; Biocontrol; Postharvest diseases; Blue mould; *Botrytis cinerea*; Gray mould; *Penicillium expansum*

1. Introduction

Some fruits, apples among them, are usually stored after harvest. During cold storage losses of economic importance are produced by several decays due to fungal rot. *Penicillium expansum* and *Botrytis cinerea* are well-known postharvest pathogens. They produce blue and gray rots, respectively (Goepfert, 1980). The use of synthetic chemicals as fungicides is a primary method of control of postharvest fungal decay of apple fruit. However, several fungicides are not used for postharvest treatment or have been removed from the market due to possible toxicological risks. Alternative methods of control are needed because of the negative public perceptions about the use of pesticides, development of resistance to fungicides among fungal pathogens, and high development costs of new chemicals. In recent years, biological control of postharvest diseases of fruits has become an important field for research. A number of yeasts and bacteria have been report to inhibit postharvest decay of fruit effectively (Janisiewicz and Korsten, 2002).

Among bacteria, a number of Gram positive and Gram negative bacteria have been evaluated as Biological Control Agents (BCAs). Several strains of the genus *Bacillus* have received much attention as BCAs. *Bacillus subtilis* isolated from citrus fruit surface was successfully evaluated for control of citrus green and blue moulds caused by *Penicillium digitatum* and *P. italicum* respectively (Obagwu and Korsten, 2003), and *Bacillus licheniformis* was reported as an effective BCA against tomato gray mould caused by *B. cinerea* (Jae Pil Lee et al., in press). Among Gram negative bacteria, *Pseudomonas cepacia* (Janisiewicz and Roitman, 1988), *Pseudomonas syringae* (Janisiewicz and Jeffers, 1997) *Pantoea agglomerans* (Nunes et al., 2001, 2002) and

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Serratia plymuthica (Meziane et al., 2006) were reported as effective BCAs against diseases of different fruits.

Searching for BCAs, in the permanent screening program of our laboratory, a coliform bacterium, identified as *Rahnella aquatilis*, was the bacterium mostly isolated from different sources. The natural habitat of *R. aquatilis* is water, but the organism has also been isolated from plant leaves (Hashidoko et al., 2002), soil (Tallgren et al., 1999), foods (Jensen et al., 2001) and non-environmental samples as blood, surgical wounds, urine, sputum, and bronchial washings. Most clinical cases have occurred in compromised hosts and young children and there have been no reports of *Rahnella*-associated mortality (Tasch, 2005).

This microorganism considered to be a little public health significance, has not yet been searched as a BCA against postharvest diseases, however it was reported as an antagonistic agent of *Xanthomonas campestri* which cause bacterial spot of cucumber and tomato (El-Hendawy et al., 2003, 2005). Then, we evaluated the bacterium *R. aquatilis* as an antagonist against the two major postharvest pathogens of apple, *P. expansum* and *B. cinerea*, because there are not any reports in this matter.

The present study was conducted to determine: (1) the potential of *R. aquatilis* for control of postharvest decays caused by *P. expansum* and *B. cinerea* on apple, (2) the influence of antagonist concentration on biocontrol efficacy and its behavior in wound apples, and (3) the putative mechanism of action.

2. Materials and methods

2.1. Bacterial antagonist and fungal pathogen strains

R. aquatilis (bSL1strain) was isolated in our laboratory from microbial consortiums obtained by picking the surface of apple fruit throughout the growing season. It was identified by using the API 20 E and API 50 CHE systems (bioMérieux, France). Additional test for motility, Voges–Proskauer (VP), Simmons citrate and methyl red were conducted according methods prescribed by Krieg and Gerhardt (1994), and Smibert and Krieg (1994). The acute oral toxicity of the bacterium was determined by the "Centro de Servicio Farmacológico" of the Universidad Nacional de San Luis. Assays were carried out by using Wistar rats. Six male and six female (between 200 g and 250 g) were starved during 4 h before the administration of a *R. aquatilis* suspension (4×10^{11} CFU kg^{-1} live weight) or the same volume of distilled water (control). Rats were observed during 14 days to evaluate mortality or any other symptom. After this time, the rats were killed and macroscopic observation of their organs was carried out.

P. expansum came from CEREMIC (Mycological Reference Center), Rosario, Argentina and *B. cinerea* was a kind gift to us from INTA (National Institute of Agricultural Technology). Both moulds were maintained on potato dextrose agar medium (PDA medium).

2.2. Preparation of bacteria and pathogen spore suspensions

R. aquatilis strain was grown on YGA (yeast extract 5 g/l, glucose 10 g/l, agar–agar 20 g/l) in Roux's bottles, suspended in sterile distilled water and concentrations adjusted according to standard curve with a Metrolab1500 UV–VIS Spectrophotometer by measuring the optical density at 635 nm.

For conidial production, *B. cinerea* were grown on PDA (Potato Dextrose Agar) at 20–25 °C. When the mycelium appeared, cultures were kept at 15 °C for inducing sporulation. After a week, spores were harvested and suspended in 10 ml of sterile distilled water containing 0.05% (v/v) Tween 80. The concentration of spore suspension was determined with a Neubauer chamber and adjusted with sterile distilled water to 1×10^6 spores/ml. Conidia of *P. expansum* were obtained from 7 day old PDA cultures grown at 20–25 °C. The concentration was also adjusted to 1×10^6 spores/ml.

2.3. Inhibition of germination of P. expansum and B. cinerea spores

In order to evaluate antagonistic activity of *R. aquatilis* against *P. expansum* and *B. cinerea*, in vitro assays on special slides for micro culture were performed. In these assays was also determined the minimum *Rahnella* concentration necessary for inhibiting the conidial germination of the phytopathogens. The composition of GP medium used in these assays was as follows: Glucose 5 g/l, $(NH_4)_2SO_4$ 0.5 g/l, Peptone 0.05 g/l, PO_4H_2K 0.2 g/l, $Cl_2Mg{\cdot}6H_2O$ 0.2 g/l, $FeSO_4$ 0.005 g/l. pH 4.5.

Aliquots of 20 μl of *R. aquatilis* suspensions in GP medium (10^2, 10^3, 10^4, 10^5, and 10^6 cells/ml) were put on the slide, immediately 20 μl of conidial suspension (10^6 spores/ml) were added. Controls were made with 20 μl of GP medium without antagonist and 20 μl of conidial suspension (10^6 spores/ml). Slides were incubated in a wet chamber at 28 °C during 48 h. After this time, these preparations were observed with a light microscope (Olympia) at a magnification of ×500. Conidial germination was assessed counting at least 100 conidia per slide. Preparations were also observed by SEM using a scanning electron microscope LEO 1450 VP in the Laboratory of Electronic Microscopy and Micro analysis. (LABMEM-UNSL).

2.4. Source of fruit material

Red Delicious apples used in the biological control assays were obtained from a commercial orchard. Fruits selected were free of wounds and rots and as much as possible homogeneous in maturity and size. The fruit were used after a short time of storage at 1 °C (no longer than 3 months).

2.5. Assays of control of P. expansum and B. cinerea on apple fruit

In order to evaluate the antagonistic activity against *P. expansum* and *B. cinerea* on apple fruit, surface apples were disinfected by immersion for 1 min in a dilute solution of sodium hypochlorite (1% active chlorine), washed two times by immersion in distilled water, and left in a dry place to remove excess water off the surface. Then, fruit were wounded ($3\times3\times3$ mm^3) in two places (midway between the calyx and the stem end) with a punch and 20 μl of *R. aquatilis* suspension (10^6 cells/ml) in sterile distilled water was put in each wound.

After 2 h, the treated wounds were inoculated with 20 μl of *P. expansum* or *Botrytis cinera* spores (1×10^6 spores/ml). The lesion diameters and infected wounds were determined 20 days after storage at 15 °C and 90 ± 5% relative humidity and 40 days after storage at 4 °C and 90 ± 5% RH.

Ten apples constituted a single replicate and each treatment was replicated four times.

2.6. Population dynamics

Population dynamics of the bacterium was evaluated on Red Delicious apples. Fruits were wounded in the equatorial zone (five wounds per fruits 3 mm diameter × 3 mm depth) and 20 μl of a bacterial suspension (1×10^7 cells/ml) was placed into each wound. Fruits were incubated at 15 °C and 90 ± 5% RH, or at 4 °C and 90 ± 5% RH. Bacterial populations were monitored at 0 (prior to storage), 3, 6, 9, 12, 15, 20, 25, 30, and 40 days on fruits stored at 4 °C and 0, 12, 24, 36, 48, and 72 h on fruits kept at 15 °C. The wounded tissue was removed with a cork borer (0.6 mm internal diameter), suspended in 50 ml of sterile distilled water and shaken on a rotatory shaker for 20 min ant 240 rpm. Serial dilutions of the washings were made and plated on Yeast Glucose Medium (YGM, Glucose 10 g/l, yeast extract 5 g/l, agar–agar 20 g/l). The colonies were counted after 48 h of incubation at 30 °C. There were three replicates of three fruits per treatment and the experiment was repeated twice.

2.7. Interaction assays between antagonist and pathogen

In order to assess probable mechanisms of action of *R. aquatilis* tests were performed using the "in vitro" method developed by Janisiewicz et al. (2000). Tissue plates and cylinder inserts provided with a hydrophilic polyethersulfone membrane (0.2 μm pore size) attached at bottom were used. Prior to making the assay, the performance of this membrane was evaluated by using a crystal violet solution to determine the movement of the liquid through it. The crystal violet solution was put inside the cylinder insert while water was in the well outside the cylinder and vice versa. The movement of the dye though the membrane was observed within 3 min of placing the cylinder into the well, irrespective of the placement of the dye solution, and the performance of the membrane was considered adequate.

The wells of the tissue plates were filled with GP medium with or without antagonists (10^6 cells/ml). The cylinders were placed in the wells and filled with a suspension of *P. expansum* or *B. cinerea* spores (10^6 spores/ml). Plate was incubated at 28 °C for 24 h, then cylinders were removed, the final concentration of antagonist was determined turbidimetrically and portions of suspension spores were mounted on slides. These preparations were observed with a light microscope (Olympia) at a magnification of × 1000. Conidial germination was assessed counting at least 100 conidia per slide. The experiment was also carried out without cylinders, in which the conidia solution was added directly to the well to study the direct interaction between pathogen and antagonist. Evaluation of conidial germination in the presence or the absence of antagonist was done as it was described above.

2.8. Production of antifungal compounds

Production of antifungal compounds was evaluated by means of two ways:

1– A cell-free filtrate from *R. aquatilis* was used for evaluating antifungal compounds production by this bacterium. The microorganism was cultivated in 100 ml of YGM broth for 48 h at 28 °C. After the incubation period, cells were centrifuged at 2500 *g* for 15 min (Sorvall centrifuge), the supernatant fluid decanted and a 2 ml aliquot filtered through a sterile 0.2 μm Acrodisc filter (Gelman UK). The cell-free filtrate was then screened for antagonism against *B. cinera* and *P. expansum*, in Petri dishes on PDA. Petri dishes were seeded with 200 μl of a phytopathogen suspension containing 10^6 spores/ml. After the dishes were seeded, they were stored at 4 °C over-night and then, holes (diameter 10 mm) were made in the medium of each Petri dish with a sterile cork borer. Holes were filled to capacity (200 μl) with the cell-free filtrate, and then the plates were incubated at 28 °C for 48 h and evaluated for inhibition zones.

2– A streak assay was done on PDA, an agar disk from *B. cinera* or *P. expansum* was placed at the centre of the dishes. *R. aquatilis* was streaked at two sides at about 2 cm from the centre. The plates were incubated at 28 for 48 h and evaluated for inhibition zones.

Table 1
Percent germination of *Penicillium expansum* and *Botrytis cinerea* spores after 24 h incubation in GP medium in presence of *Rahnella aquatilis*

Pathogen (10^6 spores/ml)	*R. aquatilis* (cells/ml)	Temperature (°C)	% Germination and length of germinal tube test[a]	% Germination and length of germinal tube control[a]
P. expansum	10^3	28	50	85
			7	10
P. expansum	10^4	28	20	85
			3	10
P. expansum	10^5	28	0	85
			0	10
P. expansum	10^6	28	0	85
			0	10
B. cinerea	10^3	28	60	100
			8	15
B. cinerea	10^4	28	15	100
			3	15
B. cinerea	10^5	28	0	100
			0	15
B. cinerea	10^6	28	0	100
			0	15
P. expansum	10^6	15	70	100
			2	5
B. cinerea	10^6	15	90	100
			5	10
P. expansum	10^6	4	95	100
			2	5
B. cinerea	10^6	4	90	100
			2	6

[a] Length of germinal tube expressed as a multiple of spore length.

2.9. Data analysis

Results of different treatments were expressed as: percentage of disease incidence and percentage of reduction in decay severity which was calculated on the basis of lesion diameter:

$$\%\ \text{reduction in decay severity} = (\phi\ \text{control} - \phi\ \text{treatment}/\phi\ \text{control}) \times 100$$

Lesion diameters and percentage of infected wound were analyzed by analysis of variance. Statistical analyses were done with MicroCal Origin 6.1 (MicroCal Software, Inc., 1999).

3. Results

The API 20 E test identified the isolated bacterium to be either *R. aquatilis* (78.5% probability) or *Pantoea* spp (42% probability). The API 50 CHE test identified it as *R. aquatilis* (95.2% probability) or *Pantoea* spp (4.7% probability). The results of additional tests confirmed the identity of the organism as *R. aquatilis*. The bacterium, which did not produce yellow pigment, was able to growth at 4 °C and 15 °C but optimal growth temperature was reached at 28 °C. With respect to the mobility of the bacterium, this characteristic was partially lost at 4 °C and the bacterium was immobile at 37 °C. When the acute toxicity test was performed, no mortality, toxicity symptoms or organ alterations of the test animals were observed.

3.1. Control of *P. expansum* and *B. cinerea* by *R. aquatilis*

3.1.1. Inhibition of germination of spores and mechanisms of inhibition

The antagonistic bacterium *R. aquatilis* inhibited completely the germination of *B. cinerea* and *P. expansum* spores at concentration of 10^6 cells/ ml at 28 °C (Table 1).

Inhibition of germination assays were also performed at 4 °C and 15 °C. These assays were made in the same way as before but at only one bacterium concentration (10^6 cells/ ml). After 24 h at 15 °C, 70% of spores of *P. expansum* and 90% of spores of *B. cinerea* germinated. In the case of *P. expansum* germ tube elongation reached 2 times the spore length in the test, and 5 times the spore length in the control, and in the case of *B. cinerea* germ tube elongation reached 5 times the spore length in the test, and 10 times in the control. At 4 °C, 95% of spores of *P. expansum* and 90% of spores of *B. cinerea* germinated but in the cases of *B. cinerea* the germ tube elongation reached only 2 times the spore length when the antagonist was present. At all temperatures assayed, agglutination of the bacterium around nongerminated and germinated spores of *B. cinerea* was observed, but this phenomenon was not so evident in the case of *P.*

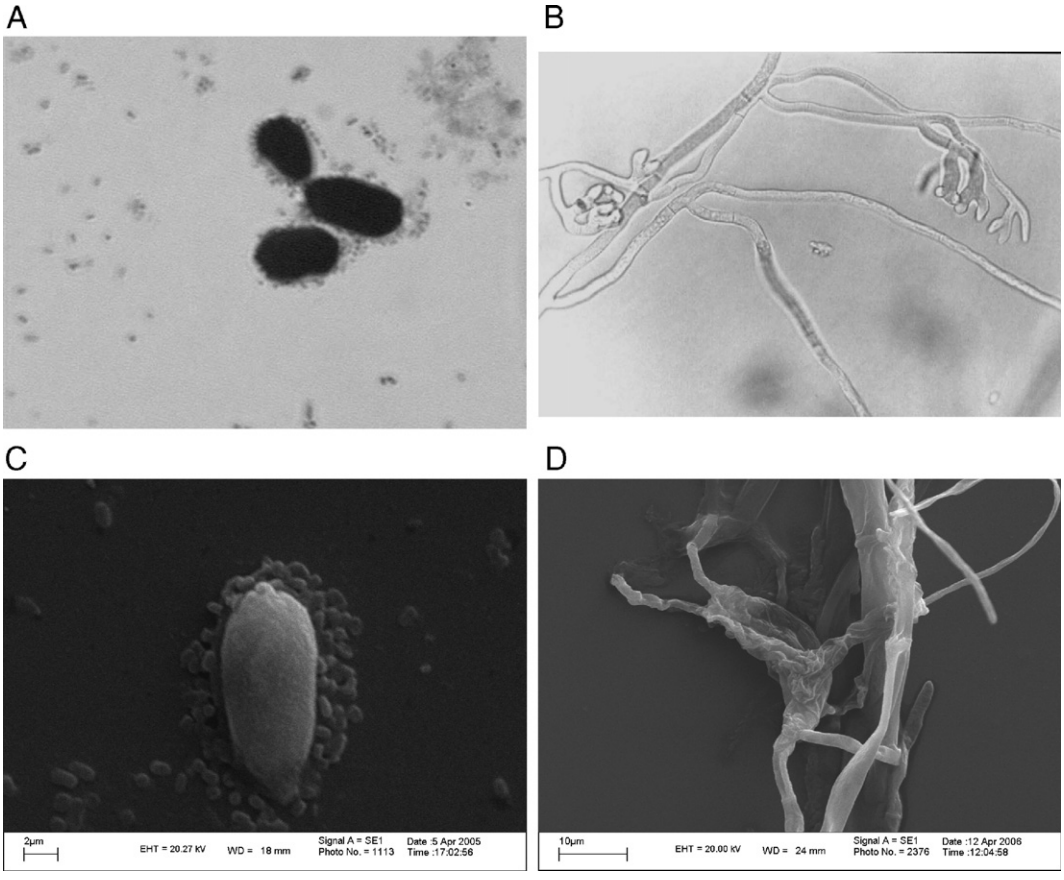

Fig. 1. Interaction between *Rahnella aquatilis* and *Botrytis cinerea*: A) and C) Light microscope micrography and SEM micrography of test with 20 µl of *Rahnella aquatilis* suspensions in GP medium (10^6 cells/ml) and 20 µl of conidial suspension (10^6 spores/ml) after 48 h incubation at 28 °C. B) and D) Light microscope micrography and SEM micrography of control with 20 µl of GP medium without antagonist and 20 µl of conidial suspension (10^6 spores/ml) after 48 h incubation at 28 °C.

expansum. Images of interaction between spores of both moulds and the bacterium at 28 °C are shown in Figs. 1 and 2.

Because the mechanism of inhibition of spore germination seemed different for both moulds, two experiments were carried out. Tests performed using a culture plate with cylinders with a hydrophilic membrane for assessing direct cell to cell interaction showed that 100% of the spores of either *B. cinerea* or *P. expansum* germinated at 24 h when the antagonist and the pathogen grew separated by the membrane. In this case, the antagonist reached a population of 10^9 cells/ml. On the other hand, when the suspension of spores was added directly into the wells with the antagonist, germination of spores was 100% inhibited by the bacterium.

Tests for evaluating the production of antifungal compounds gave negative results by both of the two ways assayed. In the assay in which the cell-free filtrate was used, no inhibition zone was observed when it was tested against *B. cinerea* or *P. expansum*. In the streak assay no inhibition zone was observed but *R. aquatilis* grew over the mycelium of the pathogen.

3.1.2. Assays of biological control of P. expansum and B. cinerea on apple fruit

Results of biological control at different storage conditions were shown in Table 2. At 28 °C and a concentration of 10^6 cells/ml, *R. aquatilis* caused the reduction of decay severity of 50% and 60% for *B. cinerea* (10^6 spores/ml) and *P. expansum* (10^6 spores/ml) respectively, while the incidence of disease was reduced by 50% in both cases. Biocontrol activity was not increased significantly by raising the concentration of antagonist to 10^7 cells/ml.

At 15 °C and 90% RH, *R. aquatilis* (10^6 cells/ml) significantly inhibited the development of *P. expansum* on apples stored for 20 days. Thus, the incidence of disease was reduced by nearly 100% In the case of *B. cinerea*, the reduction of decay severity was nearly 64% but there was no reduction in the incidence of disease (Table 2).

Assays of biological control of *P. expansum* and *B. cinerea* using *R. aquatilis* were also performed at 4 °C and 90% RH. Under these cold storage conditions, the treatment significantly inhibited the development of *B. cinerea* on apples stored for 40 days and the incidence of disease was reduced by nearly 100%. The incidence of disease caused by *P. expansum* at 4 °C was 60% and the reduction of decay severity was also 60% (Table 2). After 40 days at 4 °C, apples were maintained at room temperature (nearly 20 °C) but despite the fact that the bacterium was present in the wounds, it could not maintain control of the rots.

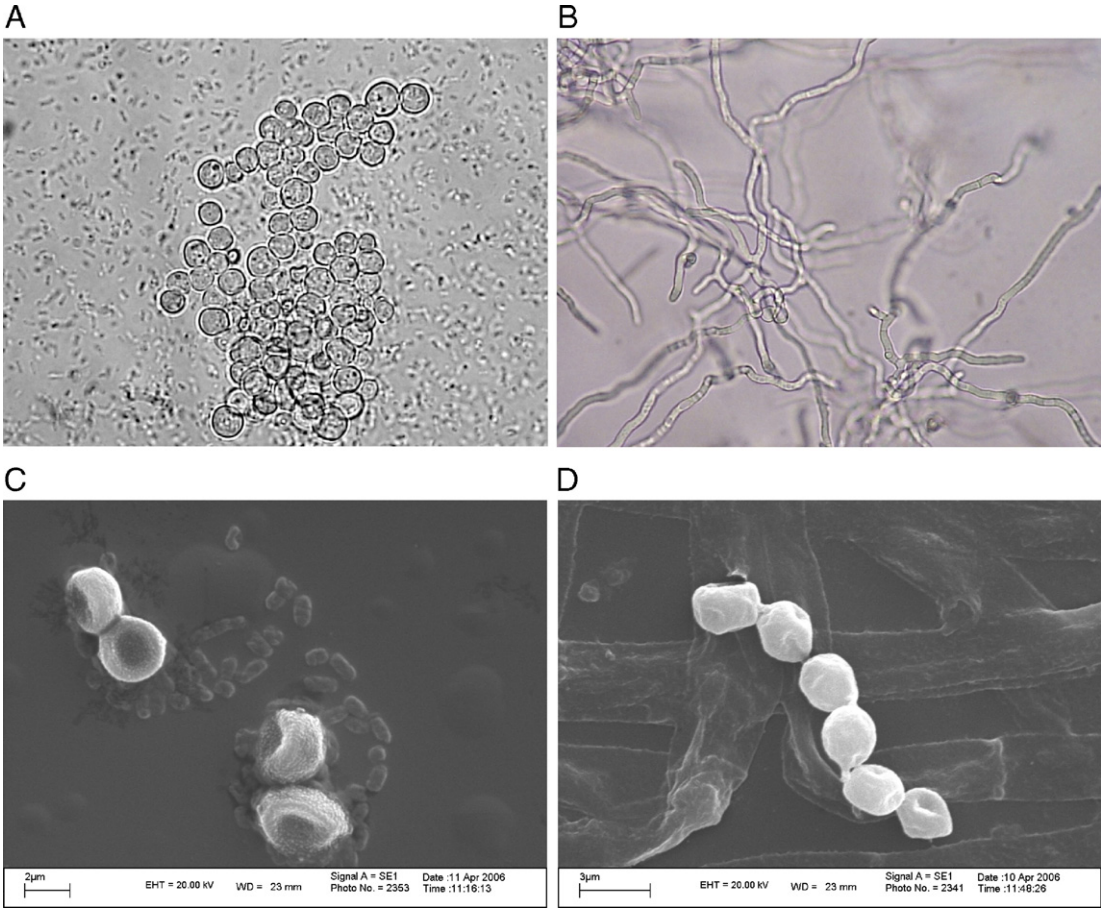

Fig. 2. Interaction between *Rahnella aquatilis* and *Penicillium expansum*: A) and C) light microscope micrography and SEM micrography of test with 20 µl of *Rahnella aquatilis* suspensions in GP medium (10^6 cells/ml) and 20 µl of conidial suspension (10^6 spores/ml) after 48 h incubation at 28 °C. B) and D) Light microscope micrography and SEM micrography of control with 20 µl of GP medium without antagonist and 20 µl of conidial suspension (10^6 spores/ml) after 48 h incubation at 28 °C.

Table 2
Disease incidence and reduction of severity in apple fruit treated with *R. aquatilis* and inoculated with *P. expansum* or *B. cinerea*

Pathogen[a]	Storage conditions[b]		Incidence %[c]		Reduction % (mean lesion diameter of control, mm)[d]
	Temperature °C	Relative humidity	Test	Control	
P. expansum	28	95	50	100	60 (40)
P. expansum	15	95	3	100	89 (33)
P. expansum	4	95	60	90	57 (32)
B. cinerea	28	95	50	100	50 (45)
B. cinerea	15	95	95	100	64 (42)
B. cinerea	4	95	5	95	93 (45)

R. aquatilis was applied by inoculation of a suspension of 10^6 cells/ml in fruit wounds.

[a] Pathogen suspensions of 10^6 spores/ml were inoculated in fruit wounds after *Rahnella*.

[b] Storage conditions were at 28 °C during 7 days, at 15 °C during 20 days and 4 °C during 40 days.

[c] Values are the mean of 4 repetition of 10 fruit per repetition. Test results significantly different to control ($P=0.05$).

[d] Reduction of severity was calculated on the basis of lesion diameters. Values between brackets correspond to lesion diameters in controls. Standard deviations <15%.

3.2. Population dynamics

In fruit incubated at 15 °C (Fig. 3A) the population of *R. aquatilis* reached a maximum after 36 h (2.5×10^7 CFU/ml), and then decreased. A lag period occurred before this time. When the same assay was carried out at 20 °C, the same maximum value was reached at 24 h and no lag period was detected. At 4 °C, the antagonist population increased gradually and reached a maximum value of 4×10^7 CFU/ml after 25 days, then it was practically stable declining slightly after 40 days (Fig. 3B).

4. Discussion

A Gram negative, psychrotrophic, coliform bacterium, identified by its phenotypic and metabolic properties as *R. aquatilis,* has been studied here as a possible control agent against phytopathogens which produce postharvest spoilage in apple fruit. The bacterium was isolated from the surface of apple fruit and showed a high efficacy in the reduction of rots produced by *B. cinerea* and *P. expansum* in apple fruit under cold storage. When the population dynamic was studied, the population of *R. aquatilis* recovered from wounds was higher than the reported for other enterobacteria (Nunes et al., 2001). In studies of population dynamics at 15 °C the bacterial population recovered after 24 h of incubation was near 100% of the population inoculated. While, in the case of cold storage (4 °C), the population reached a concentration higher than 1×10^6 CFU/ml at the third day of incubation. An important attribute of a successful biocontrol agent is the ability to be efficient at low concentrations (Wisniewski and Wilson, 1992). This attribute has relation with the ability of the antagonist to survive and growth in the wounds. *R. aquatilis* can grow at 4 °C to 10 °C probably because it has evolved to live in the soil. This fact would be an excellent indicator of adaptation of *R. aquatilis* to cold storage temperatures, a necessary feature for a postharvest biocontrol agent (Wisniewski and Wilson, 1992).

With respect to the mechanism of action involved in the biocontrol process of *B. cinerea* and *P. expansum*, we concluded that the bacterium needed cell to cell contact to inhibit the germination of pathogen spores, but the mechanisms seemed different for each pathogens. In the case of *B. cinerea* it was possible to observe that bacteria surrounded germinated and nongerminated conidia, but it was not possible to observe the same phenomenon in the case of *P. expansum.* A possible explanation of this phenomenon could be a stimulation of bacteria by endogenous nutrients leaked from the conidia of *B. cinerea* (Guinebretiere et al., 2000). Consumption of these nutrients would inhibit conidia germination. The attachment of antagonists to pathogen hyphae has been suggested as an important factor in the competition for nutrients between the antagonist *Enterobacter cloacae* and *Rhizopus stolonifer* on peach (Janisiewicz and

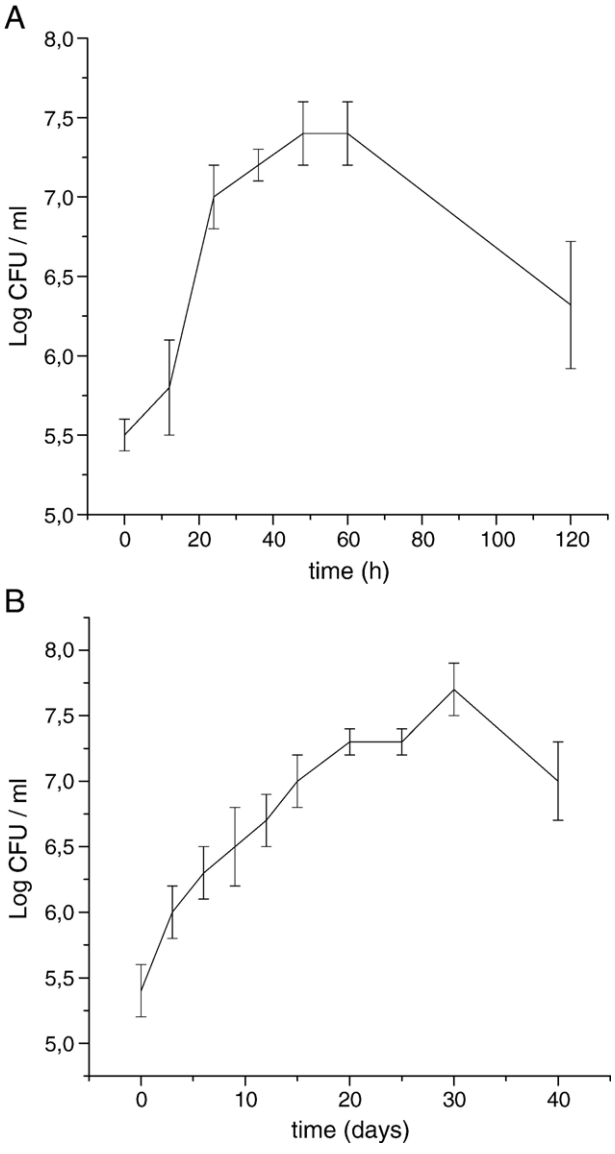

Fig. 3. Population dynamics of *R. aquatilis* on surface of wounded apples incubated at 15 °C for 120 h (A) and at 4 °C for 40 days (B).

Korsten, 2002). The tendency of adhesion to hypha and spores by *Rahnella* could be also explained by the presence of a major outer membrane protein of the bacterium which seems to be involved in its adhesion to plant roots (Achouak et al., 1998). Another important fact to be taken into account is the ability of *R. aquatilis* for levan production because as it has been hypothesized, the presence of extracellular substances helps in cellular aggregation, and inhibition of germination and lengthening of the germinative hypha.(Mari et al., 1996). On the other hand, Tallgren et al. (1999) reported that *R. aquatilis* isolated from soil showed a tendency for a higher production of levan at temperatures below 13 °C. This fact perhaps can explain the major efficiency of *R. aquatilis* in the control of *B. cinerea* at 4 °C, taking into account that in "in vitro" assays at the mentioned temperature as well as 15 °C the bacterium showed a tendency to lay around germinated and nongerminated spores of *B. cinerea* but it was not possible to observe the same phenomenon with *P. expansum* spores.

As was mentioned, the inhibition of germination of *P. expansum* spores was observed when there was a direct interaction between antagonist and pathogen, but not when a membrane filter, which permits medium nutrients and metabolite interchange, separated the bacterium and the fungus. The exact mechanism by which this antagonist inhibits spore germination is not yet clear.

Despite to the fact that it was not possible to show the production of antifungal metabolites in the culture medium used, *R. aquatilis* is able to produce siderophores if it is cultured in the adequate medium. It was reported by Hashidoko et al. (2002), and El-Hendawy et al. (2003) that these compounds had significant antimicrobial activities in vitro and they may help to regulate the microflora of plants.

We concluded that *R. aquatilis* is a interesting microorganism to be used as a biocontrol agent and at present we are evaluating mixtures of the bacterium with yeasts with the aim to improve the control of decay in apples. It was mentioned we did not detect any antifungal compound at cultural conditions established in the present study, but we believe that the production of siderophores by this bacterium would be an important tool for controlling postharvest pathogens and we are also working in this trend.

Acknowledgments

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