



Exploring the potential of lactic acid bacteria and carrot isolates as postharvest disease control agents in carrots

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ABSTRACT

Carrot (*Daucus carota* L.) production is challenged by various phytopathogens, including *Berkeleyomyces basicola*, responsible for black root rot. Current control measures are limited, prompting interest in sustainable bio-preservation approaches leveraging beneficial microorganisms. This study evaluated the biopreservation potential of several lactic acid bacteria (LAB) strains from the Agroscope Culture Collection and bacteria newly isolated from carrots against *B. basicola* and other carrot phytopathogens, namely *Alternaria radicina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Results highlighted the superior performance of strains isolated from carrots, including *Leuconostoc mesenteroides*, *Serratia plymuthica* and *Raoultella terrigena* in inhibiting *B. basicola* mycelial growth and spore germination compared to the previously isolated LAB strains from the Agroscope Culture Collection. Interestingly, non-LAB strains, particularly *Serratia plymuthica* Sp1, exhibited broad-spectrum antifungal activity and sustained protection of carrots while used as postharvest treatment. These findings emphasize the value of exploring the microbiota of the host plant to be protected to find new agents suitable for biocontrol solutions. While LAB strains showed promising results in *in vitro* assays, *S. plymuthica* Sp1 emerged as a highly effective candidate for postharvest disease management. Future research should focus on optimizing the application and formulation of *S. plymuthica* for large-scale use, ensuring its compatibility with diverse carrot varieties and storage environments. This work contributes to the development of environmentally friendly strategies to reduce postharvest losses and enhance sustainability in food production.

1. Introduction

Carrot (*Daucus carota* L.) is among the most popular vegetables worldwide [1]. This vegetable is widely grown due to favorable growth conditions in fall in tropical and subtropical regions, and from early spring to late fall in temperate regions [2]. Carrots are also prized for their excellent nutritional value, being a rich source of dietary fibers, magnesium, manganese, molybdenum, and provitamin A [3]. However, carrot producers face challenges from numerous phytopathogens, including *Sclerotinia sclerotiorum* [4], *Alternaria radicina* [5], *Rhizoctonia solani* [6], *Erysiphe heraclei* [7] and *Berkeleyomyces basicola* (syn. *Thielaviopsis basicola*, *Chalara elegans*). This latter root pathogen, an ascomycete responsible for black root rot, affects over 230 species from a

wide range of host crops and ornamental plants [8] and causes significant quality issues during storage and distribution [9]. In Switzerland, *B. basicola* poses a significant problem for producers due to its rapid development cycle and persistent chlamydospores, which can survive in the soil for many years and are prevalent in most soils within Swiss carrot production areas [10,11]. While no known treatment currently exists to control *B. basicola*-induced postharvest damage [12], studies suggest that cultivation practices such as using a crop rotation of at least four years, harvesting carrots when the soil temperature is below 10 °C and avoiding the reuse of washing water, can contribute to inhibiting the spread and growth of the phytopathogen [13,14].

The development of new biopreservation approaches aligns with the current demand for sustainable agriculture and environmental

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preservation. Biopreservation, which utilizes beneficial microbes and their metabolites to control undesirable microorganisms with the ultimate aim of preserving food and increasing shelf life, is a promising measure for the management of postharvest decay, improving food quality and safety in an environmentally friendly and economically attractive manner. Various microorganisms are being explored as sources of biopreservation. Biopreservation can also rely on biological extracts rather than living organisms. Along these lines, microbial volatile organic compounds have been reported as a promising avenue to reduce postharvest diseases [15,16], but their potential to control postharvest disease development in carrots remains to be investigated.

Compared with the intensively studied bacterial “stars” of biocontrol, which include many *Pseudomonas*, *Bacillus* and *Streptomyces* species, lactic acid bacteria (LAB) have received little attention in the biopreservation field. Nevertheless, some studies investigating the biocontrol potential of *Lactobacillus* and *Pediococcus* strains have reported that these strains can inhibit the growth of pathogenic fungi and bacteria through various mechanisms, including the production of antimicrobial compounds like organic acids, hydrogen peroxide, and bacteriocins [17]. By producing such compounds, LAB can inhibit the development of postharvest diseases and thereby contribute to extending the shelf-life of fresh fruits and vegetables [18]. While no study yet has investigated the application of LAB as biocontrol agents specifically for carrots, their broad-spectrum antimicrobial activity and potential benefits for plant health make them promising candidates for further exploration of their biopreservation potential in carrot cultivation. Moreover, numerous reports highlight the ‘generally recognized as safe’ (GRAS) status of LAB, allowing their safe use as protective treatments on vegetables, even at the postharvest stage [19].

Furthermore, Droby and M. Wisniewski (2018) [20], highlighted the crucial role of the fruit and vegetable microbiome in plant health and the prevention of postharvest diseases. Plants may have the ability to selectively recruit microorganisms based on their needs, including those with antagonistic activity against pathogens or those that promote plant growth. Given the high abundance and diversity of microorganisms identified in carrot taproots [21], this system may harbor strains with potential biocontrol activity against fungal pathogens. The aim of this study was to evaluate the biopreservation potential of lactic acid bacteria and of bacterial strains newly isolated from carrot against postharvest black root rot development on carrots. For this purpose, we analyzed the antagonistic activity of a large number of LAB strains originating from Swiss substrates (dairy product, meat, plant material) and available in the Agroscope Culture Collection (Agroscope, Bern, Switzerland) on the mycelial growth and spore germination of *B. basicola* using *in vitro* dual assays. In addition to these available strains, we newly isolated bacteria from the surface of carrots on the assumption that bacteria isolated from this specific environment would already be adapted to survive on the surface of carrots and that this particular environment might be potentially enriched in antagonists against carrot disease-causing agents.

Among these bacteria, eleven candidates showing the best results in *in vitro* tests against *B. basicola* were also tested for their protective potential against other fungal pathogens of carrots, such as *A. radicina*, *R. solani* and *S. sclerotiorum*. From these, the six best strains, of which half were indeed carrot isolates, were inoculated on carrots to evaluate their potential for biopreservation in conditions mimicking those prevailing for carrot postharvest storage.

2. Materials and methods

2.1. Isolation and selection of bacteria from the surface of carrots

Swiss carrots were bought in a supermarket (Coop), the healthy carrots were peeled, and 1g of peel was placed in a homogenizer bag (3MTM) with 9 ml physiological water (0,9 % NaCl). A stomacher (Lab-Blender 400) was used to homogenize the peel for 2 min at 200 rpm. The

homogenized solution was taken out of the bag and serially diluted five times. For each dilution, 100 µl of the solution was spread on De Man, Rogosa and Sharpe (MRS) agar [22] or Luria Bertani medium (LB), which was prepared by dissolving 20 g/L of LB Broth (Lennox) in distilled water with 15 g/L of agar (Agar-agar, Kobe I, Roth) and sterilized by autoclaving at 120 °C for 20 min. The plates were then incubated for two days at 30 °C under anaerobic (MRS) and aerobic (LB) conditions respectively. The different colonies were selected based on their morphology and purified on the corresponding medium. The resulting 27 isolates (Table S2) were tested for their activity on *B. basicola* spore germination in a 48-well plate setup as described below, and the 19 isolates showing activity were identified by Mabritec AG using the MALDI-TOF technology. After identification, 10 strains which belonged to species associated with clinical or plant infections were removed, yielding nine isolates.

2.2. Microbial strains and culture media

Fifty-nine strains isolated from Swiss plant material and dairy products were selected from the Agroscope Culture Collection (Table S1). These bacteria were routinely grown on MRS agar under anaerobic conditions at 30 °C. The nine isolates from the carrot surface were cultivated on SC agar (peptone ex casein 95 g/L, dextrose 1 g/L, yeast extract 2.5 g/L, agar 15 g/L in distilled water) under aerobic conditions at 30 °C. *Berkeleyomyces basicola* ETH D127 was provided by Prof. Dr. Monika Maurhofer (ETH Zurich). *Alternaria radicina* (DSM No. 62029) and *Sclerotinia sclerotiorum* (DSM No. 1946) were obtained from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. *Rhizoctonia solani* was taken from an internal strain collection available in the Weisskopf laboratory (University of Fribourg). All were cultivated on Potato Dextrose Agar (PDA, Sigma-Aldrich) and carrot-based agar media. PDA media was prepared by dissolving 39 g/L of PDA powder (Sigma-Aldrich) in distilled water and sterilized by autoclaving at 120 °C for 20 min. Carrot-based agar medium was prepared by mixing 10 % of mashed carrots with distilled water and agar (18 g/L), and sterilized by autoclaving for 20 min at 120 °C. The spores of the different phytopathogens were harvested by flooding the Petri dish with sterile distilled water and scratching the mycelium and the spores of the fungi. The tube containing the suspension was shaken well and the suspension was filtered through glass wool [23]. The flow-through containing the spores was centrifuged at 800 rpm for 10 min. The supernatant was removed, and the pellet resuspended in the distilled water. The spore concentration was measured using a Thoma cell counting chamber. As *R. solani* does not sporulate, no experiment was performed on spore germination for that pathogen.

2.3. Effects of the isolates on the mycelial growth of *B. basicola* and other fungal pathogens

The antagonistic activity of the 68 selected bacteria was first tested against the mycelial growth of *Berkeleyomyces basicola* using dual culture assays as described in De Vrieze et al. (2018) [24] for *Phytophthora infestans*. The 59 bacteria from the Agroscope Culture Collection were grown overnight on MRS agar plates, while the 9 carrot isolates were grown overnight on SC agar (peptone ex casein 95 g/L, dextrose 1 g/L, yeast extract 2.5 g/L, agar 15 g/L in distilled water) under aerobic conditions at 30 °C. Cells were thereafter collected by adding 5 ml of physiological solution (0,9 % NaCl) to the Petri dish (Greiner Bio-One) and scratching the colonies. The optical density (OD) of the bacterial suspensions was measured at 600 nm and was adjusted to 1 with physiological solution. Three drops of 10 µl OD₆₀₀ = 1 were then inoculated equidistantly at the border of carrot-based agar Petri dishes. Drops were air-dried and the Petri dishes were placed in a 30 °C incubator overnight, under aerobic conditions. Control plates without bacteria were inoculated with three drops of 10 µl of physiological solution. The following day, *Berkeleyomyces basicola* was inoculated by placing a

5 mm diameter plug of a one-to two-week-old culture of the pathogen growing on carrot-based agar at the center of the Petri dish. Dual assay plates were sealed with parafilm and incubated in the dark at room temperature and pictures were taken after two weeks, once the pathogens had reached the border of the Petri dishes in the control plates. The mycelial growth was measured using ImageJ by measuring the surface of the mycelium. To enable comparison between different batches of experiments, the mycelial growth quantified on control plates was set to 100 % and the mycelial area of the treated plates was expressed as percentage of the control (relative mycelial growth). Treatment efficiency was then calculated as described above ($100 - \text{relative mycelial growth}$). Statistical analysis was performed using a two-tailed Student's t-test; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, with $n = 3-5$. After the first test on *B. basicola*, a similar dual culture assay was carried out against other pathogens, namely *Alternaria radicina*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, using the same experimental setup as described above.

2.4. Effects of the isolates on the spore germination of *B. basicola*, *A. radicina* and *S. sclerotiorum*

A first test was carried out in Petri dishes. Spores were prepared as described above, and 300 μl of a spore solution of 1×10^4 spores/ml was spread on carrot-based agar and air-dried for 20 min. Thereafter, the bacteria were inoculated as described above. Five plates per treatment were prepared and sterile physiological water was used for the control plates. Plates were sealed with Parafilm and incubated in the dark at room temperature for one to two weeks depending on the tested pathogen. Pictures were taken at several time points according to the pathogen spore germination on the control plates. The germination of the spores was observed and a score was given to the activity of the strains: +++ indicating almost full inhibition of the pathogen's growth except for small patches; ++ indicating a clear inhibition zone around the bacterial spots; + indicating patches of inhibition around the bacterial spots; - indicating no inhibition of the pathogen.

A second test was carried out in liquid culture using 48-well plates (CELLSTAR®, Greiner Bio-One), following the protocol described by Louvriot et al. (2024) [25] with slight modifications. The 48-well plates were filled with 200 μl of sterile clarified carrot juice (20 % of mashed carrots with distilled water, filtered through a 0.2 μm sterile syringe filter (Filtropur S plus, Sarstedt)), 200 μl Tris-HCl (10 nM, pH = 7), 100 μl of spore suspension of *B. basicola* (1×10^4 spores/ml) and 100/10/1 μl of bacterial suspension ($\text{OD}_{600} = 1$). The volume in the wells was adjusted to 600 μl with sterile distilled water when needed. Each bacterial concentration and strain was tested in three randomly located wells. The plates were sealed and incubated at 20 °C in the dark on an agitator set at 100 rpm. Sterile physiological water was used as the control instead of the bacterial suspension. Spore germination and hyphal growth were observed visually for twelve days. The same protocol was used to test the effect of the bacteria on *A. radicina* and *S. sclerotiorum*. These experiments were not applicable to *R. solani* as it does not produce spores.

2.5. Assessment of the contribution of volatile organic compounds (VOCs) to the overall activity of the strains

This experiment was performed similarly as described in chapter 2.4 but using split Petri dishes (Greiner Bio-One). This means that three drops of 10 μl of $\text{OD}_{600} = 1$ were inoculated equidistantly at the border of one compartment of the split Petri dish and the day after, the pathogen (*Berkeleyomyces basicola*, *Alternaria radicina*, *Sclerotinia sclerotiorum* or *Rhizoctonia solani*) was inoculated by placing a 5 mm diameter plug of a one-to two-week-old culture of the pathogen at the center of the remaining compartment of the split plates. Both compartments contained carrot-based agar. Control plates without bacteria were inoculated with three drops of 10 μl of physiological solution. The split plates

were incubated in the dark at room temperature and pictures were taken after approximately eight days, according to the growth rate of the pathogens (allowing each fungus to reach the border of the Petri dishes in the control plates). The mycelial growth was recorded using ImageJ by measuring the surface of the mycelium. To enable comparison between different batches of experiments, the mycelial growth quantified on control plates was set to 100 % and the mycelial area of the treated plates was expressed as a percentage of the control (relative mycelial growth). Treatment efficiency was then calculated as described above ($100 - \text{relative mycelial growth}$). Statistical analysis was performed with five replicates using a two-tailed Student's t-test: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

2.6. In vivo – protective effect of selected bacteria on postharvest damage of carrots

Swiss carrots were bought in a supermarket (Coop) and were mixed for randomization. The carrots were dipped for 10 min in bacterial suspensions (1×10^4 CFU/ml sterile distilled water containing 0,3 % NaCl) under shaking conditions to ensure continuous and complete immersion. The bacterial solution was prepared as described in chapter 2.3. The carrots were then air-dried and placed in groups of three in plastic bags into which four holes had been previously made on the bottom using a puncher. For each treatment, nine bags of three carrots were analyzed. A solution of sterile distilled water containing 0,3 % NaCl was used as negative control and a solution of 1×10^4 CFU/ml Serenade® ASO (Bayer AG, Switzerland) in distilled water containing 0,3 % NaCl was used as positive control. The bags were kept at room temperature and observed for three weeks. The carrots were observed once a week and disease scores were given to each carrot according to their infection rate (0 = no infection, 1 = <25 % infection, 2 = 25–50 % infection, 3 = 50–75 % infection, 4 = >75 % infection). The sum of the disease score of all carrots contained in one bag was used as the disease score of the bag. Statistical analysis was performed using two-tailed Student's t-test; $p < 0.05$: *, $p < 0.01$: ** and $p < 0.001$: ***.

3. Results

3.1. Isolation and selection of bacteria

Most of the bacteria tested in this project originated from the Agroscope Culture Collection and had been previously isolated from Swiss plant material and dairy products. Most strains belonged to the genera *Lactiplantibacillus* and *Leuconostoc*, with fewer members of the genera *Lactocaseibacillus*, *Loigolactobacillus*, *Latilactobacillus*, *Lactococcus*, *Lentilactobacillus*, *Levilactobacillus*, *Pediococcus* and *Weissella* (Table S1). From these 100 strains, we further selected 59 strains which i) showed promising inhibition of *B. basicola* mycelial and spore development when grown on a carrot-based medium supplemented with malt extract, and ii) were able to grow on this carrot-based medium with neither malt extract supplementation nor incubation under anoxic conditions. In addition to those strains, we newly isolated bacteria from the surface of carrots (Table S2). From the 27 isolates we retrieved, 19 significantly inhibited *B. basicola* spore germination and were therefore selected for MALDI-TOF identification. This led to the removal of the strains belonging to species known to be associated with clinical or plant infections, leaving nine strains to be further investigated, namely one *Serratia plymuthica* (Sp1), two *Raoultella terrigena* (Rt1 and Rt2), four *Leuconostoc mesenteroides* (Lm13, Lm14, Lm15 and Lm16), and two *Leuconostoc miyukimchii* (Lmi2, Lmi3). In the end, a total of 68 strains were tested for their antagonistic potential on carrot disease-causing agents.

3.2. Inhibition of *B. basicola* mycelial growth and spore germination

As a first step towards determining the plant-protective potential of

Table 1

Effect of the selected strains on mycelial growth and spore germination of *B. basicola*. The values shown for the percentage of inhibition of mycelial growth are the means of 3–5 replicates, with standard deviation. Stars indicate significant differences between treatment and control at $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***) according to a Student's *t*-test. For the inhibition of spore germination, (+) was used for a pattern of inhibition of the spore germination around the bacterial colonies, and (++) for a clear inhibition zone around the bacterial colonies, while (–) indicates that no activity on spore germination was observed. The experiment included 3–5 replicates. The lactic acid bacteria listed in white were isolated previously and belong to the Agroscope Culture Collection and those in grey were isolated in this study from carrot peel. The isolates highlighted in bold indicate those selected for further experiments. *Lcb.* = *Lactocaseibacillus*, *Lpb.* = *Lactiplantibacillus*, *Lolb.* = *Loigolactobacillus*, *Lalb.* = *Latilactobacillus*, *Lc.* = *Lactococcus*, *Lelb.* = *Lentilactobacillus*, *Ln.* = *Leuconostoc*, *Lvlb.* = *Levilactobacillus*, *P.* = *Pediococcus*, *W.* = *Weissella*, *R.* = *Raoultella*, *S.* = *Serratia*.

Treatment	Mycelial growth inhibition (%)	Germination inhibition	Treatment	Mycelial growth inhibition (%)	Germination inhibition
<i>Lcb. paracasei</i> (Lpac1)	16,5±5 (*)	-	<i>Lelb. parabuchneri</i> (Lp4)	7,2±3 (**)	-
<i>Lcb. paracasei</i> (Lpac2)	25,6±4 (***)	-	<i>Lelb. parafarraginis</i> (Lpaf1)	14,3±4 (**)	-
<i>Lcb. rhamnosus</i> (Lr1)	16,5±3 (***)	-	<i>Ln. mesenteroides</i> (Lm1)	25,0±3 (***)	++
<i>Lcb. rhamnosus</i> (Lr2)	8,7±3 (***)	-	<i>Ln. mesenteroides</i> (Lm2)	11,3±2 (***)	-
<i>Lcb. rhamnosus</i> (Lr3)	14,4±3 (***)	-	<i>Ln. mesenteroides</i> (Lm3)	21,2±5 (*)	++
<i>Lpb. pentosus</i> (Lpe1)	9,9±9 (ns)	-	<i>Ln. mesenteroides</i> (Lm4)	16,6±2 (***)	+
<i>Lpb. pentosus</i> (Lpe2)	25,0±6 (*)	-	<i>Ln. mesenteroides</i> (Lm5)	26,3±8 (**)	+
<i>Lpb. pentosus</i> (Lpe3)	18,4±7 (***)	-	<i>Ln. mesenteroides</i> (Lm6)	8,7±4 (*)	+
<i>Lpb. pentosus</i> (Lpe4)	20,3±6 (***)	-	<i>Ln. mesenteroides</i> (Lm7)	32,9±3 (***)	+
<i>Lpb. plantarum</i> (Lpl1)	26,8±8 (***)	-	<i>Ln. mesenteroides</i> (Lm8)	22,2±2 (***)	+
<i>Lpb. plantarum</i> (Lpl2)	27,2±4 (***)	-	<i>Ln. mesenteroides</i> (Lm9)	28,3±2 (***)	++
<i>Lpb. plantarum</i> (Lpl3)	23,1±2 (***)	-	<i>Ln. mesenteroides</i> (Lm10)	18,5±3 (***)	+
<i>Lpb. plantarum</i> (Lpl4)	15,4±6 (***)	-	<i>Ln. mesenteroides</i> (Lm11)	32,6±4 (*)	+
<i>Lpb. plantarum</i> (Lpl5)	19,2±5 (***)	+	<i>Ln. mesenteroides</i> (Lm12)	25,2±3 (***)	++
<i>Lpb. plantarum</i> (Lpl6)	18,3±5 (***)	-	<i>Ln. miyukkimchii</i> (Lmi1)	22,3±4 (*)	-
<i>Lpb. plantarum</i> (Lpl7)	27,1±2 (***)	-	<i>Ln. pseudomesenteroides</i> (Lpm1)	7,2±1 (**)	+
<i>Lpb. plantarum</i> (Lpl8)	26,0±3 (***)	-	<i>Ln. pseudomesenteroides</i> (Lpm2)	7,6±5 (*)	-
<i>Lpb. plantarum</i> (Lpl9)	22,5±10 (**)	-	<i>Ln. suionicum</i> (Lsu1)	29,5±4 (***)	++
<i>Lpb. plantarum</i> (Lpl10)	18,4±4 (***)	-	<i>Lvlb. brevis</i> (Lb1)	4,6±3 (ns)	-
<i>Lpb. plantarum</i> (Lpl11)	19,6±5 (***)	+	<i>Lvlb. brevis</i> (Lb2)	14,0±3 (***)	-
<i>Lpb. plantarum</i> (Lpl12)	35,9±2 (***)	-	<i>Lvlb. brevis</i> (Lb3)	5,2±7 (ns)	-
<i>Lolb. coryniformis</i> (Lc1)	3,8±3 (ns)	-	<i>Lvlb. brevis</i> (Lb4)	5,5±3 (*)	-
<i>Lolb. coryniformis</i> (Lc2)	0,7±1 (ns)	++	<i>P. pentosaceus</i> (Pp1)	13,0±4 (***)	-
<i>Lalb. curvatus</i> (Lcu1)	7,6±3 (*)	-	<i>W. cibaria</i> (Wc1)	5,1±1 (*)	+
<i>Lalb. sakei</i> (Ls1)	4,8±5 (ns)	-	<i>W. cibaria</i> (Wc2)	9,0±1 (**)	++
<i>Lalb. sakei</i> (Ls2)	4,3±3 (*)	-	<i>Ln. mesenteroides</i> (Lm13)	45,4±4 (*)	-
<i>Lalb. sakei</i> (Ls3)	27,7±3 (***)	-	<i>Ln. mesenteroides</i> (Lm14)	49,3±6 (*)	++
<i>Lc. cremosis</i> (Lcr1)	15,9±6 (**)	-	<i>Ln. mesenteroides</i> (Lm15)	45,2±7 (***)	-
<i>Lc. cremoris</i> (Lcr2)	17,1±6 (*)	+	<i>Ln. mesenteroides</i> (Lm16)	48,0±1 (***)	++
<i>Lc. lactis</i> (Li1)	14,3±3 (***)	-	<i>Ln. miyukkimchii</i> (Lmi2)	28,4±3 (***)	-
<i>Lc. lactis</i> (Li2)	12,9±6 (**)	-	<i>Ln. miyukkimchii</i> (Lmi3)	21,4±3 (***)	-
<i>Lelb. parabuchneri</i> (Lp1)	7,2±2 (**)	-	<i>R. terrigena</i> (Rt1)	54,9±6 (***)	+
<i>Lelb. parabuchneri</i> (Lp2)	31,3±5 (***)	+	<i>R. terrigena</i> (Rt2)	52,2±6 (***)	+
<i>Lelb. parabuchneri</i> (Lp3)	6,7±7 (ns)	-	<i>S. plymuthica</i> (Sp1)	44,8±4 (***)	+

Mycelial inhibition (%)	ns	< 10%	< 20%	< 30%	< 40%	< 50%	< 60%
Germination inhibition	-			+			++

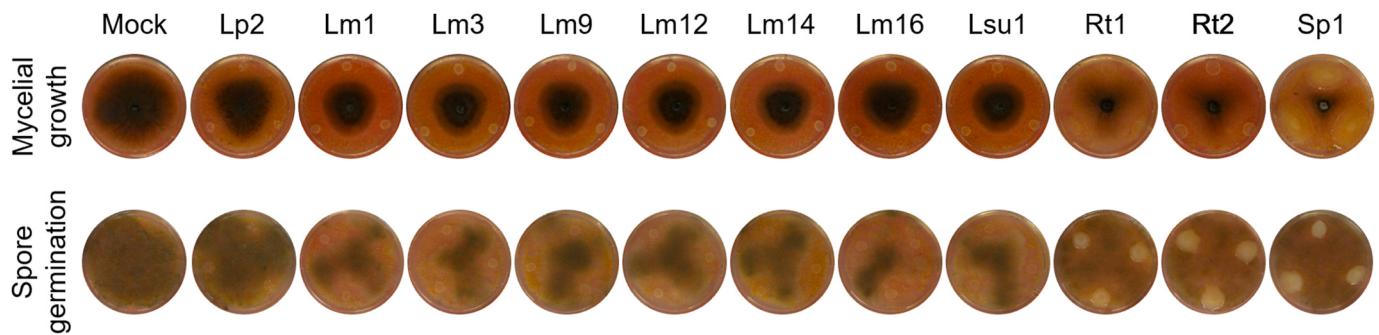


Fig. 1. Representative pictures of the antagonistic activities of selected strains against *B. basicola* mycelial growth and spore germination. The quantitative data corresponding to the representative pictures are shown in Table 1.

the 68 selected isolates, we analyzed whether they were able to inhibit two important stages of the carrot pathogen *B. basicola*, mycelial growth and spore germination. Most of the 68 strains induced a significant inhibition of mycelial growth, while less than half led to reduced spore germination (Table 1). Interestingly, the strongest inhibitors were found among carrot isolates: the two *R. terrigena* strains (Rt1 and Rt2) induced the highest mycelial growth reduction (>50 %), followed by *S. plymuthica* (Sp1) and the four *L. mesenteroides* strains (Lm13, Lm14, Lm15 and Lm16), with approximately 40 % reduction. As a comparison, the *L. mesenteroides* strains previously isolated from different environments (Lm1-Lm12) showed varied, but consistently lower activity than those isolated from carrots (Table 1). Among the strains not isolated from carrots, the best inhibitors of *B. basicola* mycelial growth were a *Lactiplantibacillus plantarum* (Lp12), two *Leuconostoc mesenteroides* (Lm7, Lm11) and a *Lentilactobacillus parabuchneri* (Lp2), at approximately 30 % reduction.

Overall, fewer strains were able to impair spore germination and subsequent growth than to impair mycelial growth. Only nine strains showed a clear inhibition halo (++), of which six belonged to the species *Leuconostoc mesenteroides*. In addition, 16 isolates also showed some growth inhibition in the direct vicinity of their colony (Fig. 1) but not a complete inhibition of spore germination, which was marked as (+) in Table 1. In this case too, half of them were *Leuconostoc mesenteroides* strains.

Based on the results shown in Table 1, eleven bacteria were selected for further experiments, which reduced *B. basicola* mycelium growth by more than 20 % and inhibited spore germination (Fig. 1). These contained six *Leuconostoc mesenteroides*, among which were two isolates from carrot (Lm1, Lm3, Lm9, Lm12, Lm14, Lm16), one *Lentilactobacillus parabuchneri* (Lp2), one *Leuconostoc suionicum* (Lsu1), two *R. terrigena* (Rt1 and Rt2), and one *S. plymuthica* (Sp1), the latter three also being carrot isolates.

To determine whether these eleven strains would still be active against *B. basicola* when applied in lower population densities, we compared their impact on spore germination and subsequent mycelial growth using a liquid culture setup. When applied at a final OD₆₀₀ of 0.25, all isolates induced very strong inhibition of spore germination (96 %–100 %) (Fig. 2A). For most isolates except for the two *Raoultella* strains, an OD of 0.025 or even 0.0025 did not inhibit spore germination, but it still induced a clear delay in mycelial growth for all strains except for *L. parabuchneri* Lp2, for which the activity was completely lost already at OD 0.025 (Fig. 2B). Remarkably, the two *R. terrigena* strains retained their full activity even at the lowest population density tested.

3.3. Antagonistic effects of the selected bacteria on mycelial growth and spore germination of other carrot pathogens

Although *B. basicola* is a major pathogen responsible for postharvest damages in carrots, other disease-causing agents such as *Alternaria radicina*, *Rhizoctonia solani* or *Sclerotinia sclerotiorum*, responsible for

black rot, crown rot and sclerotinia white mold, respectively, also often contribute to the quantitative and qualitative losses experienced by carrot producers. All eleven selected isolates caused a significant reduction in the mycelial growth of each pathogen, albeit at varying degrees (Table 2): *A. radicina* reacted most strongly to the isolates and its mycelial growth was severely affected by all strains except the two *R. terrigena* strains; *R. solani* was overall less inhibited, and only strongly so by *L. mesenteroides* strains, while *S. sclerotiorum* was only strongly inhibited by the *S. plymuthica* isolate (Table 2, Fig. 3). In contrast to their activity on mycelial growth, the eleven strains' effect on spore germination was marginal at best, which is in stark contrast to their effect on spore germination of *B. basicola*, suggesting that the inhibition of this developmental stage involves mechanisms more specific than that of mycelial growth. Only three carrot isolates, both *R. terrigena* and the *S. plymuthica* strains, were able to partially inhibit the germination of at least one of the pathogens, with the clearest inhibition observed for *S. plymuthica* Sp1 on *S. sclerotiorum* (Table 2, Fig. 3).

3.4. Contribution of volatile organic compounds to the antagonistic activity of the isolates on the different carrot pathogens

To determine whether volatile organic compounds (VOCs) might be at least partially responsible for the observed inhibitory activity of the strains on mycelial growth and spore germination, a dual cultivation assay was carried out in split plates. In contrast to the results obtained when growing the bacteria and pathogens in full plates allowing for diffusible compounds to be exchanged (Table 2), antagonistic activity mediated solely by VOCs were only observed on *B. basicola* but not on the three other pathogens, except for *R. terrigena* Rt2, whose emitted VOCs led to a reduction in *A. radicina* and *S. sclerotiorum* mycelial growth (Table 3). Among the strains which significantly reduced *B. basicola* mycelial growth through their emitted VOCs, *Lentilactobacillus parabuchneri* Lp2 and *Leuconostoc mesenteroides* Lm1 were by far the most active ones, with over 40 % of reduction (Fig. 4).

3.5. Protective effects of the six best isolates on postharvest damage of carrots

In order to quantify the potential protection that could be conferred by the inoculation of carrots with the most promising isolates against postharvest decay caused by various pathogens, we selected the six strains which performed best in the previous *in vitro* experiments to inoculate carrots and monitored their health over three weeks of storage in disease-conducive conditions (Fig. 5). After the first week, the carrots previously treated with 1×10^4 CFU/ml of *R. terrigena* Rt2 and *S. plymuthica* Sp1 showed significantly fewer symptoms of microbial spoilage than the carrots that had not received any treatments (mock). Both, the positive control Serenade ASO and the inoculation with *L. mesenteroides* Lm3 showed a trend of a positive effect, although it was not significant. One week later (W2), the significant protection provided

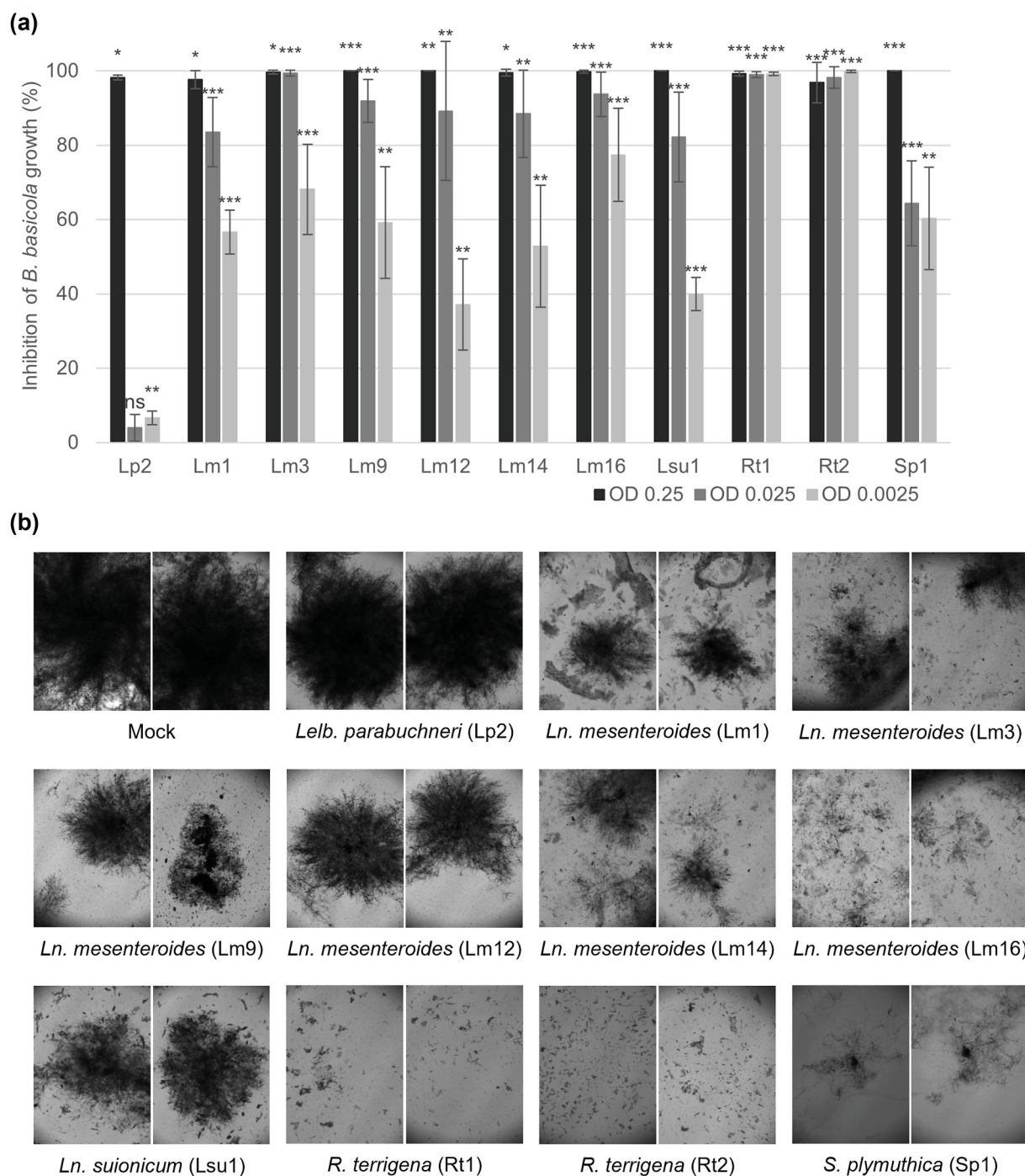


Fig. 2. Effect of different concentrations of the eleven selected strains on spore germination and subsequent mycelial growth of *B. basicola*. (A) Percentage of the wells covered by the mycelium of *B. basicola*, with three different volumes of inoculation (in a total volume of 400 μ l) leading to different final population densities (100 μ l leading to a final OD of 0.25, 10 μ l to a final OD of 0.025 and 1 μ l to a final OD of 0.0025). The results shown are the means of 3–5 replicates with standard deviation. (B) Representative pictures of *B. basicola* growth when incubated in media containing 10 μ l of bacteria suspension, i.e. OD 0.025. Pictures were taken with the Bio Tek Cytation 5 Cell Imaging Reader (Agilent) at a 4-fold magnification.

by the *R. terrigena* Rt2 strain was no longer observable, but that conferred by the *S. plymuthica* Sp1 strain was still visible. At this stage, the treatment with the positive control still showed a positive trend. Finally, after three weeks of storage, the carrots treated with the *S. plymuthica* Sp1 strain still showed significantly fewer disease symptoms than the untreated control, which was also true for the positive control Serenade ASO, although the variability within this treatment seemed higher than with *S. plymuthica* Sp1.

4. Discussion

According to the Food and Agriculture Organization, almost half of cultivated fruits and vegetables are lost or wasted between the fields and the plates [26]. This huge waste of perishable food is partially due to improper transport and storage conditions but also and mainly to spoilage by undesired microorganisms [27]. Nowadays, the control of these microorganisms mostly relies on the use of synthetic pesticides and other chemical compounds, but social acceptance towards the use of

Table 2
Effect of the eleven selected isolates on mycelial growth of *Alternaria radicina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* and on spore germination of *A. radicina* and *S. sclerotiorum*. The results shown for the percentage of mycelial growth inhibition are the means of 3–5 replicates with standard deviation. Stars indicate significant differences compared with the control at P < 0.01 (**) or P < 0.001 (***) according to a Student's *t*-test. For the inhibition of spore germination, (+) indicates a pattern of inhibition of spore germination around the bacterial colonies, (++) a clear inhibition halo around the bacterial colonies, while a (–) score means that no activity on the germination of spores was observed. No results are shown regarding spore germination of *R. solani*, as this pathogen does not produce spores. *Lelb* = *Lentilactobacillus*, *Ln.* = *Leuconostoc*, *R.* = *Raoultella*, *S.* = *Serratia*.

Species	Code	Mycelial growth inhibition			Germination	
		<i>A. rad.</i>	<i>R. sol.</i>	<i>S. scl.</i>	<i>A. rad.</i>	<i>S. scl.</i>
<i>Lelb. parabuchneri</i>	Lp2	25,4±3 (***)	7,4±1 (***)	13,6±2 (***)	-	-
<i>Ln. mesenteroides</i>	Lm1	51,2±2 (***)	27,2±6 (***)	13,6±3 (***)	-	-
<i>Ln. mesenteroides</i>	Lm3	56,7±3 (***)	34,2±16 (**)	21,2±4 (***)	-	-
<i>Ln. mesenteroides</i>	Lm9	40,0±7 (***)	41,7±18 (***)	9,9±1 (***)	-	-
<i>Ln. mesenteroides</i>	Lm12	41,2±2 (***)	21,2±7 (***)	6,5±2 (***)	-	-
<i>Ln. suionicum</i>	Lsu1	51,0±4 (***)	32,5±5 (***)	18,6±4 (***)	-	-
<i>Ln. mesenteroides</i>	Lm14	55,6±2 (***)	42,6±19 (**)	13,4±2 (***)	-	-
<i>Ln. mesenteroides</i>	Lm16	48,9±4 (***)	34,6±11 (***)	22,2±6 (***)	-	-
<i>R. terrigena</i>	Rt1	8,1±5 (**)	13,9±1 (***)	20,3±3 (***)	-	+
<i>R. terrigena</i>	Rt2	4,0±2 (**)	11,6±2 (***)	11,2±3 (***)	+	-
<i>S. plymuthica</i>	Sp1	53,0±4 (***)	13,8±3 (***)	30,8±2 (***)	+	++

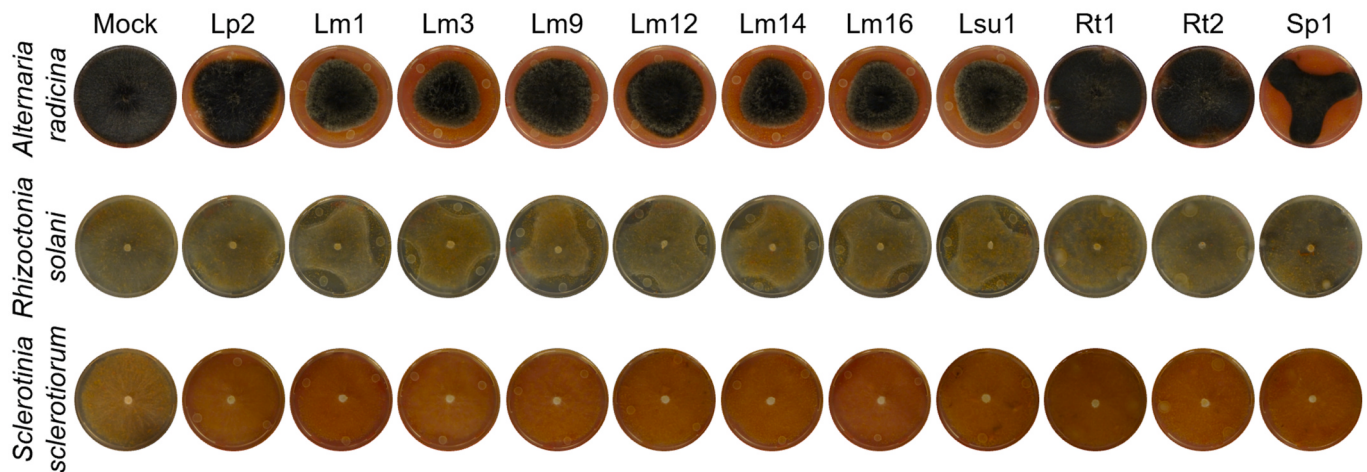
Mycelial inhibition (%)	ns	< 10%	< 20%	< 30%	< 40%	< 50%	< 60%
Germination inhibition	-			+			++

such products has significantly declined. As a more sustainable strategy, biocontrol agents (BCA) can be used as a new biopreservation approach to counteract the growth of phytopathogens [28].

Various studies have shown the potential of using bacteria as biocontrol agents to counter the development of black root rot, on tobacco plants using *Streptomyces hygroscopicus* TA21 or *Pseudomonas fluorescens* CHAO [29,30] and on cotton plants using *Paenibacillus alvei* K-165 [31]. On carrots, however, only one early study has so far analyzed whether black root rot could be controlled with biocontrol agents, using a *Penicillium* strain [32]. In the present study, we aimed to contribute to the development of a protective culture for postharvest damage caused by *B. basicola* in carrots. A vast majority of the strains we tested were lactic acid bacteria (LAB) for two main reasons: firstly, LAB have GRAS status, meaning that their application on vegetables would be facilitated; and secondly, many studies have reported the potential of this bacterial group to inhibit fungal pathogens on fruits and vegetables [17,33–35]. One draw-back of using LAB is the fact that some of them grow poorly in oxic and nutrient-poor conditions. Indeed, in our initial screen, some significant inhibitors of *B. basicola* mycelial growth and spore germination (Table S1) were not investigated further because of

their growth requirements. Still, a few LAB strains from the original Agroscope Culture Collection displayed interesting biocontrol properties across the different experiments, namely one *Lentilactobacillus parabuchneri* strain, one *Leuconostoc suionicum* and several *Leuconostoc mesenteroides* strains. *L. parabuchneri* Lp2 displayed strong mycelial growth reduction and significant inhibition of spore germination (Table 1, Fig. 1), yet this activity was not maintained when lower population densities were used (Fig. 2). However, this strain was one of the two most active emitters of *B. basicola* inhibiting VOCs, with over 40 % inhibition of mycelial growth by the sole exposure to volatiles (Table 3). The emergence of *L. parabuchneri* as a potential biocontrol agent against fungal pathogens in corn silages highlights the significance of this still poorly studied LAB species in agricultural disease management. Field experiments have validated the efficacy of selected LAB strains, including *L. parabuchneri*, in reducing the disease index of *Fusarium verticillioides* by up to 40 % [36]. Beyond *L. parabuchneri*, one *Leuconostoc suionicum* and several *Leuconostoc mesenteroides* strains showed promising inhibition of both mycelial growth and spore germination of *B. basicola* (Table 1, Fig. 1) and of other pathogens, such as *Alternaria radicina* or *Rhizoctonia solani* (Table 2, Fig. 3). This observation

Mycelial growth



Spore germination

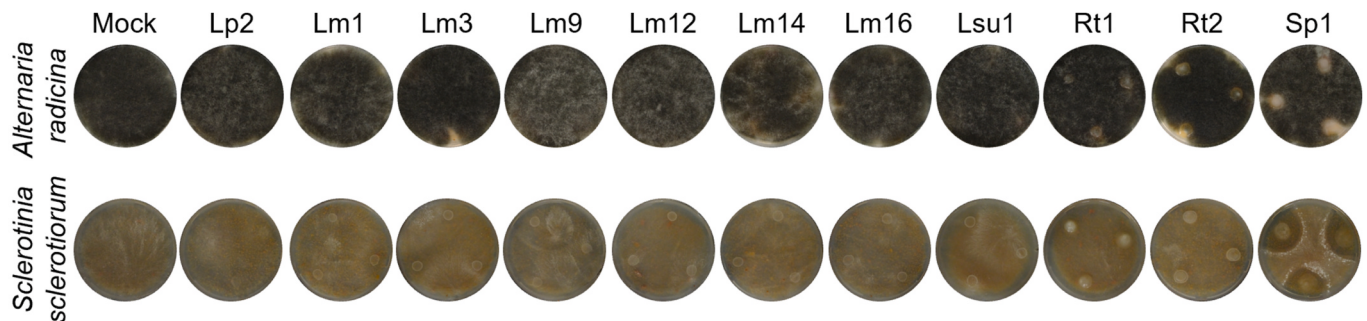


Fig. 3. Representative pictures of the antagonistic activities of the eleven selected strains against *Alternaria radicina*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* mycelial growth and *A. radicina* and *S. sclerotiorum* spore germination. The quantitative data corresponding to the representative pictures are shown in Table 2.

corroborates previous findings from the literature, which highlighted the broad range of activity of members of this species on various phytopathogenic fungi, such as *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp [37–41].

Interestingly, we also retrieved *Leuconostoc mesenteroides* strains within our carrot isolates. The motivation behind isolating strains directly from healthy carrots was that the plant microbiota is well known to contribute to the health of its host [42]. The probability of obtaining bacteria with antagonistic activity towards carrot pathogens was thus deemed higher, as well as the expectation that the strains adapted to this particular environment would survive on carrots when later inoculated as preservative culture [43,44]. And indeed, we observed this higher activity of carrot isolates compared to strains derived from other environments: the best inhibitors of *B. basicola* mycelial growth were by far the carrot isolates, and even within *L. mesenteroides* strains, carrot isolates performed better than their conspecific relatives from different environments (Table 1, Fig. 1). Strikingly, the only two strains which strongly inhibited both mycelial growth and spore germination, *L. mesenteroides* Lm14 and Lm16, were both carrot isolates. This ability to inhibit both developmental stages indicates that the same biocontrol agent can display different modes of action [45], a feature of great interest for biological control of diseases in view of its expected higher efficacy and lower risk of resistance development. A similar tendency was observed in the other carrot pathogens tested, with a higher activity of carrot isolates than non-carrot isolates within this same *L. mesenteroides* species (Table 2).

Beyond the special case of *L. mesenteroides*, we noticed that the seven strains which were able to reduce the growth of *B. basicola* by more than 40 % were all isolated from carrots. In addition to four strains of the above-mentioned *L. mesenteroides* species, these were two *Raoultella terrigena* and one *Serratia plymuthica* strain (Table 1, Fig. 1). These three strains were also the only ones able to inhibit the germination of spores from other carrot pathogens, i.e. *Alternaria radicina* and/or *Sclerotinia sclerotiorum* (Table 2). One particularly striking feature of the two *R. terrigena* isolates was their ability to keep their inhibition potential even when very strongly diluted (Fig. 2), suggesting the secretion of very potent metabolites. Recent literature on this species highlights its versatile nature ranging from a plant pathogen to a plant growth promoting lifestyle and from a gut bacterium to a potential biocontrol agent. In the agricultural context, *R. terrigena* has been implicated as a causal agent of soft rot in chili plants [46]. However, other studies have shown that when combined with other bacteria such as *Chromobacterium violaceum*, *R. terrigena* can enhance the growth of cabbage [47]. Moreover, *R. terrigena* can also live as gut symbiont in insect larvae, where it has been found to contribute to the degradation of plant toxins, facilitating insect development [48]. Furthermore, *R. terrigena* has shown efficacy as a biocontrol agent against plant pathogens such as *Phytophthora fragariae* and *P. cactorum*, responsible for diseases in strawberries [49]. Its inhibitory effect on these pathogens underlines its potential for integrated disease management strategies in agriculture. However, *R. terrigena* has also been noted as a rarely encountered opportunistic pathogen in humans, with reported cases spanning several decades [50].

Table 3
Effect of VOCs emitted by the eleven selected strains on the mycelial growth of *Alternaria radicina*, *Berkeleyomyces basicola*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The results shown for the percentage of mycelial growth inhibition are means of 3–5 replicates with standard deviation. Stars indicate significant differences compared with the control at P < 0.05 (*), P < 0.01 (**) or P < 0.001 (***) according to a Student's t-test. *Lelb.* = *Lentilactobacillus*, *Ln.* = *Leuconostoc*, *R.* = *Raoultella*, *S.* = *Serratia*.

Species	Code	Activity of VOCs			
		<i>A. rad.</i>	<i>B. basi.</i>	<i>R. sol.</i>	<i>S. scl.</i>
<i>Lelb. parabuchneri</i>	Lp2	3,9±11 (ns)	40,5±10,2 (***)	0,0±0,0 (ns)	1,9±4 (ns)
<i>Ln. mesenteroides</i>	Lm1	16,6±12 (ns)	42,6±9,0 (***)	0,0±0,0 (ns)	1,0±2 (ns)
<i>Ln. mesenteroides</i>	Lm3	21,7±12 (ns)	8,7±7,0 (ns)	0,0±0,0 (ns)	0,0±0 (ns)
<i>Ln. mesenteroides</i>	Lm9	-3,5±1 (ns)	11,8±6,3 (*)	0,0±0,0 (ns)	0,5±1 (ns)
<i>Ln. mesenteroides</i>	Lm12	16,3±18 (ns)	22,8±13,8 (*)	0,0±0,0 (ns)	0,0±0 (ns)
<i>Ln. suionicum</i>	Lsu1	-1,6±7 (ns)	11,7±5,2 (*)	0,0±0,0 (ns)	0,0±0 (ns)
<i>Ln. mesenteroides</i>	Lm14	-2,1±3 (ns)	8,5±4,3 (*)	0,0±0,0 (ns)	0,3±1 (ns)
<i>Ln. mesenteroides</i>	Lm16	24,5±5 (ns)	12,8±7,1 (*)	0,0±0,0 (ns)	0,8±1 (ns)
<i>R. terrigena</i>	Rt1	4,8±13 (ns)	15,0±5,7 (**)	0,0±0,0 (ns)	0,7±1 (ns)
<i>R. terrigena</i>	Rt2	18,3±10 (**)	13,1±6,5 (**)	0,0±0,0 (ns)	3,7±3 (*)
<i>S. plymuthica</i>	Sp1	-4,0±5 (ns)	14,5±10,3 (*)	0,0±0,0 (ns)	0,5±1 (ns)

Mycelial inhibition (%)	ns	< 10%	< 20%	< 30%	< 40%	< 50%
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Similar to the case of *Klebsiella* spp, the emergence of *Raoultella* spp. as potential human pathogens have raised concerns prompting further investigation into its epidemiology and clinical impact [51].

Members of the species *Serratia plymuthica* are well-known for their ability to suppress the growth of various plant pathogens and promote plant growth [52]. They produce a range of secondary metabolites such as prodigiosin, a tripyrrole red pigment showing antibacterial and antifungal effects on plant pathogens [53–55]. Oocydin A, produced by specific *S. plymuthica* strains, prevents plant disease caused by *Verticillium dahliae* [56]. Moreover, *S. plymuthica* strains have been found on various edible plants such as green onion, carrot, and lettuce, suggesting their potential application in postharvest disease control [57]. Indeed, patents have been filed for the use of *S. plymuthica*, including strain CL43, in controlling postharvest diseases on cabbages. However, caution is warranted in the use of this species, as rare cases of *S. plymuthica* being isolated from clinical specimens have been reported [58]. Although such occurrences are uncommon, they underline the importance of safety assessments prior to any application in food systems.

From the mycelial and spore inhibition assays performed on *B. basicola* as well as on other carrot pathogens, we selected the six best strains, containing one of the two *Raoultella terrigena* strains and the *Serratia plymuthica* strain, and tested their potential for postharvest disease control in carrots. After one week, these two strains induced significant protection of the carrots, an effect that was only maintained through time for *S. plymuthica* Sp1, which yielded protection comparable to that of the biocontrol benchmark we used (Fig. 5). This result is interesting in the sense that although this strain did not display the

highest inhibition of *B. basicola*, on which we based our screening, it was one of the strongest inhibitors of both mycelial growth and spore germination of the other carrot pathogens (Table 2), suggesting that broad range activity, which might indicate more diverse modes of action, is an important asset for efficient postharvest disease control in carrots.

Our study highlighted the potential of harnessing the plant's own microbiota resources when searching for new biopreservation agents, as the carrot peel environment was clearly enriched in strains showing higher antagonistic potential towards carrot pathogens than phylogenetically related strains from a different isolation origin. Although members of the species *L. mesenteroides* were strikingly active in laboratory experiments, the most promising postharvest disease control strains were not the LAB strains, but a *R. terrigena* and, particularly, a *S. plymuthica* strain. Although the versatile nature of *R. terrigena* and its reported occurrence as opportunistic pathogen in some cases warrant caution when considering its use in postharvest disease control, *S. plymuthica* also requires careful evaluation. While it is generally regarded as a beneficial plant-associated bacterium with strong potential for biocontrol, rare cases of *S. plymuthica* being isolated from clinical specimens have been reported. Therefore, although it remains a promising candidate for further validation—particularly through extended trials on different carrot varieties and storage conditions—its potential pathogenicity to humans and animals as well as its impact on the environment must not be overlooked. Future development should include thorough safety assessments alongside efforts to optimize its efficacy, e.g. through proper formulation.

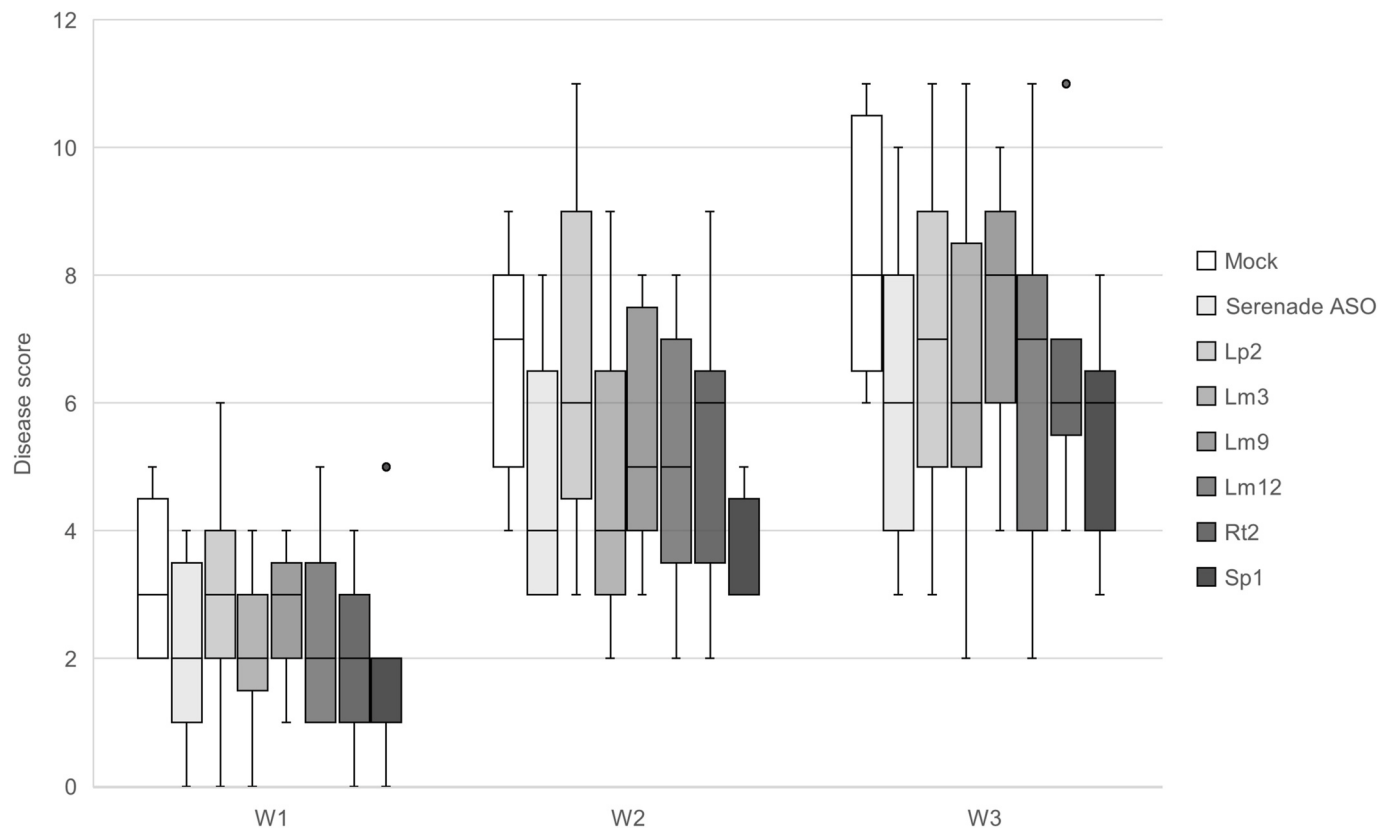


Fig. 5. Efficiency of selected isolates as postharvest treatment against carrot diseases. The lines in the boxplots represent the means of nine replicates. Each replicate corresponds to a bag containing three carrots, and the infection rates of the individual carrots were summed to calculate the disease score (DS) for each bag. The disease score (DS) was assigned based on the percentage of the carrot's surface infected by pathogens, as follows: DS0 = 0 %, DS1 = >0–25 %, DS2 = >25–50 %, DS3 = >50–75 %, and DS4 = >75 %. Stars indicate significant differences compared with the control at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) according to a Student's *t*-test. Outlier values are represented by dots.

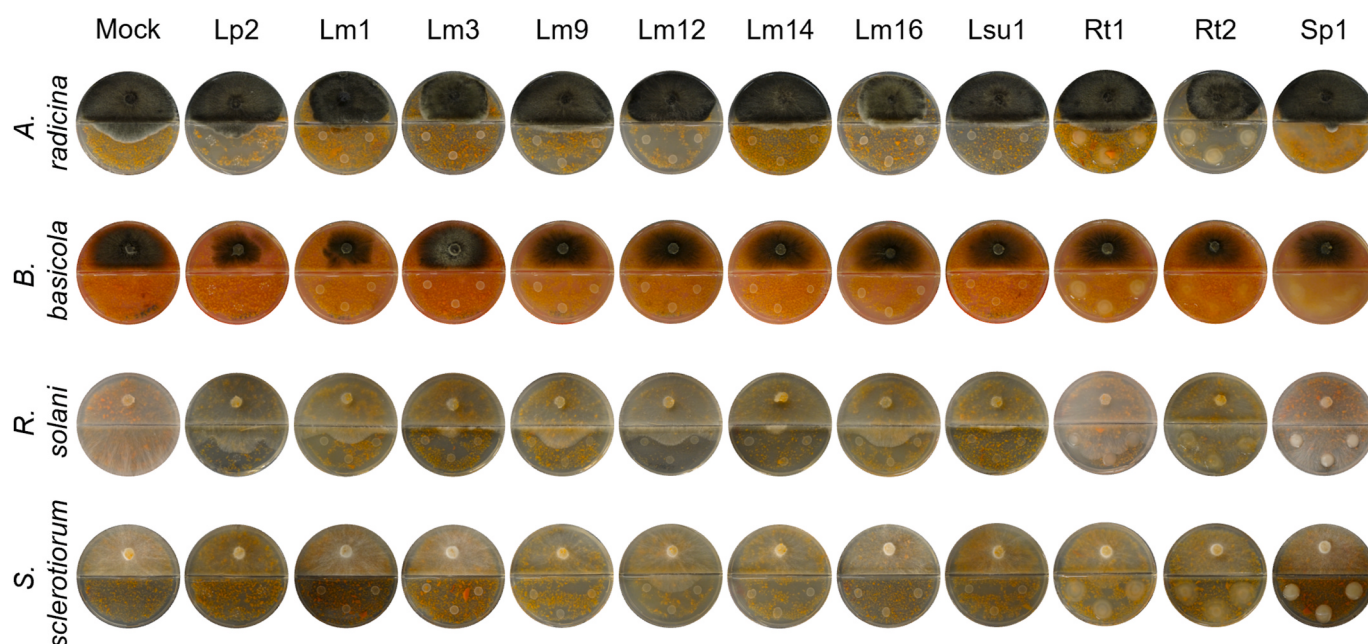


Fig. 4. Representative pictures of the antagonistic activities of VOCs emitted by the eleven selected strains on the mycelial growth of *Alternaria radicina*, *Berkeleyomyces basicola*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The quantitative data corresponding to the representative pictures are shown in Table 3.

CRedit authorship contribution statement

Fanny Louviot: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mónica Zufferey:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Emmanuelle Arias-Roth:** Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization. **Laure Weisskopf:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Elisabeth Eugster:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Elisabeth Eugster reports financial support and administrative support were provided by Federal Office for Agriculture. Laure Weisskopf reports financial support was provided by Swiss National Science Foundation. Elisabeth Eugster reports financial support was provided by Sur-la-Croix Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jafr.2025.102053>.

Data availability

Data will be made available on request.

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