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Original Research Article

Anti-oomycete potential of *Lactobacillus amylovorus* JG2 against the potato blight pathogen *Phytophthora infestans*

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Keywords

Anti-oomycete; lactic acid bacteria; *Phytophthora infestans; Lactobacillus amylovorus;* potato blight.

A B S T R A C T

Phytophthorainfestans is the oomyceteswhich causes late blight whichis the most devastating diseases in potato production today. This study was undertaken to assess the *in vitro* anti-oomycetepotential of *Lactobacillus amylovorus* JG2 against *P. infestans*. Five strains of lactic acid bacteria which initially exhibited strong antifungal activity against *Penicillium*, *Aspergillus* and human dermatophytes were selected and tested against Irish potato blight strains of *P. infestans*. All five strains caused inhibition as observed by large mycelium clearing on mMRS agar surface. The strongest inhibition was seen with *Lb. amylovorus* JG2. Cell-free culture supernatants, freeze-dried supernatants of *Lb. amylovorus* JG2 and of the non-antifungal *Lb. amylovorus* DSM20531 were used to access and compare anti-oomycete activity in agar plate assays, microtitre plate assays. When freeze-dried cell-free supernatant powder from *Lb. amylovorus* JG2 was incorporated in culture medium at concentrations greater than 1%, *P. infestans*mycelial radial growth was inhibited. Addition of the supernatant at 12.5% to liquid cultures caused complete inhibition of *P. infestans* growth on the basis of turbidity. Cell free supernatant of *Lb. amylovorus*JG2 and DSM 20531 was analysed by Liquid Chromatography Fourier Transform Mass Spectrometry (LC-FTMS) using an Accela LC coupled to an LTQ Orbitrap XL mass spectrometer.Fifteen antifungal metabolites were detected. This study has demonstrated that Biological control accomplished by beneficial microorganisms may be a viable "green approach" to reducing late blight**.**

Introduction

Potato is the fourth largest food crop, the most important vegetable in the western world, and a critical alternative to the major cereal crops for feeding the world's population (Reader, 2009). Difficulty in growing this crop commercially can be attributed to its susceptibility to diseases, of which late blight caused by *P. infestans* is considered the most important (Goodwin *et al.*, 1994; Chycoski and

Punja, 1996; Fry and Goodwin, 1997;Guenthner *et al.,* 2001; Fry, 2008).This potato diseasehas had a tremendous effect on human history, resulting in famine and population displacement during the mid-nineteenth century (Reader, 2009).For more than two decades, controlling late blight has become increasingly challengingdue to the emergence of novel strains of the pathogen (Fry *et al.,* 1993; Goodwin *et al.,* 1994; McDonald and Linde, 2002). Chemicals used to control oomycetes, which are fungus-like eukaryotic microorganisms, are also referred to as fungicides asoomycetesuse the same mechanisms as fungi to infect plants (Latijnhouwers *et al.,* 2003).Many of these oomycetestrains have developed resistance to the most commonly knownfungicides, consequently leading to an increase for the costs of crop production (Daayf and Platt, 2000; Haldar et al.,2006). In the European Union late blight caused by *P. infestans* causes annual losses (costs of control and damage) estimated at more than one billion euro (Haverkort *et al.,* 2008).Moreover, the consumers have expressed concerns over the heavy reliance on environmentally unfriendly chemicals in plant protection strategies. Despite these issues, safe and environmentally friendly products for plant protection represent an insignificant portion of the pesticide market, which is dominated by synthetic chemicals. If alternative methods, such as biological control could be developed to minimize the need for synthetic chemicals in food crop production, it could result in a more sustainable agricultural system.

Lactic acid bacteria (LAB) have previously been shown to play a crucial role in the preservation and microbial safety of food because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics, and are naturally occurring in many food systems (Caplice and Fitzgerald, 1999; Tamminen *et al.,* 2004; De Vuyst and Leroy, 2007). Numerous reports on antimicrobial activity of LAB have focused on antibacterial effects (Murry*et al.,* 2004; Topisirovic *et al.,* 2006; Jones *et al.,* 2008;Maragkoudakis *et al.,* 2009;Garde *et al*.,2011).In recent years there has been a growing interest in research on antifungal characteristics of LAB, especially with regard to their application in food systems (Dal Bello *et al.,* 2007; Ho *et al.,* 2009; Coda et al.,2011). LAB produce a variety of antifungal metabolites, which comprise organic acids, proteinaceous compounds and a variety of low molecular mass compounds (less than 1000 Da) (Ström *et al.,* 2002; Sjörgen *et al.,* 2003;Broberg *et al.,* 2007;Yang and Chang, 2010).The published studies on the antifungal activity of LAB are still considered to be limited and most studies are focused on fermented food-associated fungi such as *Fusarium* (Magnusson and Schnürer, 2001;Dal Bello *et al.,* 2007; Hassan *et al.,* 2008;Mauch *et al.,* 2010), *Aspergillus* (Strom *et al.,* 2005;Muňoz *et al.*,2010;Djossouet al.,2011), and *Penicillium* (Florianowicz, 2001;Voulgari *et* al.,2010). Very few studies were aimed at antifungal activity of LAB against other fungi such as citrus fungal pathogen *Geotrichumcitri-aurantii* (Gerez *et al.,* 2010), the human dermatophytic fungi *Trichophyton tonsurans* (Guo *et al.,* 2011), *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum* (Guo *et al.,* 2012).

This study was undertaken to assess the in vitro anti-oomycete potential of *Lactobacillus amylovorus* JG2 against *P. infestans*. Through previous work, a large bank of LAB (over 200) with a broad

antifungal spectrum has been generated (Guo *et al.,* 2011; Guo *et al*., 2012). Five strains of lactic acid bacteria which initially exhibited strong antifungal activity against *Penicillium, Aspergillus* and human dermatophytes were selected and tested against the Irish potato blight strains of *P. infestans*.LC-FTMS analysis of the cell free supernatant of *Lactobacillus amylovorus* JG2 and DSM20531 was performed with an Accela LC connected to an LTQ Orbitrap XL mass spectrometer. Chromatograms produced by the positive (JG2) and negative (DSM20531)anti-oomycetestrains were compared and quantified against a multianalyte standard chromatogram containing twenty-five known antifungal compounds found in lactic acid bacteria.

Materials and Methods

Bacterial cultures and media

Two hundred and twenty strains of lactic acid bacteria were previously isolated and identified from pigs, human infants, mice, cows, sourdough, cheeseand cereal samples. Five strains of lactic acid bacteria namely *Lactobacillus arizonensis* R13, *Lactobacillus brevis* JJ2p, *Lactobacillus amylovorus*JG2, *Weissellacibaria*FF3PR and *Lactobacillus reuteri* R2 which initially exhibited strong antifungal activity against *Penicillium, Aspergillus* and human dermatophytes were selected and tested against Irish potato blight strains of *P. infestans*. Five negative control strains (of the same species) are included (Guo *et al.,* 2011). LAB were routinely grown on MRS agar plates (FlukaChemie AG, Buchs, Switzerland) under microaerophilic conditions for 48 h at 37 °C. Long-term storage was done in 40 % glycerol at -80 °C.

P. infestans cultures and preparation

Two late blight strains*P. infestans* 05 and *P. infestans* 16 (obtained from Teagasc Crops Research Centre, Oak Park, Carlow, Ireland) were used in this research. *P. infestans*were grown on Pea agar (Montarryet al.,2006) plates at 20°C for 5-10 days and then stored at 4 °C until required. An inoculum from thepea plate was transferred into 500 ml of Synthetic-Nutrient-Poor Bouillon (SNB) (Nirenberg, 1976). The suspensions were then incubated at 20 °C for 10-15 days with stirring (120 rpm) before using.Typically the suspension was adjusted to the required concentration prior to use.

Determination of antifungal activity by dual-culture plate assays

Selected LAB were tested for anti-oomyceteactivity against *P. infestans*using a dual-culture plate assay. Two millitersof *P. infestans*suspension was mixedinto 18 ml of MRS agar modified as follows (mMRS): pH was adjusted to 6.0 and sodium acetate as well as potassium dihydrogenphosphate were omitted. After 30 min, lactic acid bacteria were inoculated as two parallel lines of 2 cm length; keeping a distance between the lines of approx. 2 cm and allowed to grow under microaerophilic conditions at 30°C for 48 h.

The plates were then incubated for 5-10 days under aerobic conditions at 20°C to promote oomycetegrowth. The anti-oomycete activity of each LAB was ascertained by measuring the size of the halo surrounding the bacterial streaks. The overall growth of the *P. infestans*was compared to that in control plates (i.e. with non-antifungal LAB present and with no LAB present).

Influence of acidic conditions on *Phytophthora infestans* growth

To evaluate the influence of acidic conditions onoomycete growth, *P.infestans* were grown on pea agar surface with the pH adjusted to 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 using either HCl or NaOH. 10µL of *P.infestans* suspension were inoculated as a spot in the centre of the pea agar plates after which they were incubated for 15 days at 20°C under aerobic conditions. Colony diameters were measured and recorded.

Preparation of lactic acid bacterial cell-free supernatant (cfs) powders

Cell-free supernatant and freeze-dried supernatant powders of *Lb.amylovorus*JG2 (cfsP), and of a non-inhibiting control strain *Lb.amylovorus*DSM 20531 (cfsN) were produced to serve as base material for the experiments describing the nature of the antifungal compounds. Briefly, overnight cultures of bacteria were inoculated in 500 ml of mMRS broth to reach an initial concentration of 10^5 CFU/ml.

The bacteria were grown for 5 days at 37 °C (temperature at which the antifungal strain showed its highest activity). Cells were separated from the supernatant by centrifuging twice at 3000 g for 15 min. The cell-free supernatant was freeze-dried and the powder stored at 4 °C. Freeze-dried mMRS broth (cfsC) powder, which was used as a control for some experiments, was obtained using same procedure. Typically, 500 ml of supernatant gave rise to 25 g of lyophilized powder. Powders were routinely reconstituted in sterile distilled water.

Effect of LAB on *P. Infestans* mycelial radial growth

A 50 % (w/w) cfsP, cfsN orcfsC working-solution was prepared by dissolving the powder in distilled water, adjusted pH to 4 using commercial D/L-lactic acid (Sigma-Aldrich, St. Louis, USA) and variable amounts of 4M NaOH, and then filter sterilized using a 0.45µm MINISART®-plus filter (Sartorius, Goettingen, Germany). Pea agar plates were prepared containing 0 (control), 0.2, 0.5, 1% (m/v) of freeze-dried cell-free supernatant of *Lb.amylovorus*JG2 (cfsP). For each concentration, negative control plates were prepared containing same amount of freeze-dried cell-free supernatant of *Lb.amylovorus*DSM20531 (cfsN), acidified control plates were prepared containing same amount of freeze-dried mMRS broth (cfsC). After cooling, 10µl of spore-mycelia suspension were inoculated as a spot in the centre of the pea agar plates. The plates were incubated for 15 days at 20°C under aerobic conditions. The *P.infestans* growth was monitored by measuring themycelial radial growth area of *P.infestans* colonies.

P.infestansgrowth in presence of Lb. amylovorus cell-free supernatant

Microtitreplate assays were used to determine the anti-*P.infestans* effect of *Lb. amylovorus* cell-free supernatant on *P. infestans* growth. The spore suspension was adjusted to 1.0×10^5 ml^{-1}. Aliquots of 50 ml were centrifuged at 3000 g for 10 min and the supernatant was discarded. The spore pellets were resuspended in 5 ml pea broth, and then 100µl of spore solution were adding to the wells of a sterile 96-well microplate (Sarstedt AG and Co, Nuembrecht, Germany). 100µl of cell-free supernatant of *Lb.*

*amylovorus*JG2 (cfsP) dilutions were adding to the wells, and the final concentrations of cfsP were 0, 0.2, 0.4, 0.8, 1.5, 3.0, 6.0, 12.5, 25.0 or 50.0(%). The microplate was sealed with optically clear seal for QPCR (Thermo Scientific, Waltham, USA). The microplates were incubated for 120h at 20°C inside a Multiskan FC microplate-reader (Thermo Scientific, Waltham, USA). The optical density at 620 nm (OD_{620}) was automatically recorded for each well every 3 h. The changes in OD_{620} over time were used to generate *P. infestans* growth curves at each cfsP concentration. Experiments were performed in duplicate.

Identification of antifungal compounds from *Lb. amylovorus* strains using LC-FTMS (LC-LTQ-Orbitrap XL)

Anti-fungal standard compounds: (A) cytidine, (B) 2-deoxycytidine, (C) D-glucuronic acid, (D) DL-ρ-hydrophenyllactic acid, (E) 1,2-dihydroxybenzene, (F) 3,4-dihydroxyhydrocinnamic acid, (G) 4-hydroxybenzoic acid, (H) caffeic acid, (I) vanillic acid, (J) (S)-(-)-2-hydroxy-isocapric acid, (K) 3-(4-hydroxyphenyl)-propionic acid, (L) 3-(4-hydroxy-3-methoxyphenyl)propionic acid, (M) ρ-coumaric acid, (N) ferulic acid, (O) azelaic acid, (Q) benzoic acid, (R) hydrocinnamic acid, (S) methylcinnamic acid, (T) 3-hydroxydecanoic acid, (U) DL-β-hydroxylauric acid, (V) decanoic acid, (W) DL-β-hydroxymyristic acid, (X) 2-hydroxydodecanoic acid, (Y) salicylic acid, Internal standard (IS) hydrocinnamic acid D9 OH and the mobile phase additive acetic acid were purchased fromSigma Aldrich(Dublin, Ireland).(P)Phenyllactic acid was obtained by Bachem (Weil am Rhein, Germany). LC-MS grade solvents and Liquid Liquid Extraction (LLE) solvent ethyl acetatewere sourced from Thermo Fisher Scientific (Dublin, Ireland).

The chromatographic profiles for the strains were obtained according to the method of Brosnanand co-workers (Brosnan *et al.*, 2012). The cell free supernatant was separated on a Gemini C_{18} (150 x 2 mm, 5 µm; Phenomenex, Macclesfield, UK) column equipped with a Security Guard cartridge (C_{18}, 4 x 2 mm; Phenomenex)on an Accela LC system (Thermo Fisher Scientific, Hemel Hempstead, UK). A stepped gradient comprising of water with 0.1% acetic acid and acetonitrile with 0.1% acetic acid was employed. Preparation of the samples for analysis involved extracting the crude *Lb. amylovorus* JG2and DSM 20531 extract, following centrifugation and filtration, by LLE with ethyl acetate.Samples were dried under nitrogen, reconstituted in initial mobile phase, filtered (0.22µm) and vialed prior to injection onto the LCMS.

The LC system was connected to aLTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). It was operated in negative ion mode with an electrospray interface (ESI). Previously optimisedion source tune method conditions were used for analysis of these strains (Brosnan *et al.*, 2012).

Result and Discussion

Determination of anti-*P.infestans* activity by dual-culture plate assays

Five antifungal strains of lactic acid bacteria namely *Lactobacillus arizonensis* R13, *Lactobacillus brevis* JJ2p, *Lactobacillus amylovorus*JG2, *Weissellacibaria* FF3PR and *Lactobacillus*

reuteri R2 were selected and tested against Irish potato blight strains of *P. infestans*05 and *P. infestans* 16. Five negative control strains (of the same species) are included. All five positive strains showed some degree of antimicrobialacitivity against *P. infestans*. The inhibition profile is summarized as sizes of clear zones of *P.infestans*inhibition and thesewere recordedin Table 1. Most strong inhibition was observed by *Lb. amylovorus*JG2, the distances between the peripheral sides of the bacterial-lines and the starting *P.infestans*growth zones were large. The negative control strain *Lb. amylovorus* DSM20531 did not appear to cause any *P.infestans*inhibition (Table 1, Fig.1). *Lb. amylovorus* DSM20531 was the most appropriate negative control strain as its growth rate and acid-producing ability in liquid medium were almost identical to that of *Lb. amylovorus*JG2 (data not shown).

Influence of acidic conditions on *Phytophthora infestans* growth

The pH tolerances of two strains of *P. infestans* were tested by inoculating fungi on pea agar with a series adjusted pHs ranging from 2.0 to 9.0. Colony diameters were measured after 15 days of growth. The growth of *P.infestans*was shown to be same at pHs 4.0 to 7.0 for both strains. At pH 3.5, *P.infestans*growth was reduced, while at pH 3.0 or less, no *P.infestans*growth occurred. The data is summarized in Table 2. This indicated that while low pH did have a slight effect on *P.infestans*inhibition, other factor(s) related to *Lb.amylovorus*JG2 was contributing to the inhibition.

Effect of LAB on *P. infestans* mycelial radial growth

To evaluate the effect of LAB on *P.infestans* mycelial radial growth, pea agar plates were prepared containing 0 (control), 0.2, 0.5, 1% (m/v) of freeze-dried cell-free supernatant of *Lb. amylovorus*JG2 (cfsP). As a negative LAB control, cfsN, a freeze-dried cell-free supernatant of the non-oomycete strain DSM20531 was used. As an acidified control to exclude the effect of acid, cfsC was used,which is essentially a freeze-dried uninoculatedmMRS broth, and whichhad been adjusted to pH 4.0 with lactic acid.Using *P. infestans* 16 as an example, addition of cfsP and cfsN at a concentration of 0.5 % slightly affected oomycetemycelial growth compared to cfsC with same concentration and also for the negative control plate with no additive. At a concentration of 1 %, complete inhibition of *P. infestans*growth was observed for the cfsP plate while growth (albeit reduced) occurred on the cfsN plate (negative control). On acidified control plates to which cfsC (freeze-dried mMRS) was added, no differences were observed when compared to the diameter on the control plate with no additives (Fig. 2). This indicated that cfsP contained a distinct anti-oomycete factor or factors.

P. infestans growth in presence of *Lb. amylovorus* cell free supernatant

Microtitreplate assays were used to determine the growth of *P. infestans* in pea broth containing different concentrations of cell-free supernatant of *Lb. amylovorus*JG2 (cfsP).Final concentrations of JG2 cell-free supernatant used were 0, 0.2, 0.4, 0.8, 1.5, 3.0, 6.0, 12.5, 25.0 or 50.0(%). When 12.5% (or greater) JG2 cell-free supernatant was added, no change in OD_{620} was detected over 120hrs. When 6% or 3% was used, *P. infestans*growth was inhibited during incubation over the same time range.

Table.1 Zone around bacterial streaks indicate antifungal activity of lactic acid bacteria against *P. infestans*. Distances between the peripheral sides of the bacterial-lines and the starting fungal growth zone were recorded in **mm**. The table includes the five antifungal positive LAB strain and a negative control strain for each, indicated by an asterisk. Negative control strains have the same growth rates and exhibit the same final culture pH as their respective antifungal partner.

Genus and Species	*P. infestans* 05	*P. infestans*16
*Lactobacillus arizonesis*R13	8	15
*Lactobacillus arizonesis*R14*	2	5
*Lactobacillus amylovorus*JG2	11	16
*Lactobacillus amylovorus*DSM20531*	0	0
*Lactobacillus brevis*JJ2P	7	8
*Lactobacillus brevis*NL*	7	2
*Weissellacibaria*FF3PR	8	8
*Weissellacibaria*E7*	6	2
*Lactobacillus reuteri*R2	10	15
*Lactobacillus reuteri*M13*	0	0

Table.2 Effect of pH on fungal growth. Fungal colony diameter (cm) after inoculation of *P.infestans*on pea agar adjusted to pHs ranging from 2.0 to 9.0.

pH of agar	2.0	2.5	3.0	3.5	4.0	5.0	6.0	7.0	8.0	9.0
Phytophthorainfestans 05	0.0	0.0	0.0	6.5	8.5	8.5	8.5	8.5	8.5	8.5
Phytophthorainfestans 16	0.0	0.0	0.0	7.0	8.5	8.5	8.5	8.5	8.0	8.0

Figure.1 Antifungal activity of *Lb. amylovorus*JG2 against *P. infestans*05:(a) *P. infestans* grown 10 days at 20°C on mMRS agar plate with no LAB present. (b) *P. infestans* grown with negative control *Lb. amylovorus*DSM20531. (c) *P. infestans* grown with *Lb. amylovorus*JG2 showing clear zone of fungal inhibition.

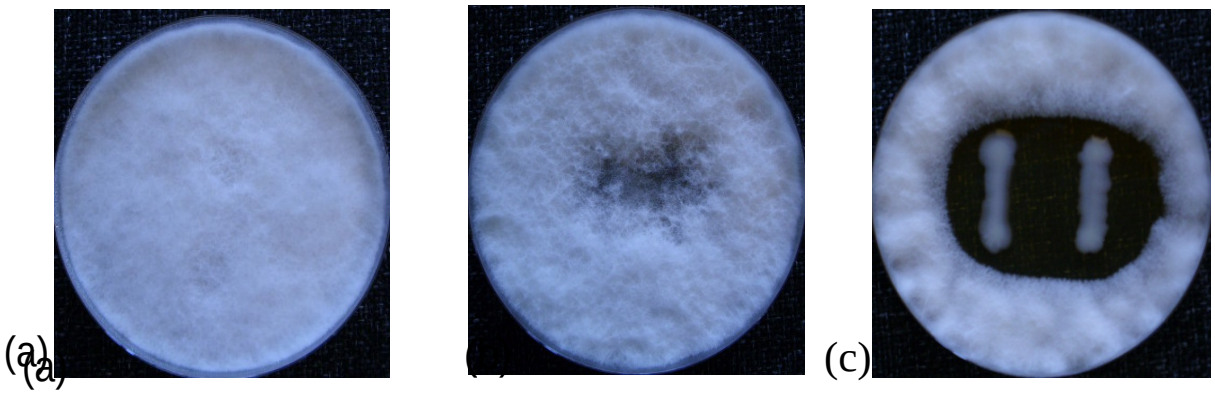
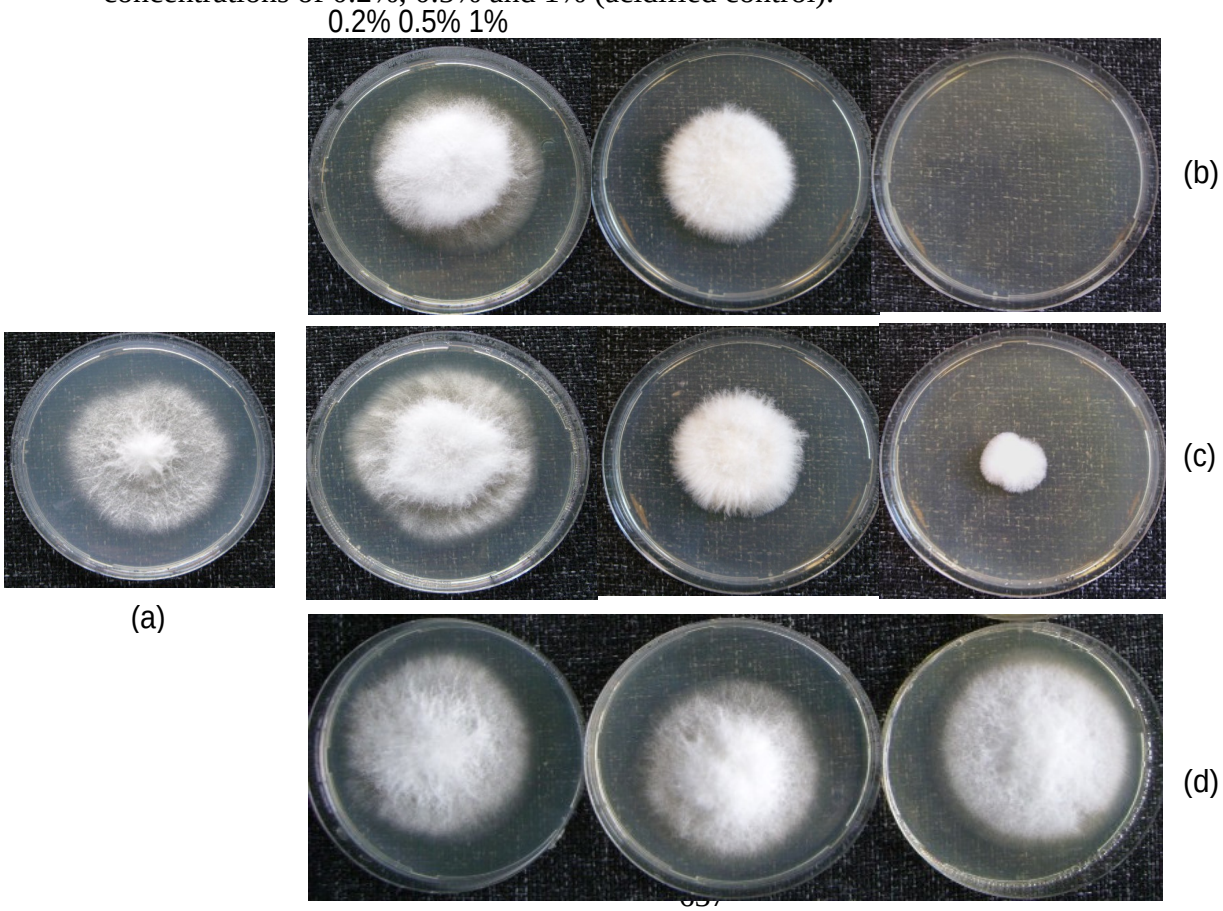


Figure.2 *P. infestans*mycelial radial growthafter 15 days incubation on pea agar plates containing (a) no freeze-dried additives (control); (b) freeze-dried cell-free supernatant of *Lb. amylovorus*JG2 at pH 4 (cfsP) with concentrations of 0.2%, 0.5% and 1%; (c) freeze-dried cell-free supernatant of *Lb. amylovorus*DSM2031 at pH 4 (cfsN) with concentrations of 0.2%, 0.5% and 1% (negative LAB control) or (d) freeze-dried mMRS at pH 4 (cfsC) with concentrations of 0.2%, 0.5% and 1% (acidified control).



Reducing the concentration of F2 cell free supernatant in pea broth to 1.5%caused in the loss of antimicrobial activity. Profiles are shown in Fig. 3. Concentrations of JG2 cell-free supernatant lower than 1.5% did not affect the growth of *P. infestans*(data not shown).

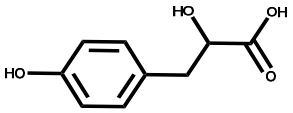
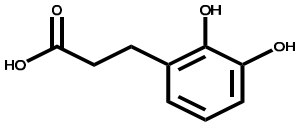
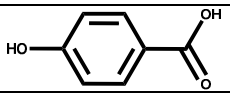
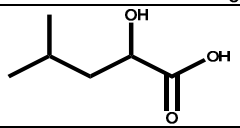
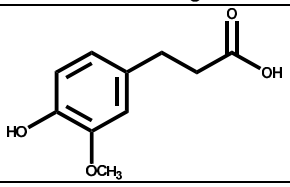
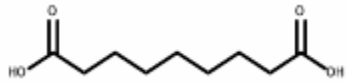
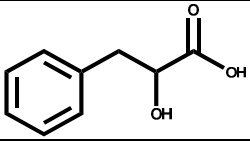
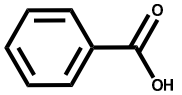
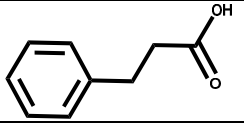
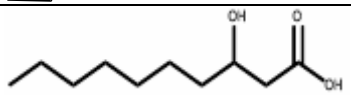
Identification of antifungal compounds in *Lb. amylovorus* JG2 using LC-FTMS

Once LLE extraction with ethyl acetate has been completed on *Lb. amylovorus*JG2and DSM 20531,the strains wereanalysed by LC-FTMS using an Accela LC instrument coupled to a LTQ Orbitrap XL mass spectrometer. Retention times and high mass accuracy spectrum were compared to that of twenty five known antifungal compounds produced by LAB. Fifteen anti-fungal compounds from the known twenty five were identified in the *Lb. amylovorus*JG2and DSM 20531 strain. Chromatographic profiles of the compounds identified are shown in Fig. 4.Compounds identified were as follows (D) DL-ρ-hydrophenyllactic acid, (F) 3,4-dihydroxyhydrocinnamic acid, (G) 4-hydroxybenzoic acid, (J) (S)-(-)-2-hydroxy-isocapric acid, (L) 3-(4-hydroxy-3-methoxyphenyl)propionic acid, (O) azelaic acid, (Q) benzoic acid, (R) hydrocinnamic acid, (T) 3-hydroxydecanoic acid, (U) DL-β-hydroxylauric acid, (V) decanoic acid, (W) DL-β-hydroxymyristic acid, (X) 2-hydroxydodecanoic acid, (Y) salicylic acid. The chemical formula, structure and quantities found of each compound detected in the positive (JG2) and the negative (DSM 20531) are shown in Table 3.All identified anti-fungal compounds were matched against their equivalent standard peak retention times and spectra. Each identified negative ion, $[M-H]^-$, mass was compared to its theoretical mass and a PPM error value was calculated. PPM errors below 3 ppm tolerance ensure that there is only one possible molecular formula for that identified compound. All identified anti-fungal compounds in the *Lb. amylovorus*JG2 strain had PPM errors between 0.5-2 PPM when compared to their equivalent standard $[M-H]^-$ions. Therefore we can unequivalently conclude that these fifteen anti-fungal compounds are present in the *Lb. amylovorus*JG2 and DSM20531 strains.

Discussion

Late blight caused by the oomycete *P.infestans*, is a major disease of potato and tomato worldwide and can cause up to 100% yield losses (Fry and Goodwin, 1997; Judelson, 1997; Fry, 2008).*Phytophthora infestans* was the causative agent of the Irish potato famine in the late 1840s (Ristaino, 2002). The disease is considered a re-emerging problem and still causes major epidemics on potato crops worldwide (Fry, 2001b; Ristaino, 2002; Slininger *et al.*, 2007). Traditionally the disease has been controlled with a "Bordeaux mixture", an environmentally very unfriendly crop protection agent consisting of copper sulphate and calcium hydroxide. Synthetic fungicides have been extensively used to control the late blight since mid-20^{th}century.Thishas been the cheapest and most effective approach for the control of plant diseases. However, these chemicals may cause toxic residues in treated products and also lead to environmental pollution, owing to their slow biodegradation (Thorstensenand Lode, 2001). Current late blight control systems are still based on the application of pesticides (Cooke et al.,2011), which have been increased in recent decades due to the emergence of new resistant strains of the

Table.3 Name, chemical formula, structure and concentration calculated of the fifteen LAB compounds detected in the antifungal strain*Lactobacillus amylovorus* JG2 and the non-antifungal strain *Lactobacillus amylovorus DSM20531* by using the LTQ Orbitrap XL hybrid mass spectrometer.

Compounds	Chemical formula	Structure	Calculated concentration (mg/L)	
			DSM 20531	JG2
D) DL-ρ-Hydroxyphenyllactic acid	$C_9H_{10}O_4$		1.97	4.15
F) 3,4-dihydroxyhydrocinnamic acid	$C_9H_{10}O_4$		0.06	0.15
G) 4-Hydroxybenzoic acid	$C_7H_6O_3$		0.03	0.03
J) (S)-(−)-2-Hydroxyisocaproic acid	$C_6H_{12}O_3$		5.73	21.63
L) 3-(4-hydroxy-3-methyphenyl)propionic acid	$C_{10}H_{12}O_4$		-	0.03
O) Azelaic acid	$C_9H_{16}O_4$		0.05	0.06
P) Phenyllactic acid	$C_{10}H_{12}O_3$		14.33	39.79
Q) Benzoic acid	$C_7H_6O_2$		0.18	0.2
R) Hydrocinnamic acid	$C_9H_{10}O_2$		0.03	0.03
T) 3-Hydroxydecanoic acid	$C_{10}H_{20}O_3$		0.3	0.45

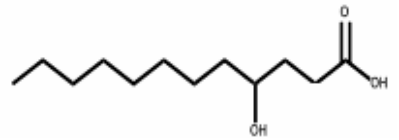
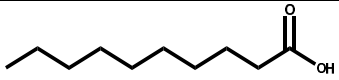
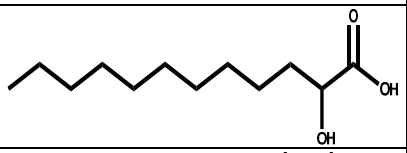
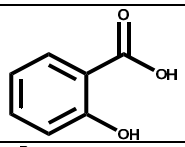
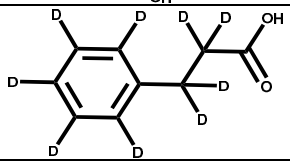
U) DL-β-hydroxylauric acid	$C_{12}H_{24}O_3$		0.02	0.10
V) Decanoic acid	$C_{10}H_{20}O_2$		-	0.64
X) 2-Hydroxydodecanoic acid	$C_{12}H_{24}O_3$		0.26	0.45
W) DL-β-Hydroxymyrstric acid	$C_{14}H_{28}O_3$		-	0.25
Y) Salicylic acid	$C_7H_6O_3$		0.26	0.46
Hydrocinnamic acid D9 (Internal standard)	$C_9HO_2D_9$		N/A	N/A

Figure.3 Growth of *P. infestans*in pea broth containing 25% (■), 12.5% (▲), 6 % (X), 3 % (+), 1.5% (●) or 0 %(◆) of cell-free supernatant of *Lb. amylovorus*JG2.

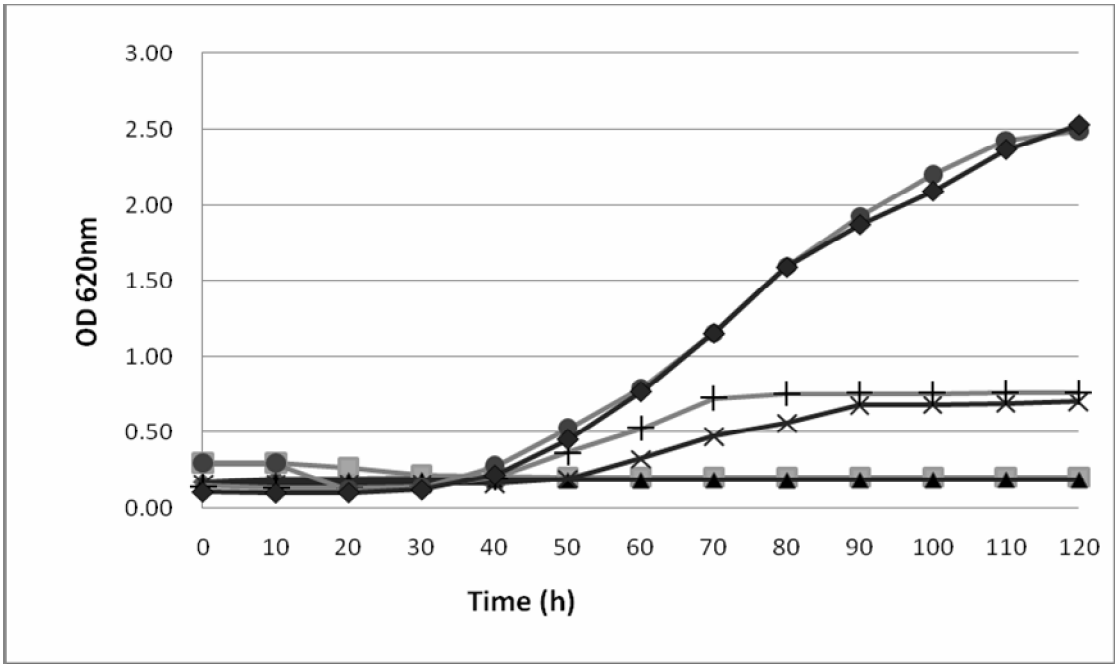
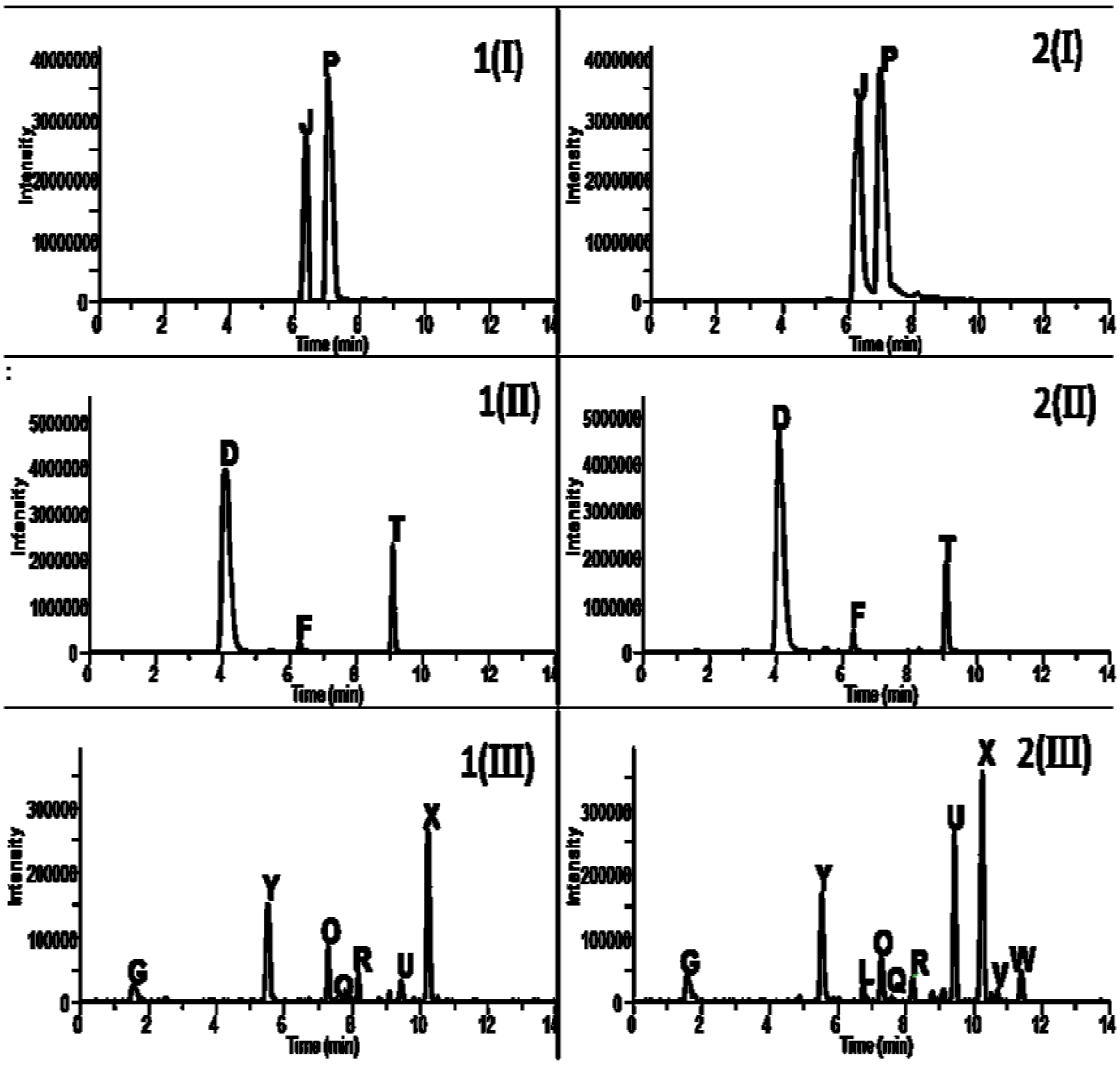


Figure.4 Chromatograms obtained for *Lactobacillus amylovorus* strains JG2 and DSM20531 separated on a Gemini C18 column as outlined in the material and methods section. Due to the varying intensities found between the compounds detected three layouts (I), (II) and (III) at different intensity ranges are used to clearly show the compounds found in each sample. 1(I), 1(II) and 1(III) corresponds to DSM20531 (non-antifungal) and 2(I), 2(II) and 2(III) correspond to JG2 (antifungal). Compounds identified included: D) DL-ρ-Hydroxyphenyllactic acid, F) 3,4-dihydroxyhydrocinnamic acid, G) 4-Hydroxybenzoic acid, J) (S)-(−)-2-Hydroxyisocaproic acid, L) 3-(4-hydroxy-3-methyphenyl) propionic acid, O) Azelaic acid, P) Phenyllactic acid, Q) Benzoic acid, R) Hydrocinnamic acid, T) 3-Hydroxydecanoic acid, U) DL-β-hydroxylauric acid,V) Decanoic acid, W) DL-β-Hydroxymyrstric acid, X) 2-Hydroxydodecanoic acid, Y) Salicylic acid. Concentrations detected for each compound can be found in Table 3.



pathogen (Fry and Goodwin, 1997). Recently, many researchers concentrated their attention on the potencialuse of alternative natural products to preserve crops from fungicide-resistant fungi. The management of pathogens with the use of microbial and plant sources is now considered to be an effective yet environmental friendly disease control technology (Copping and Menn, 2000; Kouland Dhaliwal, 2002).However, there are only few studies performed using microorganisms or microorganism metabolites as biocontrol agents to control potato late blight. Recent studies have focused on the utilization of *Bacillus*, *Enterobacter*, *Flexibacter*, *Micrococcus*, *Pseudomonas* and *Xenorhabdus* (Ajay and Sunaina, 2005; Hultberg *et al.,* 2010; Kim and Jeun, 2006; Silva *et al.,* 2004; Slininger et *al.,*2007; Yan *et al.,* 2002; Yang *et al.,*2011; Zakharchenko *et al.,* 2011). In addition fungi such as *Fusarium oxysporum* (Kim *et al.,* 2007; Son et al.,2008) and *Arbuscularmycorrhizal* (AM) fungi (Gallou *et al.,*2011) have also been evaluated. Itis important to know that these genera are generally categorized as non-food grade. To the best of our knowledge, there is neither fundamental research of Lactic acid bacteria, which have a GRAS (Generally Recognized As Safe) status being used as bio control agents against potato late blight nor published studies of identified antifungal compounds produced by LAB to inhibit the growth of *P. infestans*.

LAB have previously been used for biopreservation of a range of fermented foods and feed. (Vermeiren *et al.,* 2004;Mauch *et al.,*2010, Ryan *et al.,*2011).Earlier studies in our laboratory identified 220 LAB strains which were isolated from various environments/ Conditions and tested against food fungi such as *Aspergillus*and *Penecillium*.Strains with strong antifungal activity were selected to test against *P. infestans*in this study. All five selected LAB strains showed some degree of antimicrobial acitivity against *P. infestans*. The strongest inhibition was observed by *Lb. amylovorus*JG2, the distances between the peripheral sides of the bacterial-lines and the starting *P. infestans* growth zones were large. The most appropriate negative control strain *Lb. amylovorus* DSM20531 did not appear to cause any inhibition, suggesting that anti-*P. infestans* activity of *Lb. amylovorus*is a strain dependant trait.This observation is similar to the work of Lavermicocca *et al.,*(2000) who screened a number of LAB strains isolated from sourdough bread and found that the rate of inhibition of a number of fungal species was highly strain dependent. Another work has been done by Mauch *et al.,(*2010)who used LABagainst food spoilage fungi *Fusarium*. Mauch *et al.,* (2010) found that *Lb. brevis* was the dominant species among the most inhibitory LAB from 129 isolates, with four out of the five most inhibitory strains belonging to this species. However, they isolated a *Lb. brevis* strain among the non-active LAB, which likewisesignifying that antifungal activity of *Lb. brevis* is strain dependent.

In this study, *Lb. amylovorus*JG2 was selected toinvestigateanti-oomycete activity in depth against *P. infestans*. The metabolites produced by*Lb. amylovorus* JG2 affected *P. infestans* mycelial radial growth as well as oomycetegrowth in liquid medium.Addition of freeze dried cell free supernatant (cfsP) of *Lb. amylovorus*JG2at concentrations less than 0.2% did not affect the mycelial radial growth, the using of 0.5 % slightly affect oomycete mycelia growth. At a

concentration of 1 %, nooomycetegrowth was observed for the cfsPplate. No differencewas observed from acidified control plates when compared to the control plate without additives. Freeze dried cell free supernatant of *Lb. amylovorus*DSM20531(cfsN) at 1% did affect the *P. infestans* mycelia growth.Whilst it caused a weakened oomycetegrowth,this antifungal activity was weakerwhen compared with cfsP plate, where it caused a complete inhibition of *P. infestans* growth. This indicated that other anti-oomycete compounds must be specifically produced (or produced at higher levels) by *Lb. amylovorus*JG2.Addition of JG2 cell free supernatant into liquid medium at 3% or 6%resulted in inhibition of *P. infestans*during incubation over 120hrs. When 12.5% (or greater) JG2 cell free supernatant was added, no oomycetegrowth was detected over the same time range. This compares well with a recent study in anti-dermatophytes activity of LAB where supernatants of*Lb. reuteri*which were evaluated in microtitreassays and morphology assay (Guoet al.,2011). In that study,when freeze dried cell free supernatant of *Lb. reuteri*was added into the medium greater than 1% retarded fungal colony growth. Addition of the powder at 10% to liquid cultures caused complete inhibition of fungal growth on the basis of turbidity.

Two previous studies assessed the potential of *Lb. amylovorus* to inhibit the outgrowth of some common food-spoiling fungi. Onestudyby Ryan et al.,(2011)focused on the antifungal activity of *Lb. amylovorus*. In that study, *Lb.amylovorus* DSM19280 was shown to produce a wide spectrum of antifungal compounds active against common bread spoilage fungi *Aspergillus fumigatus* and *Fusarium culmorum.* The bread fermented with *Lb. amylovorus* DSM19280was evaluated for the ability to retard the growth of *Fusarium culmorum, Aspergillus niger, Penicillium expansum, Penicillium roqueforti* and fungal flora from the bakery environment. They also found that breads containing sourdough fermented with *Lb. amylovorus* DSM19280 were more effective in extending the shelf life of bread than the chemical preservativecalcium propionate. Another study reported by De Muynck *et al.*,(2004) involved the evaluation of the culture supernatants of 20 Lactic acid bacterial strains for antifungal activity using an agardiffusion method. *Lb.amylovorus*DSM 20532and other four LAB strains were shown to have very strong antifungal activity.It was found that the antifungal metabolites produced in their study were pH-dependent, whereas the exact chemical nature of those substances was not elucidated.The problem was addressedin thisstudy, fifteen previously identified antifungal metabolites (Strom *et al.,* 2002;Broberg*et al.,* 2007; Sjögren*et al.,* 2003; Ryan *et al.*,2011) were detected in both*Lb.amylovorus*JG2 (antifungal) and DSM20531 (non-antifungal). This information provides the first documented data with regards to compounds present in a negative non-antifungal strain in comparison with a positive antifungal strain. The data obtained shown that the same compounds are present in both strains but at varying quantities.It is likely that this concentrationvariation betweenthese compounds is responsible for the anti-*P. infestans* activity.Overall a larger quantity was detected of the compound identified by the JG2 strain. The quantities determined for the compounds ranged from 0.03-39.79 mg/L in JG2 and 0.02-14.33 mg/L in DSM

20531. Exact quantities detected are detailed in Table 3.

The development of fungicide-resistant *P. infestans* have placed significant strain on the potato production and processing industry. Biological control accomplished by beneficial microorganisms such as lactic acid bacteria and their formed metabolites may be a viable "green approach" to reducing late blight

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References

Ajay, S., and Sunaina, V., 2005. Direct inhibition of *Phytophthora infestans*, the causal organism of late blight of potato by *bacillus antagonists*. Potato J. (Shimla). 32: 179-180.

Broberg, A., K. Jacobsson, K. Ström and Schnürer, J. 2007. Metabolite profiles of lactic acid bacteria in grass silage. Appl. Environ. Microbiol. 73: 5547-52.

Brosnan, B., A. Coffey, E. Arendt and Furey, A. 2012. Rapid Identification of Anti-Fungal Compounds Produced by Lactic Acid Bacteria using the LTQ - Orbitrap XL Hybrid Mass Spectrometer. Anal. Bioanal. Chem. 403: 2983 - 2995.

Caplice, E., and Fitzgerald, G. F., 1999. Food fermentations: role of microorganisms in food production and preservation. Int. J. Food Microbiol. 50: 131 – 149.

Chycoski,C. I., and Punja, Z. K., 1996. Characteristics of populations of *Phytophthorai nfestans* from potato in British Columbia and other regions of Canada during 1993 to 1995. Plant. Dis.80:579 -589.

Coda, R., A. Cassone, C.G. Rizzello, L. Nionelli, G. Cardinali and Gobbetti, M., 2011. Antifungal activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during sourdough fermentation: identification of novel compounds and long-term effect during storage of wheat bread. Appl. Environ. Microbiol. 77: 3484-92.

Cooke, L. R., H. Schepers, A. Hermansen, R.A. Bain, N.J. Bradshaw, F. Ritchie, D.S. Shaw, A. Evenhuis, G.J.T. Kessel, et al., 2011. Epidemiology and Integrated Control of Potato Late Blight in Europe. Potato Res. 54: 183-222.

Copping, G. L., and Menn, J. L., 2000.Biopesticides: a review of their action, applications and efficacy. Pest Manag. Sci. 56: 651–676.

Daayf, F., and Platt, H. W., 2000.Changes in metalaxyl resistance among glucose phosphate isomerase genotypes of *Phytophthora infestans* in Canada during 1997-1998.Am. J. Potato Res. 77: 311-318.

Dal Bello, F., C.I. Clarke, L.A.M. Ryan, H. Ulmer, T.J. Schober, K. Strom, J. Sjogren. D. van Sinderen, J. Schnurer and Arendt, E. K. 2007. Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. J.Cereal. Sci. 45,309-318.

De Muynck, C., A.I. Leroy, S. De Maeseneire, F. Arnaut, W. Soetaert and Vandamme, E. J., 2004. Potential of selected lactic acid bacteria to produce food compatible antifungal metabolites. Microbiol.Res. 59: 339-46.

De Vuyst, L., and Leroy, F. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. J. Mol.Microbiol.Biotechnol. 13: 194-9.

Djossou, O., I. Perraud-Gaime, F.L. Mirleau, G. Rodriguez-Serrano, G. Karou, S. Niamke, I. Ouzari, A. Boudabous and Roussos, S. 2011. Robusta coffee beans post-harvest microflora: *Lactobacillus plantarum* sp. as potential antagonist of *Aspergillus carbonarius*. Anaerobe. 17: 267-72.

Florianowicz, T., 2001.Antifungal activity of some microorganisms against *Penicillium expansum*. Eur. Food. Res. Technol. 212, 282-286.

Fry, W. E., S.B. Goodwin, A.T. Dyer, J.M. Matuszak, A. Drenth, P.W. Tooley, L.S. Sujkowski, Y.J. Koh, B.A. Cohen, L.J. Spielman, K.L. Deahl, D.A. Inglis and Sandlan, K. P. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. Plant Dis. 77: 653-661.

Fry, W., 2008. *Phytophthora infestans*: the plant (and R gene) destroyer. Mol. Plant Pathol.9, 385-402.

Fry, W. E., and Goodwin, S. B., 1997. Re-emergence of potato and tomato late blight in the United States.Plant Dis. 81: 1349-1357.

Gallou, A., H.P.L. Mosquera, S. Cranenbrouck, J.P. Suarez and Declerck, S. 2011. Mycorrhiza induced resistance in potato plantlets challenged by Phytophthora infestans. Physiol. and Mole. Plant Pathol. 76: 20-26.

Garde, S., M. Avila, R. Arias, P. Gaya and Nuñez, M. 2011.Outgrowth inhibition of *Clostridium beijerinckii* spores by a bacteriocin-producing lactic culture in ovine milk cheese. Int. J. Food. Microbiol.150:59-65.

Gerez, C. L., M.S. Carbajo, G. Rollán, G. Torres Leal and Font de Valdez, G., 2010. Inhibition of citrus fungal pathogens by using lactic acid bacteria. J. Food Sci. 75:354-9.

Goodwin,S. B., B.A. Cohen, K.L. Deahl and Fry, W. E., 1994. Migration from northern Mexico as the probable cause of recent genetic changes in population of *Phytophthora infistans* in the United States and Canada. Phytopathol. 84: 553-558.

Guenther, J. F., K.C. Michael and Nolte, P. 2001.The economic impact of potato late blight on US growers. Potato Res.44: 121-125.

Guo, J., A. Mauch, S. Galle, P. Murphy, E.K. Arendt and Coffey, A. 2011.Inhibition of growth of *Trichophyton tonsurans* by *Lactobacillus reuteri*. J. Appl.Microbiol. 111:474-83.

Guo, J., B. Brosnan, A. Furey, E.K. Arendt, P. Murphy and Coffey, A., 2012. Antifungal activity of Lactobacillus against *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum*. Bioeng Bugs. 3(2): 1-10.

Haldar, K., S. Kamoun, N.L. Hiller, S. Bhattacharjee and van Ooij, C. 2006.Common infection strategies of pathogenic eukaryotes. Nat. Rev.Microbiol.4: 922–931.

Hassan, Y. I., and Bullerman, L. B., 2008. Antifungal activity of *Lactobacillus paracasei* ssp. tolerans isolated from a sourdough bread culture. Int. J. Food Microbiol.121: 112-115.

Haverkort, A. J., P.M. Boonekamp, R. Hutten, E. Jacobsen, L.A.P. Lotz, G.J.T. Kessel, R.G.F. Visser and van der Vossen, E. A. G., 2008. Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. Potato Res. 51: 47–57.

Ho, P. H., J.B. Luo and Adams, M. C., 2009. Lactobacilli and dairy propionibacterium with potential as biopreservatives against food fungi and yeast contamination. Prikl Biokhim.Mikrobiol. 45:460-4.

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

Jones, R. J., H.M. Hussein, M. Zagorec, G. Brightwell and Tagg, J. R., 2008.Isolation of lactic acid bacteria with inhibitory activity against pathogens and spoilage organisms associated with fresh meat. Food. Microbiol. 25;228-34.

Judelson, H. S., 1997. The genetics and biology of *Phytophthora infestans*: modern approaches to a historical challenge. Fungal Genet. Biol. 22: 65–76.

Kim, H. J., and Jeun, Y. C., 2006. Resistance induction and enhanced tuber production by pre-inoculation with bacterial strains in potato plants against Phytophthora infestans. Mycobiol. 34: 67-72.

Kim, H. Y., G.J. Choi, H.B. Lee, S.W. Lee, H.K. Lim, K.S. Jang, S.W. Son, S.O. Lee,

K.Y. Cho, N.D. Sung and Kim, J. C., 2007. Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. Lett. Appl.Microbiol. 44:332-7.

Koul, P., and Dhaliwal, G. S., 2002. Microbial. Biopesticides New York, NY: Taylor & Francis Inc.

Latijnhouwers, M., P.J. de Wit and Govers, F. 2003.Oomycetes and fungi: similar weaponry to attack plants. Trends in Microbiol.11: 462-469.

Lavermicocca, P., F. Valerio, A. Evidente, S. Lazzaroni, A. Corsetti and Gobbetti, M. 2000. Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl. Environ. Microbiol. 66: 4084-4090.

Magnusson, J., and Schnürer, J. 2001. *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. Appl. Environ.Microbiol.67(1): 1-5.

Maragkoudakis, P. A., K.C. Mountzouris, D. Psyrras, S. Cremonese, J. Fischer, M.D. Cantor and Tsakalidou, E. 2009.Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and Salmonella *enteritidis*. Int. J. Food Microbiol.130(3): 219-26.

Mauch, A., F. Dal Bello, A. Coffey and Arendt, E. K. 2010. The use of *Lactobacillus brevis* PS1 to in vitro inhibit the outgrowth of *Fusariumculmorum* and other common *Fusarium* species found on barley. Int. J. Food Microbiol.141: 116–121.

McDonald, B. A., and Linde, C., 2002.Pathogen population genetics,evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40: 349–379.

Montarry, J., R. Corbie`re and Lesueur, S. 2006. Does selection by resistant host trigger local adaptation in plant-pathogen systems? J.Evolu.Biol. 19: 522–531.

Muñoz, R., M.E. Arena, J. Silva and González, S. N. 2010. Inhibition of mycotoxin-producing *Aspergillus nomius* VSC 23 by lactic acid bacteria and *Saccharomyces cerevisiae*. Brazilian J.Microbiol. 41: 1019-1026.

Murry, A. C., A. Hinton and Morrison, H., 2004. Inhibition of growth of *Escherichia coli*, *Salmonella typhimurium*, and *Clostridia perfringens* on chicken feed media by *Lactobacillus salivarius* and *Lactobacillus plantarum*. Int. J. Poult. Sci. 3: 603 – 607.

Nirenberg, H., 1976. Untersuchungenüber die morphologischeDifferenzierung in der Fusarium- SektionLiseola. Mitt. Biol.Bundesanst. Land–Forstwirtsch. 169: 1–117.

Reader, J., 2009. Potato: A History of the Propitious Esculent (Yale Univ. Press).

Ristaino, J. B., 2002. Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans.* Microbes Infect. 4: 1369–1377.

Ryan, L. A., E. Zannini, F. Dal Bello, A. Pawlowska, P. Koehler and Arendt, E. K. 2011. *Lactobacillus amylovorus* DSM 19280 as a novel food-grade antifungal agent for bakery products. Int. J. Food Microbiol.146(3): 276-83.

Silva, H. S. A., R.S. Romeiro, R. Carrer, J.L.A. Pereira, E.S.G. Mizubuti and Mounteer, A., 2004. Induction of systemic resistance by Bacillus cereus against tomato foliar diseases under field conditions. J. Phytopathol. 152: 371-375.

Sjogren, J., J. Magnusson, A. Broberg, J. Schnurer and Kenne, L. 2003.Antifungal 3-hydroxy fatty acids from *Lacotobacillusplantarum*MiLAB 14. Appl. Environ. Microbiol. 69: 7554–7557.

Slininger, P. J., D.A. Schisler, L.D. Ericsson, T.L. Brandt, M.J. Frazier, L.K. Woodell, N.L. Olsen and Kleinkopf, G. E. 2007. Biologicalcontrol of post-harvest late blight ofpotatoes.Biocontrol Sci. and Technol. 17: 647–663.

Son, S. W., H.Y. Kim, G.J. Choi, H.K. Lim, K.S. Jang, S.O. Lee, S. Lee, N. Sung and Kim, J. C. 2008. Bikaverin and fusaric acid from*Fusariumoxysporum*show antioomycete activity against

Phytophthora infestans. J. Appl.Microbiol. 104(3): 692-8.

Strom, K., J. Schnurer and Melin, P., 2005. Co-cultivation of antifungal *Lactobacillus plantarum* MiLAB393 and *Aspergillus nidulans*, evaluation of effects on fungal growth and protein expression. FEMS Microbiol. Lett. 246: 119-124.

Strom, K., J. Sjogren, A. Broberg and Schnurer, J., 2002.*Lactobacillus plantarum* MiLAB393 produces the antifungal cyclic dipeptides cyclo (L-Phe-L-Pro) and cyclo(LPhe- trans-4-OH-L-Pro) and 3-phenyllactic acid. Appl. Environ.Microbiol. 68: 4322–4327.

Tamminen, M., T. Joutsjoki, M. Sjo¨blom, M. Joutsen, A. Palva, E.L. Ryha¨nen and outsjoki, V. 2004. Screening of lactic acid bacteria from fermented vegetables by carbohydrate profiling and PCR-ELISA. Lett. Appl.Microbiol. 39 439–444.

Thorstensen, C. W., and Lode, O. 2001.Laboratory degradation studies of bentazone, dichlorprop, MCPA, and propiconazole in Norwegian soils. J. Environ.Qual. 30: 947–953.

Topisirovic, L., M. Kojic, D. Fira, N. Golic, I. Strahinic and Lozo, J. 2006.Potential of lactic acid bacteria isolated from specific natural niches in food production and preservation. Int. J. Food Microbiol.112(3) 230-5.

Vermeiren, L., F. Devlieghere and Debevere, J. 2004.Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. Int J Food Microbiol.96(2): 149-64.

Voulgari, K., M. Hatzikamari, A. Delepoglou, P. Georgakopoulos, E. Litopoulou-Tzanetaki and Tzanetakis, N. 2010. Antifungal activity of non-starter lactic acid bacteria isolates from dairy products. Food Control.21: 136–142.

Yan, Z. N., M.S. Reddy, C.M. Ryu, J.A. McInroy, M. Wilson and Kloepper, J. W. 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathol. 92: 1329-1333.

Yang, E. J., and Chang, H. C., 2010.Purification of a new antifungal compound produced by *Lactobacillus plantarum* AF1 isolated from kimchi.Int. J. Food Microbiol.139(1-2): 56-63.

Yang, X. F., D.W. Qiu, W.H. Yang, Z. Liu, H.M. Zeng and Yuan, J. J. 2011. Antifungal activity of xenocoumacin 1 from *Xenorhabdus nematophilus* var. *pekingensis* against *Phytophthora infestans*. World J. Microbiol.& Biotechnol. 27: 523-528.