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Bacillus velezensis RC 218 as a biocontrol agent to reduce *Fusarium* head blight and deoxynivalenol accumulation: Genome sequencing and secondary metabolite cluster profiles

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ABSTRACT

Bacillus subtilis RC 218 was originally isolated from wheat anthers as a potential antagonist of *Fusarium graminearum*, the causal agent of *Fusarium* head blight (FHB). It was demonstrated to have antagonist activity against the plant pathogen under *in vitro* and greenhouse assays. The current study extends characterizing *B. subtilis* RC 218 with a field study and genome sequencing. The field study demonstrated that *B. subtilis* RC 218 could reduce disease severity and the associated mycotoxin (deoxynivalenol) accumulation, under field conditions. The genome sequencing allowed us to accurately determine the taxonomy of the strain using a phylogenomic approach, which places it in the *Bacillus velezensis* clade. In addition, the draft genome allowed us to use bioinformatics to mine the genome for potential metabolites. The genome mining allowed us to identify 9 active secondary metabolites conserved by all *B. velezensis* strains and one additional secondary metabolite, the lantibiotic ericin, which is unique to this strain. This study represents the first confirmed production of ericin by a *B. velezensis* strain. The genome also allowed us to do a comparative genomics with its closest relatives and compare the secondary metabolite production of the publically available *B. velezensis* genomes. The results showed that the diversity in secondary metabolites of strains in the *B. velezensis* clade is driven by strains making different antibacterials.

1. Introduction

Fusarium graminearum sensu stricto is the main causal agent of *Fusarium* head blight (FHB) in wheat in Argentina. During the last 60 years several epidemics of FHB occurred with reductions in grain yield, quality and safety due to the contamination with trichothecenes mainly deoxynivalenol (DON) (Dalcero et al., 1997; Kikot et al., 2011; Palazzini et al., 2015). This toxin, besides representing a constraint for human and animal health (Pestka, 2010), it facilitates disease development by acting as a virulence factor allowing the disease to spread during the infection process (Proctor et al., 1995; Talas et al., 2012).

Due to the toxicity of trichothecenes, the European Union has established a maximum tolerance level of 0.75 ppm (mg/kg) for DON in food commodities for human consumption (EC Regulation, 2006) and the FDA in United States has set an advisory level of DON at 1 ppm (mg/kg) in wheat products intended for human consumption (US-FDA, 2010). Strict management of FHB is required to meet these tolerance levels.

Different strategies have been used to reduce the impact of FHB including fungicides treatment, planting less susceptible cultivars, but none of them are enough to control the problem (McMullen et al., 2012; Mesterházy et al., 2011; Miedaner and Korzun, 2012). Management of the disease using biocontrol is an alternative to reduce FHB and the accumulation of DON in the frame of an integrated pest management (IPM) (Da Luz et al., 2003; Khan and Doohan, 2009; Khan et al., 2001, 2004; Schisler et al., 2002, 2004, 2006; Zhao et al., 2014). The demand of biopesticides has been increasing steadily worldwide due to increased environmental awareness and the pollution potential and health hazards from many of the chemical pesticides. Biopesticides can supplement the conventional chemical pesticides when used in IPM (Thakore, 2006).

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Members of the *Bacillus* genus are considered microbial factories for the production of a vast array of biologically active molecules potentially inhibitory of pathogenic fungi and bacteria. Among these antimicrobial compounds, cyclic lipopeptides (LPs) including surfactin, iturin and fengycin families are relevant in strains isolated as biocontrol agents (Arguelles-Arias et al., 2009; Chen et al., 2007; Dunlap et al., 2013). In addition, it has been shown that LPs induce disease resistance in plants (Jourdan et al., 2009; Kloepper et al., 2004; Yamamoto et al., 2015) and confer an advantage to the producing *Bacillus* strains in specific ecological niches (Mukherjee and Das, 2005). *Bacillus* lipopeptides are non-ribosomally synthesized via large multi-enzymes called non-ribosomal peptide synthetases (NRPSs). In addition, these *Bacillus* biocontrol strains are known to make several polyketides, such as bacillaene, difficidin and macrolactin (Chen et al., 2009). Polyketides are a large family of secondary metabolites that include many bioactive compounds with antibacterial, immunosuppressive and antitumor bioactivities. They are synthesized by type I polyketide synthases (PKSs) modularly organized assembly lines starting from acyl-CoA precursors by decarboxylative Claisen condensations. The biosynthetic pathway follows the same logic as in non-ribosomally synthesized peptides and requires at least three modular domains.

These biocontrol properties of *Bacillus* spp. have led to the isolation and characterization of many strains in this area. Unfortunately, the large and concurrent research effort has also led to confusion and inconsistencies in the taxonomic naming of these strains. However, the availability of genome sequence data and the generation of new genome sequence data of type strains has led to a definitive understanding of the taxonomy of these strains. Recent phylogenomic studies have shown that *Bacillus velezensis*, *Bacillus methylotrophicus, Bacillus amyloliquefaciens* subsp. *plantarum* and *Bacillus oryzicola* are conspecific (Dunlap et al., 2016). Under the rules of the prokaryotic naming commission, *B. velezensis* is the valid name for this species, since its description predates the other.

In previous studies we demonstrated the ability of *Bacillus* sp. RC 218 to reduce disease severity and DON accumulation *in vitro*, under greenhouse and field trials (Palazzini et al., 2007, 2009, 2013, 2016; Palazzini, 2010). The aims of this study were: (i) to evaluate under field trial the selected potential of biocontrol agent *Bacillus* sp. RC 218 to reduce FHB and deoxynivalenol accumulation, (ii) to sequence the genome of the selected agent *Bacillus* sp RC 218, (iii) to make the phylogenomic analysis of the strain, (iv) to determine the potential for secondary metabolite production.

2. Material and methods

2.1. Biocontrol strains, biomass production and formulation

Bacillus subtilis RC 218 used in this study was originally isolated from wheat anthers as potential biocontrol agents against *F. graminearum* in Argentina (Palazzini et al., 2007, 2009). Biomass of *B. subtilis* RC 218 was produced in liquid basic medium (sucrose 10 g/l, yeast extract 5 g/l) modified with NaCl (a_W 0.97) (Costa et al., 2001) with an incubation of 48 h at 28 °C in a rotatory shaker (150 rpm). Biomass was produced in a 50 l fermentor by Bio-ferm GmbH (Tulln, Austria) in order to obtain bacterial spores. These bacterial spores were freeze dried and tested in the field trial.

2.2. Pathogen inoculum production

Two strains of *F. graminearum*, RC276 and KRC7, were used in the field trials. These strains were isolated from head blight infected ears from commercial fields located in Pergamino, Buenos Aires, Argentina. Toxigenic profiles were determined in a previous study (Palazzini et al., 2007). *F. graminearum* conidia were produced in Mung bean broth (Rosewich Gale et al., 2002). After 7–10 days of incubation at 25 °C and 200 rpm on a rotatory shaker, cultures were centrifuged (7000 rpm; 5 min), re-suspended in sterile distilled water plus Tween 80 (0.05%) and filtered through sterile gauze to obtain a conidia suspension. Conidia concentration was determined using a haemocytometer and was adjusted to 5×10^5 conidia/ml (1:1 mixture of RC276 and KRC7 strains).

2.3. Field trial conditions

The field trial was conducted in Marcos Juarez, Córdoba province, Argentina, during the 2014 harvest seasons. The wheat cultivar BioInta 1005 (susceptible *to F. graminearum*) was sown at the end of July. The experimental plots consisted of 3 rows (2 m/row, 0.2 m between rows; 250 heads per plot) with three replicates per treatment. The experiments were done in a random block design with 1 m separation between plots.

The application of the biocontrol agents was done at the anthesis stage with the inoculation of *B. subtilis* RC 218 and *F. graminearum* strain mixture. The anthesis stage was considered at the period where 50% of the heads in the plots were at flowering stage (Feekes stage 10.5.2–10.5.3; Wiese, 1987). Before applications of the BCA, wheat heads were always misted with water for 2 min in order to increase the humidity in the heads. The formulated biocontrol agent were resuspended in sterile distilled water + Tween 80 (0.05%) and allowed to stabilize for 30 min before application. The viability of the biocontrol agents was evaluated by plate counting. Control negative plots were treated with sterile distilled water + Tween 80 (0.05%).

Bacterial and *F. graminearum* suspensions were applied using a commercial sprayer consisting of 5 linear sprinklers and a CO_2 pressure source. The sprayer was adjusted to 30 mbar and flow to 15 ml per second. Application was done at a rate of 15 ml per linear meter for all treatments. Wheat plots were misted with water for 5 min every 30 min from 8:00 am to 18:00 pm for six days after inoculation. Water sprinklers (fine misting) were located between the plots and also surrounding them.

2.4. FHB disease evaluation

FHB disease incidence and severity were evaluated 21 days after inoculation with *F. graminearum*. FHB incidence was determined by counting infected heads and divided from the total spikes of the plot (treatment replicate); disease severity was evaluated by observing symptomatic spikelets (decoloured, browny) and visually compared with a 0–100% scale proposed by Stack and McMullen (1995) (Supplemental Fig. S1).

2.5. Deoxynivalenol accumulation in wheat heads

At harvest, wheat heads were collected to determine DON concentration in the entire heads. Toxin extraction was done using Mycosep 225 (Romer Labs) according to the manufacturer conditions. Briefly, 25 g of grains were milled and extracted with 100 ml acetonitrile:water (84:16 v/v) and shaken for 30 min. The suspension was then filtered through Whatman N°1 and 5 ml were transferred to the column and cleaned by push-through. After cleaning the filtered, 2 ml were taken and evaporated to dryness by N_2. The sample was then redissolved in 400 μl of mobile phase (methanol:water, 12:88) for HPLC quantification. Quantification was relative to external standards of DON (Biopure, Romer; 0.5, 1, 2 and 4 μg/ml). DON concentration was determined by liquid chromatography using the methodology described by Palazzini et al. (2007).

Table 1
Biocontrol activity of *Bacillus velezensis* RC 218.

Treatments	*Fusarium* Head Blight		
	Incidence (%)[a]	Severity (%)[b]	Deoxynivalenol (ppm)[c]
B. velezensis RC 218 + *F. graminearum*[d]	21.66 ± 10.4a	27.6 ± 21.9a	Nd[e]
F. graminearum	21.63 ± 8.15a	37.2 ± 23.2b	1.59 ± 0.53

[a] Incidence data were subjected Kruskal-Wallis ANOVA on ranks, no statistical differences were observed (P = 0.821).
[b] Severity data were subjected Kruskal-Wallis ANOVA on ranks, means were separated by Dunnís test. Different letters indicate significant differences (P < 0.05).
[c] Deoxynivalenol content was assessed by HPLC (Section 2.5).
[d] *F. graminearum: F. graminearum* mixture of strains applied at anthesis stage at a concentration of 5×10^5 spores/ml.
[e] Nd: not detected; Detection Limit = 0.05 ppm.

2.6. Statistical analyses

Disease incidence data was subjected to a one way ANOVA and means were separated by Holm-Sidakís method. Disease severity data were subjected to Kruskal-Wallis one way analysis of variance on ranks and means were separated with the multiple comparison Dunnís test. Deoxynivalenol accumulation was expressed as the plot means ± standard deviation. All statistical analyses were performed using Sigma Stat for Windows version 3.5 (SPSS Inc.).

2.7. Bacillus subtilis *RC 218 genome sequencing and analysis*

Biomass of *B. subtilis* RC 218 was obtained from an overnight culture grown on nutrient broth at 28 °C. DNA extraction was done using Wizard genomic DNA purification kit (Promega, USA) according to manufacturer instructions. The genome was sequenced using MiSeq DNA sequencer using the MiSeq V3 2 × 300 sequencing kit following the manufacturer's suggested protocols. The resulting reads were quality trimmed to the Q30 confidence level. The draft genome was assembled using CLC bioGenomics Workbench 8.0 (Qiagen Inc., Cambridge, MA) using default parameters. The sequences were deposited in NCBI GenBank under accession number LQCL00000000. Genome comparisons and alignments for phylogenetic trees were made using BIGSdb software(Jolley and Maiden, 2010). Comparative genomics were based on gene annotations based on RAST server (Overbeek et al., 2014). Secondary metabolite clusters were identified with antiSMASH3.0 (Weber et al., 2015) or direct blasting. The phylogenetic tree is based on the core genome of the 73 strains analyzed and consisted of 2740 genes. The phylogenetic tree was constructed using MEGA 6.06 software (Tamura et al., 2013). The neighbour-joining tree were reconstructed using the Tamura-Nei model (Tamura and Nei, 1993) with a gamma correction (alpha value = 0.5); this model was chosen on the basis of the likelihood test implemented in MEGA 6.06. Measures of bootstrap support for internal branches were obtained from 1500 pseudoreplicates. The determination of genes unique to each of the three analyzed biocontrol strain genomes (RC218, AS 43.3, TrigoCor 1448), was determined by blasting (>70% homology and >50% coverage) each respective annotated genome against the other two. Genes not found in the other two strains were considered unique.

2.8. Ericin analysis

Mass spectrometry samples (2 μl injection) were analyzed by LC–MS (Thermo Acella HPLC) through a narrow-bore (2.1 mm × 150 mm, 3 μm particle size) C18 column (Inertsil, GL Sciences, Inc., Torrance, CA) running a gradient elution of 95% A:5% B (buffer A 0.1% formic acid, buffer B 100% acetonitrile) to 5% A:95% B over 65 min at a flow rate of 250 μl/min, followed by a 5 min B washout and 10 min re-equilibration, while maintaining a constant column temperature of 30 °C. Electrospray positive mode ionization data were collected with a linear ion trap-Orbitrap mass spectrometer (Thermo LTQ-Orbitrap Discovery) under Xcalibur 2.1 control. Prior to LC–MS^n experiments the instrument was calibrated using the LTQ positive ion calibration solution.

3. Results and discussion

3.1. Biocontrol activity

In the 2014 field trial, the application of *B. velezensis* RC 218 (see Section 3.2, previously named as *B. subtilis* RC 218) during anthesis at an inoculum rate of 10^6 cfu/ml, showed that FHB disease incidence was not reduced in comparison with the control treatment. Disease severity was significantly reduced by 25.8% when compared with the control treatments ($P \leq 0.05$). The treatment with the biocontrol agent reduced also deoxynivalenol accumulation to a non-detectable level (<0.05 ppm) (Table 1). In previous studies, the antagonist activity of *B. velezensis* RC 218 on disease severity and DON accumulation was observed. The reduction in disease severity and DON accumulation were 55 and 57%, respectively, under greenhouse conditions when a susceptible cultivar (ProINTA Gaucho) was co-inoculated with *F. graminearum* and *B. velezensis* RC 218 (Palazzini, 2010). The reductions were similar to those obtained by the application of *B. velezensis* AS 43.3 controlling FHB on wheat (Schisler et al., 2002). Other bacteria belonging to the genera *Streptomyces, Pseudomonas* and *Lysobacter,* and yeasts from *Cryptococcus* genus were also effective in controlling FHB at field level (Palazzini et al., 2016). In addition, *B. velezensis* RC 218 showed a good antagonist activity under field trials during two harvest seasons (2010/2011 and 2011/2012) with reduction in disease severity between 42 and 76% and also reduction in DON accumulation to undetected level (<0.05 ppm) during both periods evaluated (Palazzini et al., 2016). Similar results in DON reduction (up to 69%) were observed by using *B. subtilis* SG6 by Zhao et al. (2014). Under field trials, the antagonist activity of *B. velezensis* RC 218 was effective at a lower inoculum dose (10^4–10^6 cfu/ml) than reported for similar strains in controlling FHB. For example *B. velezensis* strain TrigoCor only provide field protection when applied at >10^8cfu/ml and up to three applications (Crane et al., 2013), *B. amyloliquefaciens* at 10^8cfu/ml (Khan et al., 2004) or *P. fluorescens* at 10^7 cfu/ml (Khan and Doohan, 2009).

3.2. Bacillus subtilis *RC 218 genome sequencing and taxonomy*

Bacillus subtilis RC 218 was sequenced to an average coverage depth of 177×. The sequencing reads assembled into 19 contigs. A summary of the results are provided in Table 2. In order to confirm the taxonomy of the strain RC218, a phylogenomic analysis was done including the publically available genomes of *B. velezensis*, *B. amyloliquefaciens* and *Bacillus siamensis*. The phylogenomic tree of the strains confirmed that strain RC 218 is a member of the *B. velezensis* species clade (Fig. 1). In addition, the tree shows strain RC 218 was most closely related to *B. velezensis* AS 43.3 and *B. velezensis* TrigoCor. Interestingly, both *B. velezensis* AS 43.3 and *B. velezensis*

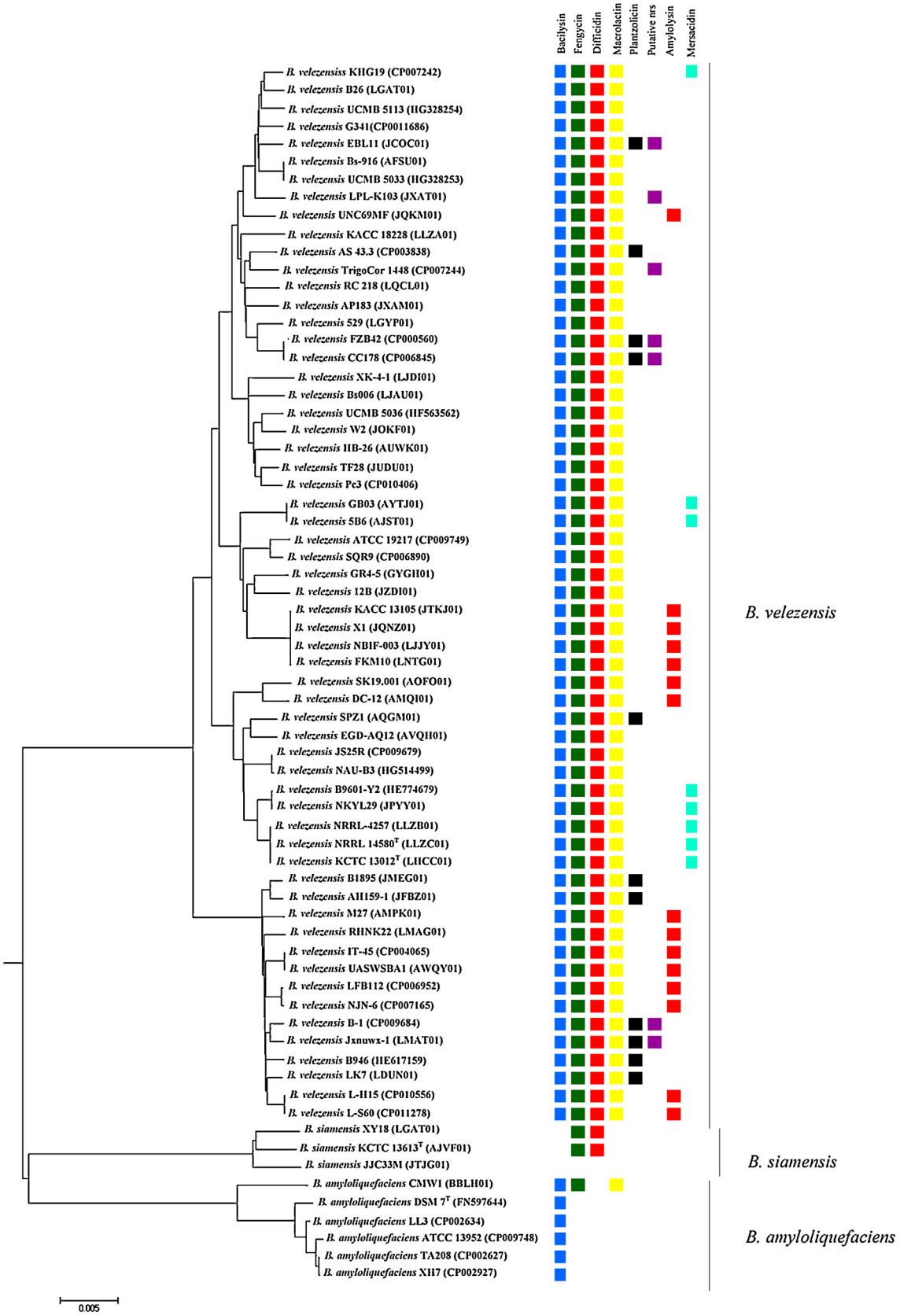

Fig. 1. Phylogenomic neighbour-joining tree constructed from the core genomes of strains from *B. amyloliquefaciens*, *B. siamensis*, and *B. velezensis* strains. The box on the right, represent the presence of the listed secondary metabolite cluster. In addition to the listed metabolites, all strains contained clusters to produce an iturinic compound (*e.g.* iturin or bacillomycin), the lipopeptidesurfactin, the siderophorebacillibactin, the antibacterialsbacillaene and amylocyclicin.

Table 2
Genome statistics of *Bacillus velezensis* RC 218.

Size	3873821 bp
Number of genes	3744
Protein-coding genes	3603
Pseudo genes	48
tRNAs	82
GC mole content	46.5%
Average coverage	177×

TrigoCor 1448 were also isolated from wheat hosts (Khan et al., 2001; Nelson et al., 2014).

3.3. Secondary metabolite clusters in B. velezensis RC 218

Bacillus velezensis RC 218 possesses ten secondary metabolite clusters within its genome (Table 3). The genome contains five non-ribosomal peptide synthetase clusters, which are conserved in all *B. velezensis* members. This group of NRPS clusters comprises surfactin, a representative of the iturin group (iturin A), a representative of the plipastatin group (fengycin), the siderophore bacillibactin and the antibacterial bacilysin. The genome also harbors three polyketidesynthetase (PKS) gene clusters. These clusters encode the antibacterial polyketides bacillaene, difficidin and macrolactin. The genome also contains the operon for the production of the circular bacteriocin amylocyclicin, which is ribosomally synthesized (Scholz et al., 2014). This bacteriocin was demonstrated to have antimicrobial activity against Gram-positive bacteria, but not Gram-negatives ones (Scholz et al., 2014). This operon is found in all of the genomes listed in Fig. 1.

A lantibiotic gene cluster for the production of ericin was also detected in *B. velezensis* RC 218. The ericin gene cluster had 11 open reading frames (Table 3) and shared 100% protein homology to the cluster from its original description (Stein et al., 2002). Interestingly, the ericin cluster is not seen in the genomes of other *B. velezensis* strains. The only other strain known to make ericin is the strain originally described as *Bacillus subtilis* A1/3 (Stein et al., 2002). A closer look at *Bacillus subtilis* A1/3 suggests it is likely a member of *B. velezensis*. This based on blasting the available nucleotide sequencing data for *Bacillus subtilis* A1/3 (GenBank accessions; AF499447.1, AF396778.1), which shows the highest homology to the subject strain and its close relatives (data not shown). To confirm the ericin cluster was functional, we screened culture supernatants during different growth stages for the presence of the metabolites. Samples from the exponential growth phase (12 h), early stationary phase (24 h) and the late stationary phase (36 h) were analyzed by LC–MS to identify ericin S (calculated mass 3351.543 Da, Fig. 2A) or ericin A. The ion extracted chromatogram corresponding to the 4^+chargestate of ericin S (m/z 836–837 using a 1 da extraction width) reveals a distinct peak at 20 min during the chromatographic analysis (Fig. 2B). The mass spectrum of the 20 min peak contains ions for the 4^+(m/z 836.393 observed; 836.385 calculated for the 4^+ ion, Fig. 2C) and 3^+ charge states of ericin S, where the 4^+ charge state is the dominant ericin S signal. Tandem mass spectra of ericin S (Fig. 2D) yielded a small number of fragments due to the cyclic components of the lantibiotic class; however, fragments from sections consisting of linear portions adjacent to cyclizing amino acids were observed. Specifically, the c13, b19, y13 ions are consistent with the ericin S structure. The mass accuracy of the mass spectrometer allows the identification of the peak as ericin S based on the 0.7 ppm mass error between the calculated and observed masses. Ericin S was not detected at the 12 h time point of the culture; however the intensity of the peak increased from 24 to 36 h consistent with expression of the gene cluster during the stationary phase.

The genome of *B. velezensis* RC 218 allowed us to do a comparative analysis with closely related strains. *B. velezensis* AS 43.3 and *B. velezensis* TrigoCor 1448, which are two strains isolated from wheat as antagonists of FHB and have genomes in the public domain (Dunlap et al., 2013; Nelson et al., 2014). These strains are also the closest relatives to *B. velezensis* RC 218. The genomes were compared to each other to determine the genes unique to each strain. The genomes of *B. velezensis* RC 218, *B. velezensis* AS 43.3 and *B. velezensis* TrigoCor 1448, contained 100, 120, and 82 unique genes, respectively (Supplemental Tables S1–S3). As expected, many of the unique genes are known phages, appear to phage proteins or are unknown "hypothetical proteins". Interestingly, all three have a unique secondary metabolite cluster that may play role in their biological activity. *B. velezensis* RC 218 contains ericin cluster (RC218-U017–RC218-U025), which has activity against closely related *Bacillus* spp. (Stein et al., 2002). *B. velezensis* AS 43.3 has plantazolicin cluster (B938-03385–B938-03550) that has been shown to have activity against closely related *Bacillus* spp. (Scholz et al., 2011) and nematodes (Liu et al., 2013). While *B. velezensis* TrigoCor 1448 contains an unknown NRPS cluster (AJ82-15550–AJ82-15580). The other notable observations are *B. velezensis* RC 218 contains several unique genes associated with thymidine metabolism (Thymidylate synthase RC218_U033, Hydroxymethylpyrimidine phosphate kinase, ThiD RC218-U035, Deoxyguanosine-triphosphate-triphosphohydrolase RC218-U047). *B. velezensis* TrigoCor 1448 contains a gene for maltose metabolism. The ability of strains of *B. velezensis* to metabolize maltose has previously been shown to be variable (Dunlap et al., 2015).

To better understand the possible role of active secondary metabolites in this clade, we determined the availability of common secondary metabolite clusters of all the strains in Fig. 1. All the strains in Fig. 1 contained clusters to produce an iturinic compound (Iturin or Bacillomycin), the lipopeptide surfactin, the siderophore bacillibactin, the antibacterials bacillaene and amylocyclicin. All *B. velezensis* strains contain clusters for the antibacterial bacilysin, the lipopeptide fengycin, and the polyketides macrolactin and difficidin. The *B. siamensis* strains lack the clusters for macrolactin and bacilysin, which may be a useful diagnostic trait. Interestingly,

Table 3
Secondary metabolite clusters identified in the genome of *B. velezensis* RC 218.

Compound	Synthetase type	Genes	Size (kb)	Bioactivity
Surfactin	NRPS	srfAA, AB,AC,AD	26.2	multiple
Iturin	NRPS	ituD,A,B,C	37.2	antifungal
Fengycin	NRPS	fenA,B,C.D.E	37.7	antifungal
Bacillibactin	NRPS	besA,dhbA,C,E,B,F	12.7	siderophore
Bacilysin/chlorotetaine	NRPS	bacA,B,C,D,E, F, ywfH	6.7	antibacterial
Amylocyclicin	bacteriocin	acnB,A,C,D,E,F	4.5	antibacterial
Ericin	lantibiotic	LanB,T,C,A,S,I,F,E,G,R,K	14.2	antibacterial
Macrolactin	PKS	mlnA,B,C,D,E,F,G,H,I	53.2	antibacterial
Bacillaene	PKS	baeB,C,D,E,acpK, baeG,H,I,J,L,M,N,R,S	72.5	antibacterial
Difficidin	PKS	dfnA,Y,X,B,C,D,E,F,G,H,I,J,K,M,L	69.5	antibacterial

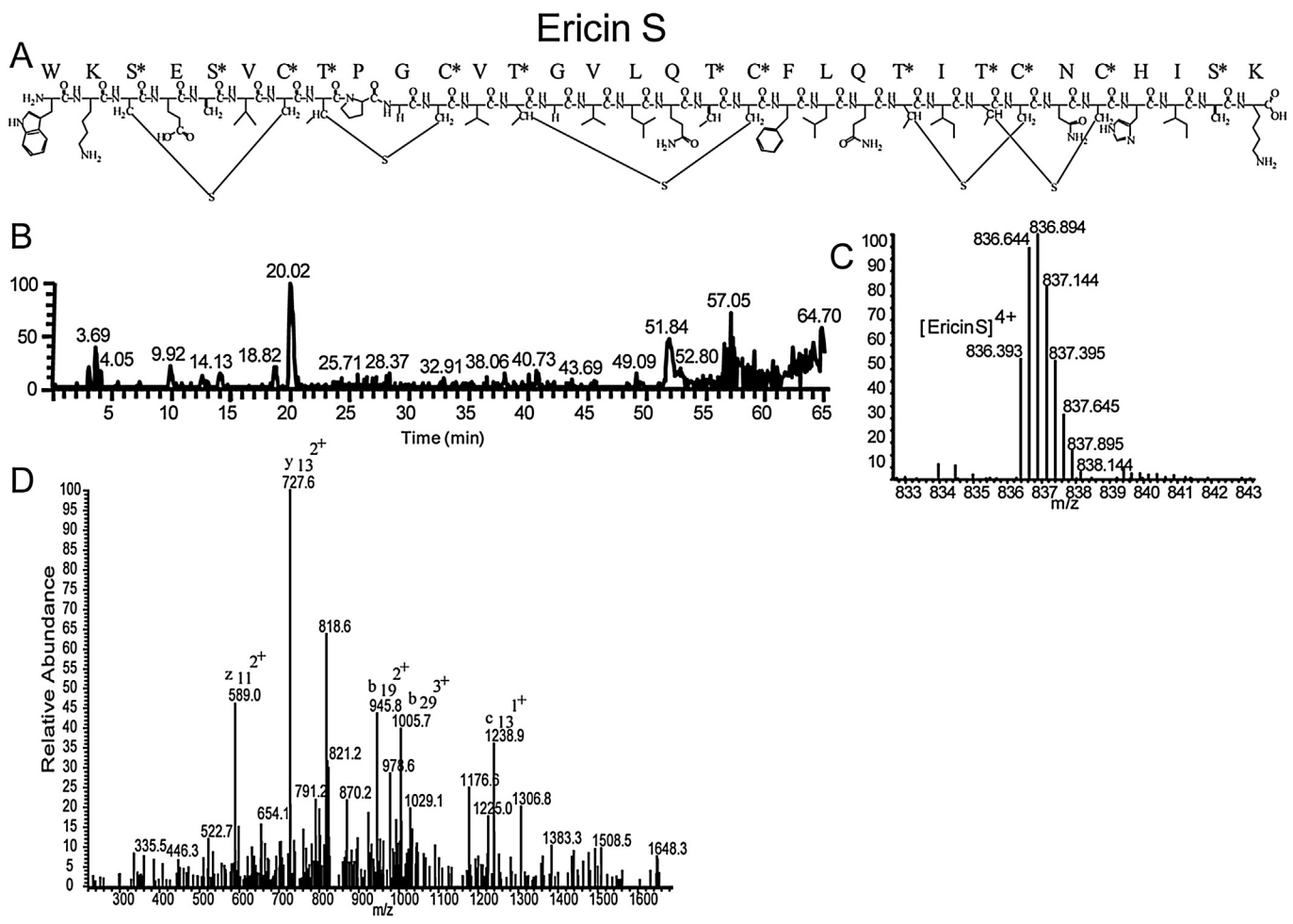

Fig. 2. (A) Structure of ericin S (calculated mass 3341.543 Da). One letter amino acid codes are used. Modified amino acids are designated as S*(dehydroalanine), T*(dehydrobutyrine), or C*(thioether linked cysteine). (B) Extracted ion-chromatograms from RP-C_{18} LC–MS analysis of culture supernatant: ericin S $[M+4H]^{4+}$peak. (C) High-resolution Orbitrap positive mode mass spectrum of ericin S (*m/z* observed: 836.393^{4+}, calculated 836.385^{4+}, 0.7 ppm error). (D) Ion-Trap tandem mass spectrum of 836.3^{4+} ion from ericin S. Fragmentation patterns are labeled according to Roepstorff and Fohlman (1984).

the greatest diversity occurs within the antibacterial secondary metabolites that primarily control Gram-positives and other members of the *Bacillus* genus, these include; plantazolicin (Scholz et al., 2011), amylolysin (Halimi et al., 2010) and mersacidin (Niu and Neu, 1991). The product of the nrs clusters is not known yet (Chen et al., 2007). These results showed that the primary differences in secondary metabolite cluster occurrence in these 59 *B. velezensis* genomes are related to the production of antibacterials. This is somewhat surprising given that most of these strains were isolated for their ability to control fungal plant pathogens. These results suggest there is significant evolutionary pressure for these strains to develop antibacterials to compete against bacteria that occupy the same ecological niche.

4. Conclusions

Bacillus subtilis RC 218 was reclassified as *Bacillus velezensis* according to the phylogenomic analysis. The biocontrol activity of this agent was demonstrated under field conditions and was successful in reducing disease severity and deoxynivalenol accumulation. The biocontrol activity can be related to the ability of the strain to produce several lipopeptides from the surfactin, fengycin and iturin families since the gene cluster for these compounds were detected. In addition, *B. velezensis* RC 218 was the first strain of this clade confirmed to produce the lantibiotic ericin. Among strains belonging to the *B. velezensis* clade, the primary driver of diversity of secondary metabolites are compounds that demonstrate antibacterial activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micres.2016.06.002.

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