# Killing effect of *Bacillus velezensis* FZB42 on a *Xanthomonas campestris* pv. *campestris* strain newly isolated from cabbage: a metabolomic study


**Čeněk Novotný[1,3]*, Helena Marešová[2], Hynek Mácha[2], Oldřich Benada[2], Andrea Palyzová[2]**

[1] Laboratory of Environmental Biotechnology and [2] Laboratory of Characterization of Molecular Structures, Institute of Microbiology of the Czech Academy of Sciences, v.v.i., Vídeňská 1083, 142 20 Prague 4, Czech Republic;

[3] Department of Horticulture, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic; * e-mail: novotny@biomed.cas.cz



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## Abstract

**Potential of *Bacillus velezensis* for biological control of various phytopathogens has been documented over the past few years but its antagonistic interactions with xanthomonads have not been studied in detail. The findings documented a strong killing effect on *Xanthomonas campestris* pv. *campestris* (Xcc) cells in a co-culture with *B. velezensis*. Lipopeptides and the siderophore bacillibactin involved in the killing process were quantified. A new robust Xcc-SU isolate tolerating high concentrations of ferric ions was used. In a co-culture with the antagonist, the population of Xcc-SU was annihilated within 24–48 h, depending on the number of antagonist cells used for inoculation. No inhibitory effect of Xcc-SU on *B. velezensis* was observed. Bacillibactin and lipopeptides (surfactin, fengycin, bacillomycin) were present in both the co-culture and the monoculture of *B. velezensis*. Except for bacillibactin, the maximum contents of lipopeptides were higher in the antagonist monoculture compared with the co-culture. Scanning electron microscopy showed that the death of Xcc-SU bacteria in the co-culture was caused by cell lysis. The analysis by mass spectrometry showed four major compounds, bacillibactin, surfactin, fengycin, and bacillomycin D. Different forms of surfactin and fengycin with variations in their side-chain length were detected. The results demonstrated the ability of *B. velezensis* FZB42 to act as a powerful antagonistic strain against Xcc.**


- **Background: *Xanthomonas campestris* pv. *campestris* (Xcc) causing black rot of crucifers is a phytopathogen responsible for serious damage of vegetable crops. Besides using pesticides and breeding plant cultivars with an increased resistance to Xcc, the bioprotection employing antagonistic microorganisms can be a promising approach that can decrease the infection and reduce the damage on the crops.**
- **Objective: The aim was to analyze the inhibitory action of *B. velezensis* FZB42 on the growth of Xcc under *in vitro* conditions in dual liquid-medium culture, to characterize the metabolites involved in destruction of the Xcc population, and to measure the kinetics of bacterial elimination.**
- **Methods: Growth inhibition was measured in dual cultures on BH agar medium (pH 7.0) at $Fe^{3+}$ concentrations of 8 and 200 μM where Xcc was spread on the agar surface to form a biofilm. Simultaneously, *B. velezensis* was inoculated using 10 μL of a fresh overnight Luria Broth culture. For antagonistic studies where the metabolome was analyzed, mineral M9 medium containing glucose and trace elements was used in the dual liquid culture. Fresh suspensions of *B. velezensis* and Xcc grown in M9 medium and harvested in exponential phase were adjusted to a final concentration of $10^8$ CFU/ml and used for inoculation of the dual culture using respective ratios 1:3, 1:15, or 1:100 CFU/ml. The dual culture was incubated at 28˚C under shaking (200 rpm) for 48 h. Selective, solid MCS medium supplemented with bile salt was used to identify Xcc in the dual culture (Mácha et al., 2021).**
  **Supernatants from the dual cultures were lyophilized and used for metabolome analysis using HPLC-MS.**
- **SEM: Samples were fixed with glutaraldehyde, postfixed with 1% OsO4, critical-point dried (K850, Quorum Technologies Ltd.), and finally sputter-coated with 3 nm of platinum (Q150T, Quorum Technologies Ltd). They were examined using FEI Nova Nano SEM scanning electron microcope (Mácha et al., 2021).**
- **Microorganisms: New strain Xcc-SU, was isolated from a cabbage field massively attacked by an Xcc infection. Molecular identification was by sequencing the 16S rRNA gene, physiological and biochemical characterization was carried out by Czech Collection of Microorganisms, Brno. The strains Xcc 3811,1279A, and 3871A were purchased from NCTC collection (University of Warwick, UK). *B. velezensis* FZB42 (DSM 23117) was purchased from DSMZ collection (Braunschweig, Germany).**

## CONCLUSIONS

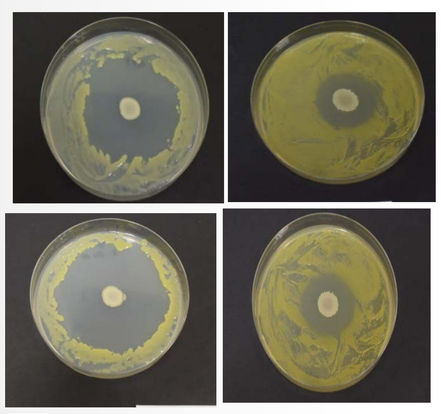
**Characteristics of Xcc strains:**

- **Two different groups of Xcc strains were described: Group 1 included 1279A and SU strains, Group 2 included 3811 and 3871A strains. Growth of Group 2 strains was more sensitive to inhibition by high $Fe^{3+}$ concentration (200 μM) (results not shown).**

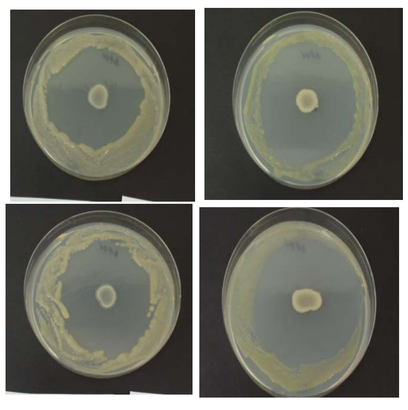
**Antagonist study with *B. velezensis*:**

- **In contrast to Group 2 strains, higher growth inhibition by *B. velezensis* was found in Group 1 strains at 8 μM compared to 200 μM $Fe^{3+}$, which indicated a role of siderophore(s) in the antagonistic inhibition.**
- **The metabolom of *B. velezensis* comprised bacillibactin, bacillomycin D, fengycin A and surfactin.**
- **In the presence of Xcc in the dual culture, the levels of bacillomycin D, fengycin A and surfactin were significantly lower.**
- **Doubling times of *B. velezensis* and Xcc-SU were 3 and 6 h, respectively.**
- **In the dual culture, a killing of Xcc-SU by *B. velezensis* was observed that was proportional to the amount of *B. velezensis* used to inoculate the dual culture.**
- **SEM observations indicated that the killing effect was due to a damage to the cell surface of Xcc-SU, including the outer wall and cellular membrane, that resulted in shrinkage and distortion of Xcc-SU cells and their ultimate collapse showing a leakage of the cytoplasm.**
- **No inhibitory or killing effect of Xcc-SU on *B. velezensis* was observed.**

## Inhibition by *Bacillus velezensis* at 8 and 200 μM $Fe^{3+}$

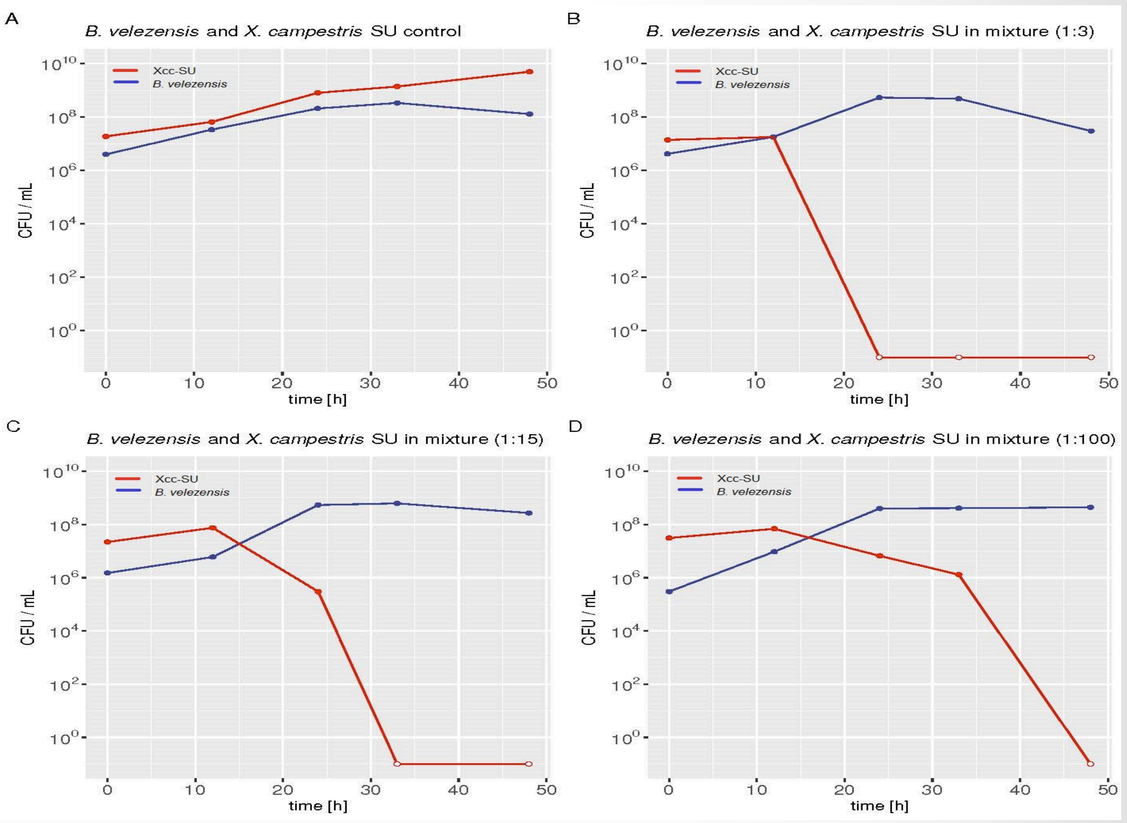


**Inhibition of growth of Xcc strains at 8 μM $Fe^{3+}$ (left column) and 200 μM $Fe^{3+}$ (right column) on Day 7: top, Xcc 1279A; bottom, Xcc SU.**



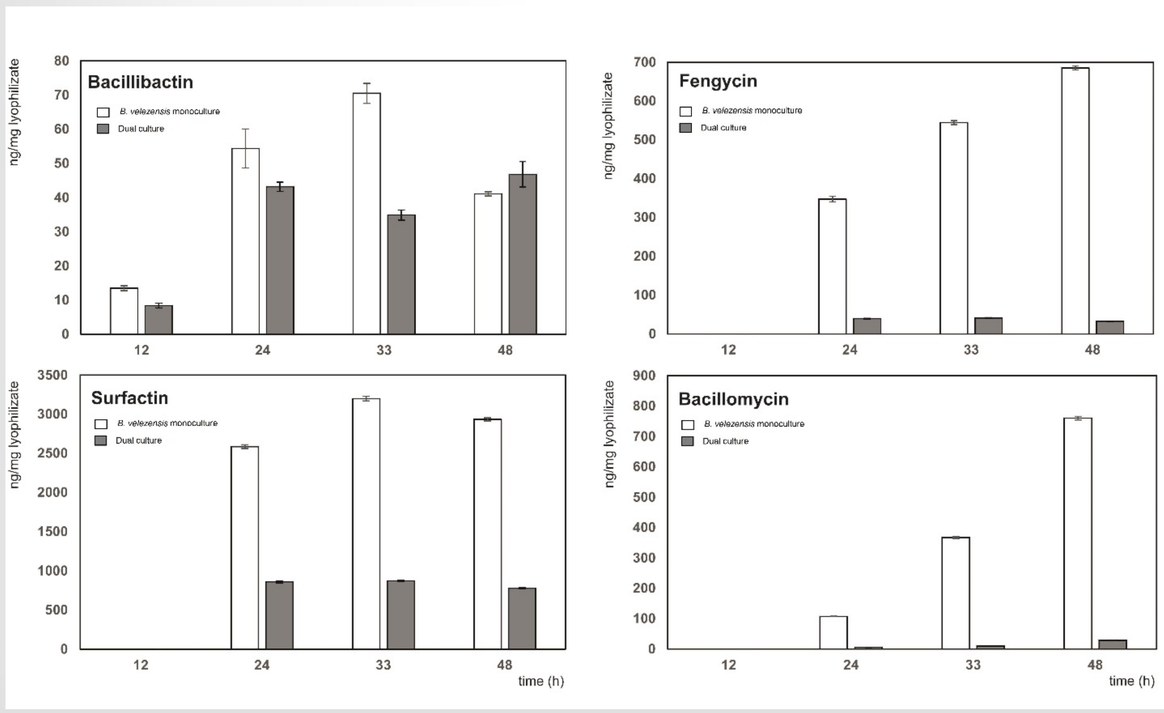
**Inhibition of growth of Xcc strains at 8 μM $Fe^{3+}$ (left column) and 200 μM $Fe^{3+}$ (right column) on Day 7:**
**top, Xcc 3811; bottom, Xcc 3871A.**

## Killing of Xcc-SU strain by *B. velezensis* in dual liquid culture




**Xcc-SU dying rates in dual cultures with *B. velezensis* grown in liquid medium. The ratios of *B. velezensis* to Xcc-SU at the time of inoculation were 1:3 (B), 1:15 (C), and 1:100 (D) in CFU/mL. The individual values in diagrams show the arithmetic means of CFU/mL measured at a given time in two independent cultures. The open points in *Xcc* plots indicate that no living Xcc bacteria were detected in the dual culture. R Core Team (2021) [46] free software environment for statistical computing and graphics was used for presentation of the data. *B. velezensis*, blue line; Xcc-SU, red line.**

## Biocontrol metabolites in dual liquid culture of *B. velezensis* and Xcc-SU




**Production of biocontrol metabolites in a dual culture of *B. velezensis* and Xcc-SU grown in liquid medium. The ratio of *B. velezensis* to Xcc-SU at the time of inoculation was 1:100 (CFU/mL). Dual culture, full columns; *B. velezensis* monoculture, empty columns. P**

## Interaction of *B. velezensis* and Xcc-Su growing in dual liquid culture