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Effect of iron oxide nanoparticles on antioxidant responses and secretome-driven antimicrobial activity in *Bacillus* strains

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Key words: nanoparticles; *Bacillus* spp.; antioxidant; antibacterial activity.

Abstract

Iron oxide nanoparticles (Fe_3O_4 NPs) have emerged as promising modulators of microbial metabolism, yet their biochemical effects on *Bacillus* strains with biocontrol potential remain poorly characterized. In this study we investigated the impact of Fe_3O_4 nanoparticles on the antioxidant response and antimicrobial activity of two *Bacillus* strains: *B. subtilis* CNMN-BB-10 and *B. velezensis* CNMN-BB-12. Both strains were cultivated in Mueller–Hinton and Luria–Bertani broths supplemented with Fe_3O_4 NPs at 5, 10 and 15 mg/L. Catalase specific activity, ABTS (2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging capacity, protein content and inhibitory activity against four phytopathogenic bacteria and two *Aspergillus* species were assessed in cell-free supernatants and partially purified peptide fractions. The results of the study suggest that the combination of sub-inhibitory Fe_3O_4 NPs supplementation (10 mg/L) and appropriate culture medium represent a viable strategy to modulate and potentially enhance the bioactive compound profile of *Bacillus* strains.

Introduction

Integrating nanotechnology with microbiology provides new opportunities to understand microbial responses to engineered nanomaterials and to exploit these interactions for biotechnological applications. Magnetite (Fe_3O_4) nanoparticles are especially attractive due to their magnetic properties, biocompatibility and broad applicability in biomedicine, environmental remediation and industrial biotechnology.^{1,2} At the same time, bacteria of the genus *Bacillus* are among the most extensively studied microbial groups, recognized for their metabolic versatility, spore-forming ability and important roles in industrial processes, agriculture and antimicrobial compound production.³⁻⁵

Interactions between *Bacillus* strains and nanomaterials have been extensively investigated, reflecting the complexity of nano-bio interfaces. Researchers have focused mainly on two aspects: the use of *Bacillus* species in the biosynthesis of nanoparticles^{6,7} and the evaluation of antimicrobial activity of engineered nanomaterials on Gram-positive bacteria.⁸⁻¹⁰ Beyond these fundamental interactions, applied studies have demonstrated synergistic effects arising from the combined use of

nanoparticles and beneficial *Bacillus* strains.¹¹⁻¹³ In addition, nanostructured systems, such as nanoencapsulation and nanoparticle-mediated immobilization improve the operational stability and efficiency of *Bacillus*-based biotechnological processes. Magnetite-assisted capture demonstrated strong interaction with *Bacillus cereus* (90.34% efficiency), supporting the feasibility of integrating members of the genus *Bacillus* with iron oxide NPs for agricultural applications.¹⁴ Self-healing bioconcrete systems have been developed using *Bacillus subtilis* encapsulated within iron oxide NPs.¹⁵ Furthermore, bacterial cells immobilized on Fe₃O₄ NPs significantly enhanced the antibiotic removal in treatment wetlands.^{16,17} Emerging evidence also indicates that nanoparticles can modulate biosynthetic pathways in *Bacillus* spp., thereby contributing to bioprocess intensification strategies.¹⁸

However, the biological effects of nanoparticles on *Bacillus* spp. remain strongly dependent on particle characteristics and environmental conditions. Nanomaterials can influence cell viability, biofilm formation, genetic competence and metabolic activity in a material-dependent manner, with nanoscale formulations exerting stronger effects than bulk counterparts.^{10,19} Reported responses to iron oxide nanoparticles vary from minimal physiological impact to reduced viability, selective biofilm inhibition and membrane damage driven by electrostatic interactions.²⁰⁻²² These observations highlight the need for systematic investigations of nano-bio interactions involving *Bacillus* spp. strains under controlled experimental conditions.

In this context, the present study evaluates nanoparticle-induced biochemical responses in *B. subtilis* CNMN-BB-10 and *B. velezensis* CNMN-BB-12 cultivated in two culture media and exposed to different concentrations of Fe₃O₄ NPs, aiming to advance the understanding of iron oxide nanoparticle–bacterial interactions and to clarify their relevance for biotechnological applications.

Materials and Methods

Bacterial strains, culture media, and experimental conditions

The study was conducted using *Bacillus subtilis* CNMN-BB-10 strain, originally isolated from the rhizosphere of tomato plants (Catalog CNMN, https://cnmn.utm.md/collection_page/153) and *Bacillus velezensis* CNMN-BB-12 strain, originally isolated from the water of a municipal lake (Catalog CNMN, https://cnmn.utm.md/collection_page/155). Both strains were obtained from the National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Technical University of Moldova.

Both bacterial strains were cultivated in two different media: i) Mueller-Hinton Broth (MH), composed of peptones (19.5 g/L) and soluble starch (1.5 g/L); and ii) Luria-Bertani Broth (LB), composed of casein peptone (10.0 g/L), yeast extract (5.0 g/L) and sodium chloride (5.0 g/L). The inoculum density was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The experiments were performed in 250 mL Erlenmeyer flasks containing 50 mL of working volume. Cultures were incubated at 30±1°C, with a pH of 7.2–7.4, under continuous lighting and 200 rpm agitation for 48 h. Fe₃O₄ nanoparticles were added to the culture media at final concentrations of 5, 10 and 15 mg/L.

Nanoparticles: origin and physicochemical characteristics

Iron (II, III) oxide nanoparticles (Fe₃O₄ NPs), Product No. 637106, were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). According to the manufacturer, the nanoparticles

had: primary particle size: 50–100 nm, measured by Scanning Electron Microscopy, (SEM), purity: 97% (trace metals basis).

Preparation of cell-free supernatant and dry mass determination

At the end of the cultivation period, bacterial biomass was separated by centrifugation (Relative Centrifugal Force (RCF) $8000 \times g$, for 10 min, at 4°C) to obtain the Cell-Free Supernatant (CFS). The dry mass of the CFS was determined gravimetrically by using the analytical balance AXIS ALN 220 (Gdansk, Poland), after evaporation to constant weight at 105°C in the drying oven POL-EKO SLW 115 Smart (Wodzisław Śląski, Poland).

Peptide fraction preparation

To achieve partial purification of the peptide fraction, the filtered CFS was subjected to precipitation with 40% (w/v) Ammonium Sulfate (AS) under continuous stirring overnight at +4°C. The precipitated proteins were collected by centrifugation ($12000 \times g$ for 10 min at 4°C) and subsequently resuspended in phosphate-buffered saline (PBS, pH 7.4). The resulting solution was then evaluated for protein concentration.²³

Determination of protein content

Total protein concentration in CFS was determined using the Lowry method, with Bovine Serum Albumin (BSA) as the standard. An aliquot of sample was mixed with Folin–Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, USA) and, after incubation at room temperature for 30 min, the absorbance was measured at 750 nm, using a T60V Spectrometer (Wibtoft, Leicestershire, UK)

Protein concentration was calculated from the standard calibration curve and expressed as mg/mL.²⁴

Determination of catalase activity

Catalase activity of CFS was determined spectrophotometrically based on the decomposition of hydrogen peroxide (H_2O_2) and subsequent formation of a yellow complex with ammonium molybdate. The absorbance was measured at 410 nm, by using T60V Spectrometer (Wibtoft, Leicestershire, UK) against a blank sample containing the same reagents, except biological material. Specific catalase activity was obtained by normalizing total activity to protein concentration and expressed as $mmol/min^{-1}/mg^{-1}$ protein.²⁵

Determination of antioxidant activity

Antioxidant activity of CFS and Peptide Fraction was evaluated using the ABTS (2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay. ABTS⁺ radicals were generated by mixing 7 mM ABTS and 2.45 mM potassium persulfate. Absorbance was recorded at 734 nm, using T60V Spectrometer (Wibtoft, Leicestershire, UK). Antioxidant activity was expressed as percentage inhibition of ABTS radicals.²⁶

Determination of antimicrobial activity

The antimicrobial activity of CFS and Peptide Fraction was assessed, using the agar well diffusion method, against four phytopathogenic bacteria (*Agrobacterium tumefaciens* CNMN-AB-01, *Clavibacter michiganense* CNMN-CrB-01, *Erwinia carotovora* CNMN-BE-01, *Xanthomonas*

campestris CNMN-BX-01) on nutrient agar (ISO 21528, BioLab, Budapest, Hungary) and two fungal strains (*Aspergillus fumigatus* 47 and *Aspergillus flavus* 40) on 6.0°Blg brewer's wort medium, pH 5.5 (SRL Rivex, Gratiesti Brewery, Republic of Moldova).

A 0.1 mL of CFS or peptide fraction volume was placed into wells in Petri dishes preinoculated with the test microorganisms. The plates were left at room temperature for 1 hour to permit the diffusion of the CFS. Then the bacteria were incubated at 35°C and the fungi at 27°C. Inhibition zone diameters were measured after 24 hours of incubation for bacteria and 4-5 days for fungi.²⁷

Statistical analysis

All experiments were conducted in triplicate using three biologically independent replicates. Results are presented as mean $\pm$ Standard Deviation (SD). Statistical significance among groups was evaluated using analysis of variance (ANOVA), with Tukey's post hoc test applied for pairwise multiple comparisons. Statistical significance was defined as follows: * $p \leq 0.05$ was considered significant, ** $p \leq 0.01$ highly significant, and *** $p \leq 0.001$ very highly significant.

Results

Protein content and catalase activity of cell-free supernatants

The effect of Fe₃O₄ NPs on protein content and catalase specific activity was evaluated in the CFS of two *Bacillus* strains cultivated in MH and LB media (Figure 1). As shown in the Figure 1 a, in MH medium, catalase activity of CFS after *Bacillus subtilis* CNMN-BB-10 cultivation at concentrations of 5-10 mg/L of Fe₃O₄ NPs remained relatively stable, declining by 41.4% at 15 mg/L. This reduction coincided with a rise in total protein content to 7.2 mg/mL. In LB medium, catalase specific activity of the same strain peaked at 5 mg/L Fe₃O₄ NPs and declined moderately at higher concentrations (10 -15 mg/L). Overall, LB medium was associated with higher protein content but lower catalase activity than MH medium.

For *B. velezensis* CNMN-BB-12 (Figure 1b), a different response pattern was observed. The strain demonstrated a gradual dose dependent decline in catalase activity from 81.17 U/mL to 69.79 U/mL on MH medium and insignificant variations on LB medium.

Antioxidant activity of cell-free supernatants and peptide fraction

The effect of Fe₃O₄ NPs on antioxidant activity was evaluated in the CFS of both *Bacillus* strains cultivated in MH and LB media. The results are shown in Figure 2.

In *B. subtilis* CNMN-BB-10, antioxidant activity in MH medium increased substantially and dose-dependently with nanoparticle concentration, from 38.15% (control) to 88.29% at 15 mg/L Fe₃O₄NPs (a 2.3-fold increase). In LB medium, baseline antioxidant activity was higher (62.13%) and remained relatively stable across all concentrations (62.13–65.94%). In *B. velezensis* CNMN-BB-12, antioxidant activity in MH medium declined from 64.98% (control) to 46.50% at 10 mg/L. In LB medium, antioxidant values were uniformly high (67.62–75.11%) across all conditions. ABTS radical scavenging activity was measured in CFS and peptide fractions isolated after the cultivation of both *Bacillus* strains on LB medium, supplemented with 10 mg/L Fe₃O₄NPs. Results are presented in Figure 3.

Peptide fractions from *B. subtilis* CNMN-BB-10 showed a reduction in both antioxidant activity (66.3% $\rightarrow$ 54.5%) and protein content (517.8 $\rightarrow$ 444.1 mg/mL) in the presence of 10 mg/L Fe₃O₄

NPs. By contrast, peptide fractions from *B. velezensis* CNMN-BB-12 exhibited an increase in protein content (575.1 → 677.0 mg/mL) at the same nanoparticle dose, while antioxidant activity decreased moderately (78.5% → 67.3%).

Antimicrobial activity of cell-free supernatants against phytopathogenic bacteria and fungi

The antimicrobial activity of CFS from both strains was assessed against four phytopathogenic bacteria and two fungal strains. Inhibition zones (mm) are presented in Table 1.

According to the obtained results, the CFS after the cultivation of *B. subtilis* CNMN-BB-10 in MH medium, showed limited antibacterial spectrum, displaying activity only against *E. carotovora* (15.3–16.3 mm) and *X. campestris* (15.0–15.3 mm), not being essentially influenced by the presence of Fe₃O₄ NPs at concentrations of 5 and 10 mg/L, and inhibited at the concentration of 15 mg/L.

CFS from *B. velezensis* CNMN-BB-12 showed broad and strong antibacterial activity against all four tested phytopathogenic bacteria in both media. The largest inhibition zones were recorded against *C. michiganense* (31.7 ± 0.7 mm) and against *A. tumefaciens* (28.3 ± 0.7 mm) in MH medium at the control condition.

The antifungal activity of *B. subtilis* CNMN-BB-10 CFS was restricted to *A. fumigatus* in LB medium at 10 mg/L Fe₃O₄NPs (21.7 ± 1.7 mm), with no activity detected in MH medium or against *A. flavus* at any concentration. The antifungal activity of *B. velezensis* CNMN-BB-12 was markedly superior and more consistent: *A. fumigatus* was inhibited in MH medium at all tested concentrations (23.3–33.0 mm). Activity against *A. flavus* was detected at 5 and 10 mg/L in MH medium (18.7–26.0 mm) but was absent in LB medium.

Antimicrobial activity of peptide fraction

To assess the contribution of peptide fraction to the overall antimicrobial potential, antibacterial and antifungal activities were evaluated for peptides isolated from the CFS of studied *Bacillus* strains cultivated in LB medium supplemented with 10.0 mg/L Fe₃O₄. Inhibition zones (mm) are presented in Table 2.

Bioactive peptides from *B. subtilis* CNMN-BB-10 showed no antibacterial activity against any of the tested pathogens, but displayed potent antifungal activity against *A. fumigatus*—increasing from 37.7 ± 2.8 mm (control) to 46.7 ± 1.7 mm at 10 mg/L Fe₃O₄ (+24%). No activity against *A. flavus* was detected in this strain.

Bioactive peptides from *B. velezensis* CNMN-BB-12 demonstrated a different profile: antibacterial activity was retained against *C. michiganense* (15.7 ± 0.7 mm control, 15.7 ± 1.3 mm at 10 mg/L), *E. carotovora* (19.0 ± 3.0 mm control, 20.0 ± 3.9 mm at 10 mg/L), and *X. campestris* (15.7 ± 1.7 mm control, 15.3 ± 0.7 mm at 10 mg/L), remaining largely unchanged regardless of nanoparticle treatment. Remarkably, complete inhibition (Ti) of *A. fumigatus* was observed both in the control and at 10 mg/L Fe₃O₄ NPs, indicating constitutive high-level antifungal activity.

Discussion

The findings of this study demonstrate complex responses of the investigated *Bacillus* strains to exposure to Fe₃O₄ NPs. First, the results revealed divergent catalase activity patterns between the two strains. *Bacillus subtilis* CNMN-BB-10 exhibited a biphasic response in MH medium: catalase activity in CFS remained relatively stable at Fe₃O₄ NPs concentrations of 5–10 mg/L, followed by a

41.4% decrease at 15 mg/L. This threshold effect may indicate that 15 mg/L exceeds the strain's oxidative stress management capacity, resulting in enzyme inactivation or metabolic dysfunction.²⁸ In contrast, *Bacillus velezensis* CNMN-12 demonstrated a gradual dose-dependent decline of catalase activity on MH medium. This response pattern suggests greater stress tolerance and more controlled adaptive response mechanisms.

The medium composition also appeared to influence the nanoparticle-related effects. The nutrient-rich LB medium was associated with lower stress-related manifestations and less pronounced dose-dependent enzymatic variations. Previous results obtained from cultivating the same strains in nutrient broth support this observation.²⁹ The contrasting trends between the studied strains suggest that catalase modulation by iron oxide nanoparticles is both strain- and medium-dependent.

The dramatic increase in ABTS radical scavenging activity for *Bacillus subtilis* CNMN-BB-10 in MH medium (from 38.15 to 88.29%) at 15 mg/L Fe₃O₄, when catalase activity was at its lowest level, may be associated with stress-induced synthesis of non-enzymatic antioxidants and secondary metabolites.³⁰⁻³² This compensatory mechanism may represent a survival-related adaptation rather than a state of healthy metabolism, potentially explaining the complete loss of antimicrobial activity at this concentration despite the high ABTS values. In contrast, *Bacillus velezensis* CNMN-12 exhibited high baseline ABTS activity (64.98-75.11%) that decreased moderately with nanoparticle exposure. This pattern suggests a constitutively high level of antioxidant capacity, consistent with the strain's superior overall tolerance profile.

The antimicrobial assays revealed fundamental differences in the biological activities of two strains. *Bacillus velezensis* CNMN-12 demonstrated broad-spectrum activity (persisting across most nanoparticle concentrations and both media) against all tested bacterial and fungal pathogens, with inhibition zones ranging from 16.0 to 33.0 mm. This aligns with the well-documented antimicrobial capabilities of *B. velezensis*, which produces a rich arsenal of bioactive lipopeptides and polyketides.³³⁻³⁵ In contrast, *Bacillus subtilis* CNMN-BB-10 showed limited activity, affecting only *E. carotovora* and *X. campestris* on MH medium, suggesting a narrower metabolite profile or lower production level. The complete loss of *B. subtilis* antimicrobial activity at 15 mg/L Fe₃O₄ NPs correlates with the catalase crash and confirms this concentration as a toxicity threshold for this strain.

The study revealed differences between the crude CFS and purified peptide preparations, with the latter exhibiting lower antioxidant activity. This observation suggests that the antioxidant activity of the CFS may be associated with synergistic interactions among multiple components, including peptides, lipopeptides, polyketides, and exopolysaccharides, which could be affected during purification.³⁶ Notably, despite reduced overall antioxidant activity, at 10 mg/L Fe₃O₄ NPs, antifungal activity of *Bacillus subtilis* CNMN-BB-10 peptides against *Aspergillus fumigatus* increased by 24%, while the bioactive peptides of *Bacillus velezensis* CNMN-12 maintained complete inhibition of *A. fumigatus* and showed enhanced activity against *A. flavus* (38.7 → 43.3 mm). Based on these findings it can be hypothesized that sub-inhibitory iron oxide nanoparticle concentrations can stimulate specific antifungal peptides, potentially through iron-responsive regulatory pathways such as ferric uptake regulator system, coordinating metal homeostasis and secondary metabolite production in *Bacillus* spp.³⁷ The antibacterial activity of *Bacillus velezensis* CNMN-12 peptides, targeting *C. michiganense*, *E. carotovora*, and *X. campestris*, remained stable regardless of nanoparticle supplementation, indicating constitutive production of antibacterial peptides in this strain.

Overall, the present findings provide a foundation for further investigation of *Bacillus* strains, their bioactive metabolites, and their interactions with iron oxide nanoparticles.

Conclusions

This study demonstrates that Fe₃O₄ NPs effects on *Bacillus* strains are complex, concentration-dependent, strain-specific and medium-modulated. Nanoparticle concentration of 15 mg/L was identified as a toxicity threshold for *Bacillus subtilis* CNMN-BB-10, characterized by catalase collapse and complete loss of antimicrobial activity. In contrast, *Bacillus velezensis* CNMN-12 demonstrated superior stress tolerance and maintained antimicrobial production across the tested NPs concentration range. The increase in ABTS antioxidant activity concurrent with catalase loss revealed the importance of non-enzymatic antioxidant compensation mechanisms, while the divergence between cell free supernatant and purified peptides activities highlighted the synergistic complexity of bacterial secretomes. Collectively, the results suggest that the combination of sub-inhibitory Fe₃O₄ NPs supplementation (10 mg/L) and appropriate culture medium represents a viable strategy to modulate and potentially enhance the bioactive compound profile of *Bacillus* strains. The identification of strain-specific safe concentration ranges may therefore support the safe and effective application of magnetic nanoparticles in microbial biotechnology.

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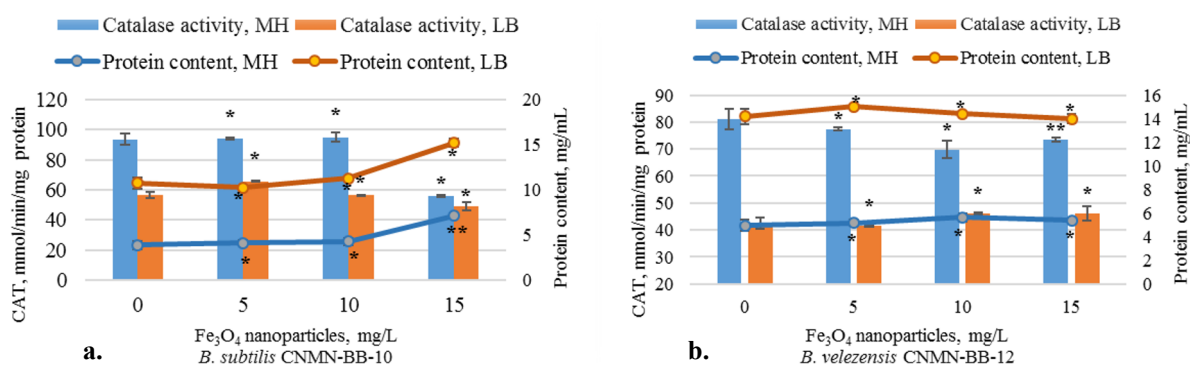


Figure 1. Catalase specific activity and protein content of Cell-Free Supernatant (CFS) from *Bacillus* strains, cultivated on Mueller–Hinton Broth (MH) and Luria–Bertani Broth (LB), with addition of 5, 10, 15 mg/L Fe₃O₄ Nanoparticles (NPs), compared to the control (0 mg/L), * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$: a) *Bacillus subtilis* CNMN-BB-10, b) *Bacillus velezensis* CNMN-BB-12.

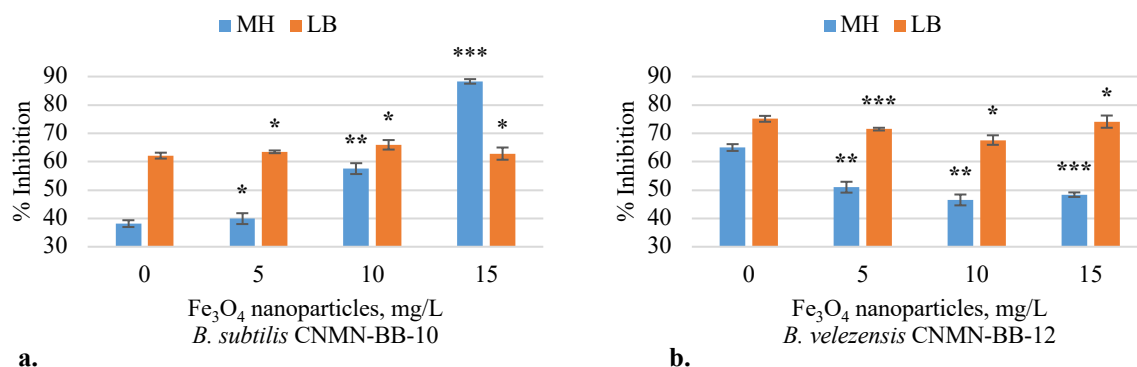


Figure 2. Changes in antioxidant activity of cell-free supernatant (CFS) from *Bacillus* strains cultivated on Mueller–Hinton Broth (MH) and Luria–Bertani Broth (LB), supplemented with Fe₃O₄ nanoparticles (NPs), compared to the control (0 mg/L), $p < 0.05$, expressed as 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical inhibition %, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. a) *Bacillus subtilis* CNMN-BB-10, b) *Bacillus velezensis* CNMN-BB-12.

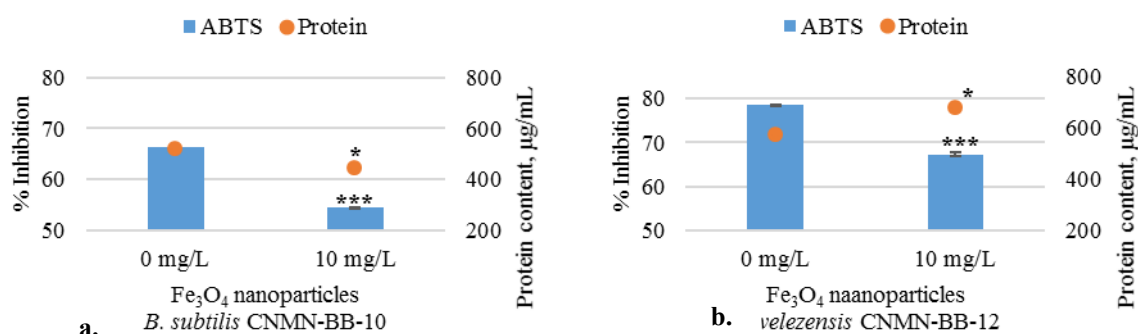


Figure 3. Antioxidant activity and protein content of peptide fraction from *Bacillus* strains cultivated on Luria–Bertani Broth (LB) medium, with addition of 10 mg/L Fe₃O₄ nanoparticles (NPs), compared to the control (0 mg/L), * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. a) *Bacillus subtilis* CNMN-BB-10, b) *Bacillus velezensis* CNMN-BB-12.

Table 1. Antibacterial and antifungal activity (inhibition zone, mm) of CFS from *Bacillus* strains cultivated in MH and LB media containing Fe₃O₄ NPs.

Pathogens	Me	<i>B. subtilis</i> CNMN-BB-10				<i>B. velezensis</i> CNMN-BB-12			
	-	Fe ₃ O ₄ NPs (mg/L)				Fe ₃ O ₄ NPs (mg/L)			
	dia	0	5	10	15	0	5	10	15
<i>Agrobacterium tumefaciens</i> CNMN-AB-01	M	Ni	Ni	Ni	Ni	28.3±0.7	25.0±1.1**	27.3±1.7*	Ni
	H								
	LB	Ni	Ni	Ni	Ni	26.0±2.0	23.7±0.7*	25.0±2.3*	26.0±1.1*
<i>Corynebacterium michiganense</i> CNMN-CB-01	M	Ni	Ni	Ni	Ni	31.7±0.7	24.3±1.7***	16.0±2.3***	24.3±0.7***
	H								
	LB	Ni	Ni	Ni	Ni	16.0±1.1	18.0±2.3*	18.0±2.0*	22.7±2.6**
<i>Erwinia carotovora</i> CNMN-BE-01	M	16.3±0.7	15.3±0.7*	15.7±0.7*	Ni	17.0±1.1	16.0±1.1*	16.7±0.7*	20.0±1.1*
	H								
	LB	Ni	Ni	Ni	Ni	22.7±1.7	16.3±1.7**	26.3±1.3*	21.0±2.0*
<i>Xanthomonas campestris</i> CNMN-BX-01	M	15.0±1.1	15.3±0.7*	15.3±0.7*	Ni	16.7±1.3	17.7±0.7*	15.7±0.7*	22.3±2.4**
	H								
	LB	Ni	Ni	Ni	Ni	21.3±1.7	18.3±1.7*	23.3±1.7*	24.3±1.7*
<i>Aspergillus fumigatus</i> 47	M	Ni	Ni	Ni	Ni	33.0±3.0	Ni	25.3±3.5*	23.3±1.7**
	H								
	LB	21.7±1.1	Ni	21.7±1.1	Ni	26.3±4.4	19.3±1.1	Ni	23.3±3.3

		.7		.7*		.6	.3*		.3*
<i>Aspergillus</i>	M	Ni	Ni	Ni	Ni	Ni	26.0±1	18.7±1	Ni
<i>flavus</i> 40	H						.1*	.3*	
	LB	Ni	Ni	Ni	Ni	Ni	Ni	Ni	Ni

Note: 1. Control (0 mg/L), cell-free supernatant obtained from cultures grown in the absence of nanoparticles; 2. The suspensions of nanoparticles in sterile culture medium without microorganisms, used as negative control, demonstrated no inhibitory effect against the tested pathogens, in all variants; Ni, no inhibition; CFS, cell-free supernatant, MH, Mueller–Hinton culture medium, LB, Luria–Bertani culture medium; ± Standard Deviation, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table 2. Antibacterial and antifungal activity (inhibition zone, mm) of peptide fractions from CFS of *Bacillus* strains cultivated in LB medium with 0 and 10 mg/L Fe₃O₄NPs

Pathogens	<i>B. subtilis</i> CNMN-BB-10		<i>B. velezensis</i> CNMN-BB-12	
	Control	10 mg/L Fe ₃ O ₄ NPs	Control	10 mg/L Fe ₃ O ₄ NPs
<i>Agrobacterium tumefaciens</i> CNMN-AB-01	Ni	Ni	Ni	Ni
<i>Corynebacterium michiganense</i> CNMN-CrB-01	Ni	Ni	15.7±0.7	15.7±1.3*
<i>Erwinia carotovora</i> CNMN-BE-01	Ni	Ni	19.0±3.0	20.0±3.9*
<i>Xanthomonas campestris</i> CNMN-BX-01	Ni	Ni	15.7±1.7	15.3±0.7*
<i>Aspergillus fumigatus</i> 47	37.7±2.8	46.7±1.7**	Ti	Ti
<i>Aspergillus flavus</i> 40	Ni	Ni	38.7±3.5	43.3±1.7*

Note: CFS, cell-free supernatant, NPs, nanoparticles, LB, Luria–Bertani culture medium; Ni, no inhibition; Ti, total inhibition, ± Standard Deviation, * $p \leq 0.05$, ** $p \leq 0.01$.

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