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Production of Anti-Methicillin-Resistant *Staphylococcus* Activity from *Bacillus subtilis* sp. Strain B38 Newly Isolated from Soil

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Abstract B38 bacterial strain, isolated from Tunisian soil showed a strong antimicrobial activity. Based on biochemical characterization and 16S rDNA sequence analysis, B38 strain was identified as *Bacillus subtilis*. Cell culture supernatant showed antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus* species and several Gram-positive and Gram-negative bacteria. Antifungal activity against phytopathogenic fungi was also observed. Antibacterial activity production started at early exponential growth phase, and maximum activity was reached at the stationary phase. This antibacterial activity was neither affected by proteases, lipase, and organic solvents, nor by surfactants. It was stable over a wide pH range and still active after autoclaving at 121 °C during 20 min. Thin layer chromatography followed by bioautography assay allowed the detection of four active spots with R_f values of 0.30, 0.47, 0.70, and 0.82. The single spot with R_f 0.30 showed antifungal activity, whereas the spots with R_f values of 0.47, 0.70, and 0.82 exhibited antibacterial activity.

Keywords Antibacterial activity · *Bacillus subtilis* · Multidrug-resistant pathogenic bacteria · TLC · Bioautography · Peptides antibiotics

Abbreviations
AU Activity unit
CFU Colony forming unit

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CS	Culture supernatant
LB	Luria–Bertani broth
MTT	Methylthiazolyltetrazolium bromide
NB	Nutrient broth
PCR	Polymerase chain reaction
PDB	Potato dextrose broth
rDNA	ribosomal DNA
R_f	Retention factor
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TSB	Trypticasein soy broth
TY	Tryptic Yeast
UV	Ultra violet

Introduction

The dissemination of multidrug-resistant pathogenic bacteria represents a serious public health problem worldwide. Methicillin-resistant *Staphylococcus* species, especially *Staphylococcus aureus* strains, appeared in the hospital environment and acquired resistance not only to ß-lactam antibiotics but also to flouroquinolones, chloramphenicol, clindamycin, tetracycline, and aminoglycosides [1]. Recently, a decrease in the susceptibility of methicillin-resistant *Staphylococcus* species to vancomycin and teicoplanin has also been reported in several hospitals around the world [2–4]. With the recent appearance of vancomycin- and teicoplanin-resistant clinical isolates, which are antibiotics of last resorts, no available antibiotic class is effective against multiresistant *Staphylococcus* infections. Therefore, the development of alternative antibiotics and therapy options is urgently needed to improve the management of bacterial infections.

Many bacterial species from different taxonomic branches produce antimicrobial activity against other bacteria. *Bacillus* is an interesting genus to investigate for antimicrobial activity since *Bacillus* species produce a large number of antibiotics with different chemical structures [5–8]. Some of these substances are active only against the same or closely related species [9–11], while others have a broad activity spectrum, including some pathogens responsible for food spoilage microorganisms such as *Listeria monocytogenes* [6, 12, 13]. Antifungal properties were also reported for *Bacillus* species against *Aspergillus*, *Penicillium*, and *Fusarium* species, as well as some yeast [14]. Only few antimicrobial compounds produced by the genus *Bacillus* exhibit inhibitory activity against methicillin-resistant *Staphylococcus* species [15]. The antimicrobial activity against methicillin-resistant *S. aureus* species has only been described for *Bacillus subtilis* producing macrolactin antibiotic [3] and *Bacillus pumilis* strain producing pumilicin 4 [15].

A screening program for anti-methicillin-resistant *Staphylococcus* activity producing bacteria was conducted. One strain designated B38 and identified as *B. subtilis* secreted in culture medium a wide antimicrobial activity spectrum including hospital pathogenic isolates of methicillin-resistant *Staphylococcus* species. Production and biological and biochemical properties of the antimicrobial activity produced by *B. subtilis* sp. B38 were described.

Materials and Methods

Strains and Media

Bacillus strains isolated from soil were grown at 30 °C in Trypticasein soy broth (TSB). Bacteria and fungi used as indicator strains for the determination of the antimicrobial activity spectrum were grown in appropriate culture conditions, as indicated in Table 1. Stock cultures were maintained at −80 °C in broth supplemented with 25% (*v*/*v*) glycerol.

Sample Preparation

B. subtilis B38 strain was grown in 100 ml of TSB medium at 30 °C with constant shaking at 150 rpm. Cells were harvested by centrifugation at 12,000×*g* for 15 min at 4 °C. The resulted supernatant was filtered through 0.45-μm membranes and concentrated tenfold by lyophilization. For thin-layer chromatography (TLC)-bioautography analysis of the antimicrobial activity produced at 8 and 24 h, concentrated culture supernatant (CS) was submitted to Sep-pak C18 cartridge, and elution was performed with 100% methanol. The active fraction was dried on a rotary vacuum evaporator and dissolved in a minimal volume of milliQ water.

Disc Diffusion Assay

B. subtilis B38 strain was tested for antibacterial activity by disc diffusion assay as previously described [6, 16]. An aliquot (100 μl) of culture supernatant was applied onto discs (6-mm diameter) on agar plates previously inoculated with the indicator strain suspension. Plates were incubated for 24 h at the optimal temperature of the tested microorganisms, and diameter of inhibition zone was evaluated. To determine the antibacterial activity titer, serial dilutions of CS were performed as previously described [6, 16, 17]. The antibacterial activity was defined as the reciprocal of the last serial dilution giving an inhibition zone and expressed as activity units per milliliter (AU/ml). Antifungal activity was also carried out by the disc diffusion assay in Petri plates containing potato dextrose agar as previously described [18]. After the mycelial colony had developed, sterile filter discs (6-mm in diameter) were disposed at 1.0 cm away from the rim of the mycelial colony. An aliquot (100 μl) of CS was then added to the disc. Plates were incubated at 25 °C until mycelial growth had formed inhibition crescents around discs. The antifungal activity was evaluated by measuring the diameter of the inhibition zone observed around the disc.

Identification of the Selected Strain

B38 strain was characterized using the API 50CHB multitest system (BioMerieux, Marcy l'Etoile, France) complemented with microscopic observation and biochemical tests as previously described [19, 20]. In order to confirm biochemical identification, a partial sequence analysis of the 16S rDNA was performed. Genomic DNA from *Bacillus* strains was prepared as previously described [21]. The 16S rDNA gene fragments were amplified by polymerase chain reaction (PCR) using the universal primers 20F (5′-AGAGTTT GATCCTGGCTCAG-3′) and 1500R (5′-GGTTACCTTGTTACGACTT-3′). PCR reaction and DNA sequencing were performed as previously described [21]. Databases (GenBank) were used for sequence similarity comparison with the 16S rDNA sequence obtained.

Table 1 Antimicrobial activity spectrum of CS from B38 strain

Indicator organism	Media	Temperature (°C)	Inhibition zone (mm)
Gram-positive bacteria			
B. mycoides LMT	TSB	30	20
B. licheniformis LMT	TSB	30	20
B. subtilis CIP 5162	TSB	30	10
B. subtilis ATCC 6633	TSB	30	8
B. thuringiensis LMT	TSB	30	12
B. thuringiensis sp. 15 LILM	TSB	30	26
S. aureus ATCC 29213	LB	30	20
S. aureus ATCC 25923	LB	30	16
S. aureus CIP 53154	LB	30	12
S. aureus CDC 103	LB	30	10
Staphylococcus hominis[a] LM	LB	30	8
Staphylococcus epidermidis[a] LM	LB	30	12
Staphylococcus haemolyticus[a] LM	LB	30	10
Staphylococcus warneri[a] LM	LB	30	12
Staphylococcus cohinii[a] LM	LB	30	8
Staphylococcus scuiri[a] LM	LB	30	14
E. faecalis ATCC 29212	LB	30	10
E. faecalis ATCC 29219	LB	30	10
L. innocua CIP 8011T	LB	30	12
L. monocytogenes CIP 82110T	LB	30	14
Gram-negative bacteria			
E. coli ATCC 25922	LB	30	12
E. coli ATCC 35214	LB	30	16
P. aeruginosa ATCC 27853	LB	30	8
Salmonella enteridis ATCC 13076	LB	30	12
Klebsiella pneumonia CIP 104727	LB	30	8
Klebsiella pneumonia CIP 105705	LB	30	8
Indicator fungi			
F. oxysporum sp. LPV	PDA	25	22
F. culmorum sp. LPV	PDA	25	20
Botryospheria sp. LPV	PDA	25	15
S. sclerotiorum sp. LPV	PDA	25	–
Phoma sp. LPV	PDA	25	16
A. solani sp. LPV	PDA	25	16

ATCC American Type Culture Collection, USA; *CDC* Center for Disease Control and Prevention; *CIP* Collection de l'Institut Pasteur, Paris, France; *LILM* Laboratoire Interactions Légumineuses-Microorganismes, Centre de Biotechnologie, Technopole de Borj Cedria, Tunisia; *LMT* Laboratoire de Microbiologie, Faculté des Sciences, Tunis, Tunisia; *LPV* Laboratoire de protection des végétaux, Institut National des Recherches Agronomiques, Tunisia; *LM* Laboratoire de Microbiologie, Centre National de Greffe de Moelle Osseuse, Tunis, Tunisia

[a] Methicillin-resistant clinical isolates

Selection of the Best Medium for Antibacterial Activity Production

In order to select the best culture medium for optimal antibacterial activity production, several broth media were tested: TSB, Luria–Bertani broth (LB), nutrient broth (NB), and tryptic yeast (TY). The pH of medium was adjusted to 7.0 before autoclaving (121 °C, 20 min). Cultures were realized in 250 ml Erlenmeyer flasks containing 50 ml of the

medium. After inoculation with 1 ml of bacterial culture, incubation was performed at 30 °C with shaking at 150 rpm. Cell growth was monitored by optical density measurement at 600 nm, and secreted antibacterial activity found in CS was tested by the disc diffusion assay.

Kinetic Production of Antibacterial Activity

Antibacterial activity detection was realized after incubating the producer strain into TSB medium at 30 °C during 52 h. Aliquots were aseptically removed every 4 h for the determination of the viable cells as well as heat-resistant spores (heated at 80 °C during 10 min). For each incubation period, the number of colony-forming units per milliliter (CFU/ml) was evaluated by the average number of colonies in three independent plates per dilution. The antibacterial activity titer of CS was determined against *S. aureus* ATCC 29213 as previously described. The pH of the medium was regularly monitored throughout the culture period. The percentage of sporulation was defined as the ratio of the number of heat-resistant spores on the number of viable colonies.

Effect of pH and Heat Treatment

Thermal stability of the antibacterial activity was evaluated after incubation of CS at different temperatures for 30 min or after autoclaving at 121 °C during 20 min. After cooling at room temperature, residual inhibitory activity was determined by disc diffusion assay against *S. aureus* ATCC 29213. The effect of pH was determined after adjustment of the CS pH between 2 and 10 with diluted HCl or NaOH. After incubation for 2 h at 25 °C and neutralization to pH 7, residual activity was tested [7].

Effect of Proteolytic and Lipolytic Enzymes

The effect of proteolytic and lipolytic enzymes as proteinase K, α-chymotrypsin, trypsin, or lipase A on antibacterial activity was also investigated. Enzymes were dissolved in 50 mM Tris–HCl buffer pH 7.8 and were added to the CS at a mass ratio of 1:10 and 1:5. After an overnight incubation at 37 °C, solutions were incubated at 90 °C for 5 min, cooled at room temperature, and centrifuged at 12,000×*g* for 15 min and 4 °C. The residual antibacterial activity in the supernatant was tested against the indicator strain. Untreated CS and enzyme alone in buffer were used as controls.

Solubility in Organic Solvents

CS was also treated with organic solvents as methanol or acetone used at 5:1 ratio (*v*/*v*). After storing at −20 °C for 2 h followed by centrifugation at 12,000×*g* for 15 min and 4 °C, both soluble and insoluble fractions were evaporated to dryness under speed-vacuum, suspended in milliQ water, and tested against the indicator strain.

Effect of Surfactants

Sensitivity towards surfactants was evaluated by incubating CS with sodium dodecyl sulphate, Tween 20, Triton X-100, and urea for 5 h at 37 °C. Surfactants were prepared as 10% stock solution in milliQ water, sterilized with 0.45 μm filter and used at 1% final concentration [22]. Surfactants alone in TSB were used as controls.

Thin Layer Chromatography

Precoated silica gel 60 F_{254} plates (20×20 cm; layer thickness, 0.20 mm; Merck) on aluminum supports were activated by heating at 110 °C for 30 min before use. Samples were spotted onto TLC plates. Chromatography was conducted using *n*-butanol-methanol-H_2O (39:10:20, *v*/*v*/*v*) as mobile phase. Developed plates were extensively dried for complete removal of the solvents and used for bioautography assay. The R_f of the detected spots is defined as the ratio between the distance traveled by the compound divided by the distance traveled by the solvent.

Bioautography Assay

This technique was used to determine active compounds found in CS of B38 strain from 24 h culture as previously described [23]. Developed TLC plates were placed on TSB agar base previously poured into Petri dishes. Pre-grown indicator strains were transferred into the molten agar containing TSB. The inoculated medium was poured onto TLC plates and incubated at 28 °C for 24 h. Active compounds were transferred from the TLC plates to the agar layer by a diffusion process. Plates were sprayed with an aqueous solution of 5 mg/ml methylthiazolyltetrazolium bromide (MTT). After 2 h incubation at room temperature, inhibition zones were visualized as clear spots in a purple background.

For antifungal activity detection, TLC plates were covered with a spore suspension of *Fusarium oxysporum* in potato dextrose broth soft agar. Clear inhibition zone appeared after incubation at 25 °C for about 72 h [24].

Antimicrobial Peptide Staining Method

Dried plates were also treated with 0.3% (*w*/*v*) ninhydrin spray reagent dissolved in ethanol. Red spots were detected after incubation at 80 °C for 10 min. Ninhydrin is the most widely used reagent for staining peptide with free N-terminal amino group. However, this reagent does not stain cyclic peptides with blocked α-amino group at the N terminus [25]. Cyclic peptides were visualized by immersion of the developed plate in 20% (*w*/*v*) trichloroacetic acid solution as previously described [25]. White spots were visible in about 5 min. For permanent visualization of these spots, the plate was immersed in 0.3% (*w*/*v*) Serva Blue W solution. Dark blue spots became easily visible on a very light blue background.

Results and Discussion

Identification of the Antimicrobial-Producing Strain

Screening for antimicrobial activity-producing bacteria collected from different soils allowed the selection of B38 strain. In fact, this strain exhibited a broad antagonistic activity spectrum by the agar spot assay and showed antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus* species (data not shown). A preliminary identification of this strain through phenotypical and biochemical characteristics showed that this isolate belongs to *Bacillus* genus. It is a Gram-positive bacilli, motile spore-forming organism, catalase-positive and oxidase-negative. The use of an API 50CHB kit with the APILAB Plus software indicated 99.83% identity with *B. subtilis*. Partial sequence alignment of 16S rDNA confirmed biochemical data and identified B38 strain as *B. subtilis* species.

Optimization of Antimicrobial Activity Production

Production of the antibacterial activity by *B. subtilis* B38 strain was tested in various media (Fig. 1). TSB medium allowed maximum biomass accumulation and high antibacterial activity level reaching 80 UA/ml against *S. aureus* ATCC 29213. Culturing B38 strain in LB medium dropped by fourfold the activity obtained in TSB medium reaching 20 AU/ml. Culture of B38 strain either in NB or TY broth media resulted in prominent growth, but no antibacterial activity was observed. It is noteworthy that optimal growth does not necessarily lead to a high level in antimicrobial activity [26, 27]. Variation in culture medium composition was previously shown to affect the production and/or secretion of the antimicrobial activity [10, 28]. Moreover, antimicrobial activity of B38 strain could not be related to organic acid effect as the pH of culture medium was in the neutral range.

Kinetic Production of the Antimicrobial Activity and TLC Analysis

In order to study the kinetic production of the antimicrobial activity during the growth period of the B38 producer strain in TSB medium, aliquots of culture medium were sampled at various time intervals and assayed against the indicator strain *S. aureus* ATCC 29213. An antibacterial activity level of 10 AU/ml was detected during logarithmic growth phase (8 h after inoculation) and reached an optimum level of 80 AU/ml during the stationary phase (24 h after inoculation) (Fig. 2). Medium pH was monitored throughout the experiment and was near 7. Synthesis of antimicrobial compounds by *Bacillus* species generally starts at the end of the exponential phase and reaches its maximum level during the stationary phase [6, 29–31]. We also observed that production of the antimicrobial activity started before the sporulation process, and maximum antibacterial activity level was attained 24 h after inoculation when sporulation was about 2% (Fig. 2). Antibacterial activity of CS sampled at 8 and 24 h after inoculation was analyzed by TLC-bioautography assay (Fig. 3). Active antibacterial compounds towards the indicator strain *S. aureus* ATCC 29213 are detectable from 8 h after inoculation as a single spot with R_f value of 0.82. At 24 h after inoculation, three distinct active spots with R_f values of 0.47, 0.70, and 0.82 were observed. These results suggest that the production of active compounds by B38 strain is highly dependent on the growth phase period. They also showed that B38 strain is able to secrete a bioactive compound as soon as the early growth phase. Antimicrobial compounds synthesized during the early logarithmic growth phase might be considered as primary metabolites [32]. The resulted antimicrobial activity was observed before sporulation

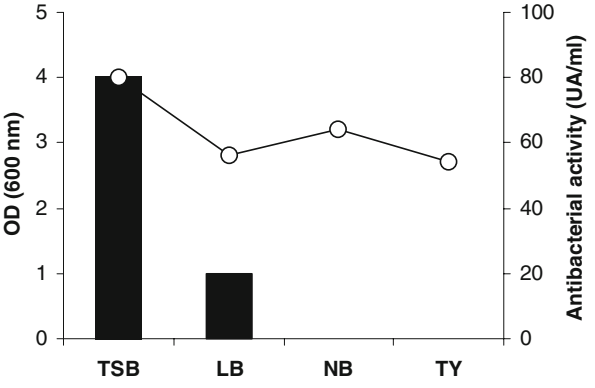

Fig. 1 Effect of various growth media on biomass and antibacterial activity production by *Bacillus* B38 strain. TSB, LB, NB, and TY media were tested. At 24 h after inoculation, bacterial growth was evaluated by optical density measurement at 600 nm (*open circles*). Antibacterial activity against *S. aureus* ATCC 29213 was expressed as activity units per ml (AU/ml; *black bars*)

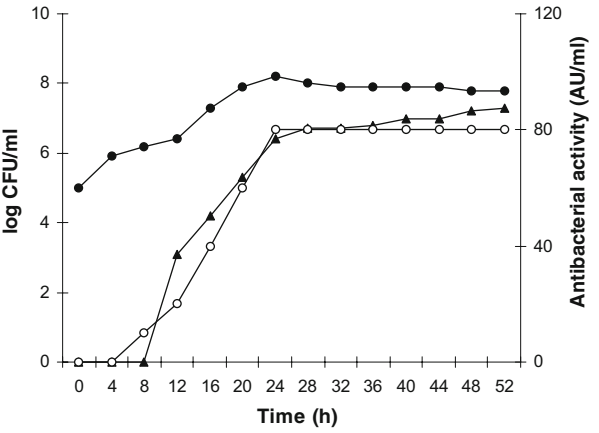

Fig. 2 Kinetic study of antimicrobial activity production by B38 strain. Aliquots were sampled every 4 h. Viable cells as well as heat-resistant spores (80 °C during 10 min) were expressed as the number of colony-forming units per ml (log CFU/ml). Antibacterial activity against *S. aureus* ATCC 29213 was expressed as activity units per ml (AU/ml). *Closed circles* Viable cell counts; *closed triangles* heat-resistant spores; *open circles* antibacterial activity

process and seemed to be induced under non sporulating conditions. The same kind of expression has already been reported for some bioactive compounds produced by *Bacillus* species [10, 33].

Antimicrobial Activity Spectrum

The antimicrobial activity spectrum of CS from B38 strain was evaluated by disc diffusion assay. The host range was determined from various strain collections including Gram-positive and Gram-negative bacteria and phytopathogenic fungi (Table 1). CS prevented the growth of several *Bacillus* species such as *Bacillus thuringiensis*, *Bacillus mycoides*, *B. subtilis*, and *Bacillus licheniformis*. Antimicrobial activity was also efficient against Gram-positive pathogenic bacteria such as methicillin-resistant *Staphylococcus* species (clinical

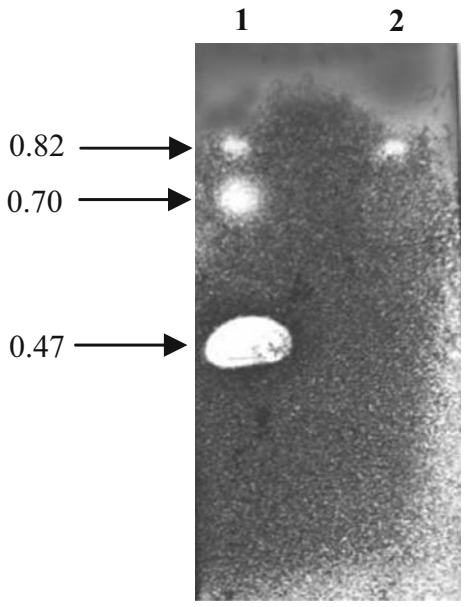

Fig. 3 TLC-bioautography analysis of CS produced by B38 strain at 8 h (4 μg of proteins, *lane 1*) and 24 h (0.8 μg of proteins, *lane 2*) after inoculation. *Arrows* indicated R_f values of bioactive compounds against *S. aureus* ATCC 29213

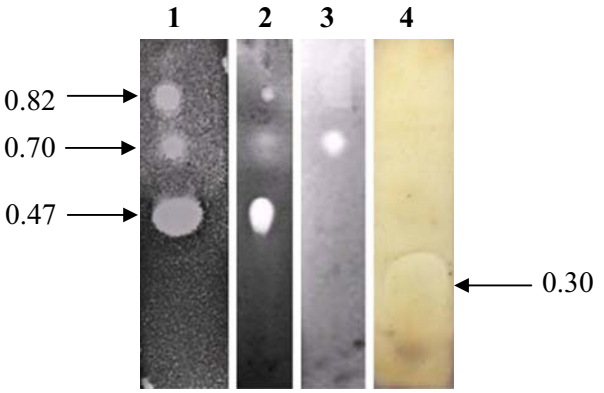

Fig. 4 TLC-bioautography assay of antimicrobial activity produced by B38 strain against *S. aureus* ATCC 29213 (*lane 1*), methicillin-resistant *Staphylococcus cohinii* (*lane 2*), *L. monocytogenes* CIP 82110 T (*lane 3*) and *F. oxysporum* sp. (*lane 4*). For detection of antibacterial and antifungal activities, 0.8 μg and 2.4 μg of proteins were used, respectively. *Arrows* indicated R_f values of bioactive compounds

isolates), *Listeria innocua*, *L. monocytogenes*, and *Enterococcus faecalis* strains which emphasize its putative use in human therapeutics. The growth of Gram-negative pathogenic bacteria such as *Salmonella enteritidis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* was also inhibited. It was previously reported that *Bacillus* species produced antimicrobial activity against both Gram-positive and Gram-negative pathogenic bacteria [13, 34, 35]. The producer strain might be used as a potential agent in biological control as it exhibited antifungal activity against phytopathogenic fungi such as *F. oxysporum* and *Fusarium culmorum*, *Alternaria solani* sp., *Botryospheria* sp and *Phoma* sp. However, no antimicrobial activity was detected against *Sclerotinia sclerotiorum* sp. *Bacillus* species, especially *B. subtilis* strains, are well known to produce antimicrobial metabolites with antifungal properties [7, 14, 36, 37].

Table 2 Effect of heat and pH treatment on the antimicrobial activity from B38 strain

Treatments	Antimicrobial activity (%)
None (control)	100
Heat	
40 °C for 30 min	100
50 °C for 30 min	100
60 °C for 30 min	100
70 °C for 30 min	100
80 °C for 30 min	100
90 °C for 30 min	100
100 °C for 30 min	80
Autoclaving 121 °C for 20 min	80
pH	
2	25
3	50
4	100
5	100
6	100
7	100
8	100
9	100
10	100
11	16.66
12	0

Table 3 Effect of hydrolytic enzymes, surfactants, and organic solvents on the antimicrobial activity from B38 strain

Treatments	Residual activity (%)
None (control)	100
Enzymes	
Proteinase K	100
Trypsine	100
α-Chymotrypsine	100
Lipase A	100
Surfactants	
Sodium dodecyl sulfate	100
Tween 20	100
Triton X-100	100
Urea	100
Organic solvents	
Methanol	100
Acetone	100

To further characterize the antibacterial activity, CS from 24 h culture was subjected to TLC analysis, and biological properties of the separated bioactive compounds were investigated. At least four bioactive spots were observed. Three of them displayed different antibacterial activity spectrum, and one spot exhibited antifungal activity. Spots with R_f values of 0.47, 0.70, and 0.82 inhibited *S. aureus* ATCC 29213 and clinical isolates of methicillin-resistant *Staphylococcus* species (Fig. 4). Only one spot with R_f value of 0.70 showed a broad inhibitory activity spectrum against all tested Gram-positive and Gram-negative strains: *L. innocua*, *L. monocytogenes*, *E. faecalis*, *Escherichia coli*, *S. enteritidis*, and *P. aeruginosa* (Fig. 4). Bioautography assay also showed antifungal activity materialized as a single spot with R_f value of 0.30 against *F. oxysporum* spores (Fig. 4).

Physico-chemical Properties of the Antibacterial Activity

Antibacterial activity was also tested for its sensitivity towards heat and pH treatment (Table 2), organic solvents, proteolytic and lipolytic enzymes as well as surfactants (Table 3). The antibacterial activity, which was conserved till 90 °C, lost 20% of its initial

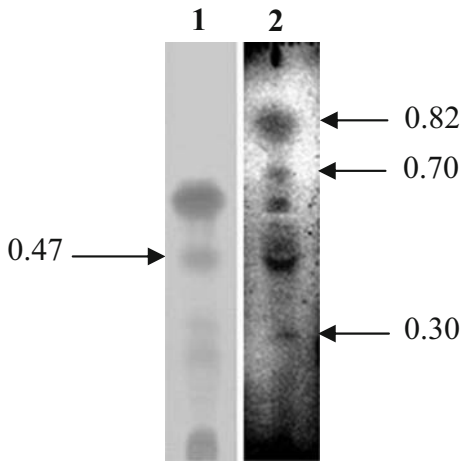

Fig. 5 TLC analysis of antimicrobial compounds from CS of B38 strain. Staining was realized either with ninhydrin (*lane 1*) or Serva blue W (*lane 2*). Two microliters (0.8 μg of proteins) of concentrated CS was applied onto TLC plates. *Arrows* indicated R_f values of stained spots with antibacterial activity

value after autoclaving at 121 °C during 20 min (Table 2). In addition, it was stable within a wide range of pH (4–10). The antibacterial activity was reduced to 25% and 50% at pH 2 and 3, respectively, and was severely altered at pH higher than 10 (Table 2). The heat-stable property was also observed in other antimicrobial metabolites produced by *Bacillus* species [14, 15, 38, 39]. The stability of this antibacterial activity over a wide range of pH and after heat treatment might be useful in several industrial applications. The antibacterial activity was soluble in organic solvents such as acetone and methanol (Table 3) and was not inhibited by surfactants at 1% final concentration (Table 3), suggesting a hydrophobic character of the compound.

The antibacterial activity was resistant to proteolytic enzymes, even at mass ratio 1:5. This resistance to proteolysis might be due to the presence of N- and/or C- terminally blocked peptides or the presence of uncommon constituents like D-amino acids [6, 40–42]. No loss of activity was also observed upon exposure of the CS to lipase A (Table 3) which indicates that active compounds do not require a lipidic moiety for their biological activity.

The separated active spots were stained with both ninhydrin and Serva bleu W (Fig. 5). The spot with R_f value of 0.47 was both ninhydrin- and Serva blue W-positive, which indicates that this compound might have a peptidic nature with a free amino group, whereas the spots with R_f values of 0.30, 0.70, and 0.82 might be cyclic peptides as they are only stained by Serva bleu W. The antifungal compound with the R_f value of 0.30 which formed a white spot when sprayed with water (data not shown) indicated a lipophilic nature of this compound. Biochemical characterization showed that the produced antimicrobial metabolites might belong to peptide antibiotics. *B. subtilis* species are well known as producers of a great number of antimicrobial compounds with different structures [41, 43, 44] in which peptide antibiotics represent the predominant class [40]. For instance, *B. subtilis* 168 strain produced both ribosomal peptides and nonribosomal lipopeptide antibiotics [45]. Other *B. subtilis* strains produced antimicrobial compounds belonging to the same group of antibiotics [14, 46–48].

In conclusion, *B. subtilis* B38 strain isolated from soil produced a variety of antimicrobial metabolites with promising features in the biocontrol of pathogenic microorganisms including methicillin-resistant *Staphylococcus* species. They displayed a wide spectrum of activity and showed remarkable stability towards proteolytic enzymes, temperature, and pH. They might be useful in the development of new anti-methicillin-resistant *Staphylococcus* activity antibiotics. Further investigations should be performed to purify at homogeneity the bioactive compounds in order to establish their exact chemical structures with special interest in optimizing their production.

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