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Antimicrobial Activity of *Bacillus* Strains Isolated from Spring Water and A Novel Bacteriocin: RS108

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The aim of this research was to investigate the bacteriocins produced by *Bacillus* strains isolated from the spring water of Rize, in Turkey. *Bacillus* cereus RS108 was identified by both conventional and molecular methods, bacteriocin RS108 which was produced by RS108, was partially characterized. A broad range of indicator strains, including several species of bacteria and yeast like fungi, was inhibited by a crude bacteriocin obtained from culture supernatant fluid. The best antimicrobial activity of RS108 was detected on *S. pyogenes*, *L. monocitogenes*, and another *B. cereus* strains, and at the late exponential growth phase. RS108 was stable at 90°C, but the activity was lost when the temperature reached 100 °C. It was inactivated by proteinase K. It was resistant 10% ration of some solvent (chloroform, ethanol etc.), but sensitive high concentration. Bacteriocin activity was observed in the pH range of 3.0-9.0. SDS-PAGE analysis of the partially purified bacteriocin shows that the molecular weight of bacteriocin RS108 is approximately 4 kDa.

Key words: *Bacillus cereus*, Bacteriocin, Spring water, Partial Purification.

Bacteriocins are ribosomally synthesized bacterial proteins or peptides, which are produced by different groups of bacteria, that inhibit strains and species, which that are usually, but not always, closely related to the producing bacteria[1].

According to Klaenhammer[2] bacteriocins can be classified into four groups, on the basis of their molecular mass, termostability, enzymatic sensitivity and presence of posttranslationally modified amino acids. Class I bacteriocins, also called the lantibiotics, are characterised by the presence of unusual thioether amino acids. Class II bacteriocins represent small (<10 kDa), heat-stable, membrane-active peptides. This class is further subdivided into three groups. Bacteriocins belonging to class III consist of large (>30 kDa), heat labile proteins, while class IV represents complex bacteriocins that contain essential lipid or carbohydrate moieties in addition to a protein component.

Bacillus genus is Gram-positive, spore forming bacteria that are ubiquitous in the environment. Strains of Bacillus have been used in food and agricultural industries for the manufacture of enzymes and bioinsecticides. Bacillus genus has received considerable attention, as a source of antimicrobials, due to the abundance of antimicrobial substance production; these substances include bacteriocin, lipopeptide, polyketide, phospholipid, and aminosugar[3]. The production of a large number of bacteriocins or bacteriocin-like substances has been already described for many Bacillus species, and has a

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history of safe use in both food and industry such as Bacillus cereus, Bacillus subtilis and Bacillus thuringiensis[4, 5, 6].

In this study we report the identification, partial purification and characterization of bacteriocin RS108, a novel bacteriocin, produced by B. cereus RS108 isolated from spring water of Rize in Turkey. The antimicrobial spectrum and some properties of the bacteriocin are described. Bacteriocin RS108 may have a potential use as food biopreservative, because of its promising thermostable properties and broad antimicrobial spectrum.

MATERIALS AND METHODS

Identification of Bacillus strains

Bacillus strains from different spring water samples were taken from Rize in 2001. For identification, morphological, biochemical and physiological tests were used. According to the results, the isolates were determined to belong the genus Bacillus[7].

16S rRNA and total protein analysis of B. cereus RS108 strain

The genomic DNA was isolated from strain RS108 with genomic DNA purification kit (Promega). The 16S rRNA gene was selectively amplified from purified genomic DNA by using primers of UNI16S-L (5'-ATTCTAGAGTTTGATC ATGGCTCA-3') and UNI16S-R (5'-ATGGT ACCGTGTGA CGGGCGGTGTGTA-3')[8]. Approximately 1400 bp PCR product was cloned to pGEM-T Easy (Promega) vector system and then the right products were sent to Macrogen (The Netherlands) for sequencing. The obtained sequences were analyzed by BLAST searches using the NCBI GenBank database[9,10]. Finally, the sequences were used to construct a phylogenetic tree to verify isolate identification.

The total protein profiles of strain RS108 together with B. cereus 702 Roma and B. subtilis ATCC 6633 were demonstrated by SDS-PAGE analysis performed by the buffer system described by Laemmli[11]. Protein bands were stained with Coomassie Brillant Blue.

Determination of bacteriocin activity

Forty one isolates were screened to produce bacteriocin like antimicrobial activity by the agar spot test[12]. The test was performed as follows: 200 µl of each Bacillus cultures at early exponential growth phase (OD 600 of 0.2-0.3) in Nutrient Broth was mixed with 4 ml of Nutrient agar soft (0.6%) agar and poured on the Nutrient agar plate. Then, the 5 µl of each cell free supernatant (CFS) was dropped onto the solidified soft agar. The plates were incubated for 48 h at 35ºC. Bacteriocin inhibition was indicated by a clear zone in the soft agar layer.

Determination of best bacteriocin activity in different medium

To chosen the best conditions of bacteriocin producing, B. cereus RS108 was grown in 10 ml of MHB (Mueller Hinton Broth), BHI (Brain Heart Infision), TSB (Triptic Soy Broth), NB (Nutrient Broth) medium 35°C in a rotary shaker. The cells were removed by centrifugation (8000Xg for 10 min.) and CFS was obtained. Also CFS's were filtered through 0.45 µm membrane filter (Milipore). CFS's were used to determine the best condition for production of antimicrobial activity by using the agar-well diffusion method[13]. The amount of indicator strain Bacillus sp. RS314 was adjusted to 0.5 MacFarland and this suspension was spread on MHB agar and allowed to dry. A cork borer was used to open well of 5mm diameter on the agar plates. The wells were filled-up with 50 µl of cell-free supernatant of B. cereus RS108. The inhibition zones were measured after incubation at 35°C for overninght and the best condition was determined according to the inhibition zone.

Antimicrobial spectrum of RS108 bacteriocin

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: Bacillus subtilis ATCC 1264, B. cereus 702 Roma, Staphylococcus aureus ATCC 25923, Streptococcus pyogenes ATCC 19615, Listeria mon?ocitogenes ATCC 43251, Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 29212, Acinetobacter iwofii ATCC 19002, Pseudomonas aeruginosa ATCC 10145, Klebsiella pneumoniae ATCC 13883, Yersinia pseudotuberculosis ATCC 911, Candida albicans ATCC 60193, Candida tropicalis ATCC 13803 and Candida glabrata ATCC 66032.

The agar well-diffusion method was used to examine the antibacterial activity of bacteriocin RS108 to several Gram-positive and negative bacteria. For bacteria, the plates were incubated

for 24 h at 35 ºC and diameters of inhibition zones were measured. Candida species were maintained in PDA for 48 h at 25 ºC.

Sensitivity to enzymes, solvent, heat, and pH

To determine the biochemical and biophysical properties of the inhibitory substance, samples were tested for sensitivity to enzymes, solvents, different temperatures and pH values. Bacillus sp. RS314 was used as an indicator organism. Aliquots (500 µl) of CFS were exposed to heat treatments of 60, 70, 80, 90 and 100 ºC for 10 min. The effect of pH on bacteriocin activity of RS108 was determined chancing pH of CFS's. The pH of CFS's were adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with HCl and NaOH. Then all samples were incubated at room temperature for 4 hours. After incubation, pH of CFS's were adjusted again to pH 7.0. The CFS's were treated with proteinase K, trypsine, lysozyme, amylase, lipase and catalase each at a final concentration at 0.5-5 mg/ml. The samples with and without enzymes were incubated at 30 ºC for 1 h, and residual activity were determined. To carry out tests of organic solvent (Chloroform, Methanol, Ethanol, Dimethyl sulfoxide, Acetone, Xylene, N-hexane) sensitivity, the CFS was mixed with 10-50% final volume of solvent and kept at room temperature for 4 h before the antimicrobial activity. All of the experiments were finished in duplicate.

Growth and Bacteriocin production

For production assays, B. cereus RS108, was inoculated (1% v/v) into 100 ml of sterile BHI broth and incubated in a rotary shaker at 35ºC for 24 h. Two milliliter of samples were aseptically removed every 2 hours. Cell growth was monitored spectrophotometrically (OD600), and bacteriocin activity of the CFS was evaluated by the well-diffusion method. Spore accumulation assay was performed by the protocol described by[14]. To determine the activity units per milliliter of culture (AU ml-1), serial two-fold dilution of the filter sterilized CSF were performed and used in the well-diffusion test against the indicator sensitive strain. One unit was defined as the reciprocal of the highest dilution that produced a detectable zone of inhibition.

For bacteriocin R108 purification, B. cereus RS108 was grown in a liquid medium until reaching the stationary phase (24 h, at 35 ºC), then cells were removed by centrifugation, and proteins in the CFS were precipitated with ammonium sulfate at 70% of saturation. The precipitate was dissolved in one hundredth of the original volume, in 20 mM sodium phosphate buffered saline (PBS), pH 6.8. Ultra filtration was used for removal of ammonium sulfate.

For detection of molecular weight of partial purified RS108, SDS-PAGE analysis was performed by standart procedure[11]. After electrophoresis the gel was fixed for 30 min in sterilled distile water and was cut two parts vertically. The one part of gel was stained Coomassie Brillant Blue R-250. Another portion was laid in MHA petri dishes and overlaid with soft agar containing indicator strain RS314. The petri plate was incubated at 35°C for 24 h. Molecular weight of separated proteins was determined by using protein weight marker (Promega).

Nucleotide sequence accession numbers

The GenBank database accession numbers for the 16S rRNA nucleotide sequences of Bacillus cereus RS108 is DQ288144.

RESULTS AND DISCUSSION

Identification of Bacillus strains

Forty one aerobic and mesophilic gram-positive bacilli-shaped bacteria were isolated from spring water samples from five different regions. The strains were identified according to morphological and biochemical test results (Table 1). Determinated strains were given in Table 2.

The preliminary identification of RS108 strain showing best bacteriocin activity was based on phenotypical characteristics, indicating B. cereus; and identified in species level as B. cereus by the total protein profiles obtained by SDS-PAGE analysis (Figure 1) and sequence analysis of 16S rDNA. In SDS-PAGE analysis, no similarity to the protein bands of B. subtilis ATCC 6633 was detected, but there was a complete resemblance to the B. cereus 702 Roma strain.

Based on BLAST search using the 16S rRNA gene sequencing, RS108 strain showed 99% similarity to B. cereus and B. thuringiensis. But, according to physiological and biochemical tests, the RS108 was more similar to B. cereus than B. thuringiensis. Phylogenetic analysis also supports this identification (Fig 2). So, the RS108 strain was identified as Bacillus cereus.

Table1. Morphological, biochemical and physiological properties of Bacillus strains

	Gram stain	Spor form	Motility	Catalase	Colony shape	Lecitinase reduction	Proskauer	Indol	Nitrate hydrolysis	Voges hydrolysis	Citrate	Gelatin	Starch	Gas	Acid from D-glucose	Acid from maltose	H2S production	Growth 15 ℃	25 ℃	35 ℃	45 ℃
RS1	+	C	-	+	R	+	-	+	+	-	+	+	+	-	-	-	+	+	+	-
RS6	+	C	-	+	R	+	-	-	+	-	+	+	+	-	-	-	+	+	+	-
RS8	+	C	+	+	R	+	-	+	+	-	+	-	+	+	+	-	+	+	+	+
RS13	+	C	-	+	R	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
RS21	+	T	+	+	R	+	-	-	+	-	+	-	+	+	+	-	+	+	+	+
RS26	+	C	-	+	R	-	-	+	-	-	+	+	-	+	-	-	+	+	+	-
RS35	+	C	-	+	R	-	-	-	-	-	+	+	-	+	-	-	+	+	+	-
RS39	+	T	-	+	R	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-
RS40	+	C	+	+	R	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
RS41	+	C	-	+	R	-	-	+	-	-	-	+	-	+	-	-	+	+	+	-
RS42	+	C	-	+	R	-	-	+	-	-	-	+	-	+	-	-	+	+	+	-
RS44	+	C	-	+	R	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
RS46	+	C	-	+	R	+	-	+	+	-	-	-	+	-	-	-	+	+	+	-
RS53	+	C	+	+	R	+	-	-	+	-	+	-	+	+	+	-	+	+	+	+
RS65	+	C	-	+	R	+	-	+	+	-	+	+	+	-	-	-	+	+	+	-
RS69	+	T	-	+	R	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
RS73	+	C	-	+	R	-	-	-	-	-	+	+	-	+	-	-	+	+	+	-
RS75	+	C	+	+	R	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
RS78	+	C	-	+	R	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
RS86	+	C	+	+	R	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+
RS105	+	C	+	+	R	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+
RS107	+	C	-	+	R	-	-	+	-	-	-	+	-	+	-	-	+	+	+	-
RS108	+	C	+	+	R	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+
RS109	+	C	+	+	R	+	-	+	+	-	+	-	+	+	+	-	+	+	+	+
RS128	+	C	-	+	R	+	-	-	+	-	-	+	+	-	-	-	+	+	+	-
RS159	+	C	-	+	R	-	-	-	-	-	+	+	-	+	-	-	+	+	+	-
RS161	+	T	-	+	R	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-
RS164	+	C	-	+	R	+	-	-	+	-	-	+	+	-	-	-	+	+	+	-
RS220	+	T	+	+	R	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+
RS221	+	C	-	+	R	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
RS225	+	C	+	+	R	+	-	-	+	-	+	-	+	+	+	-	+	+	+	+
RS261	+	T	-	+	R	-	-	+	+	-	+	+	-	-	-	-	+	+	+	-
RS314	+	C	+	+	R	+	-	+	+	-	+	+	-	+	-	-	+	+	+	+
RS337	+	C	+	+	R	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+
RS340	+	C	+	+	R	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+
RS343	+	T	+	+	R	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+
RS344	+	C	+	+	R	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+
RS355	+	T	-	+	R	-	-	+	+	-	-	-	-	-	-	-	+	+	+	+
RS356	+	C	+	+	R	+	-	-	+	-	+	+	+	-	+	-	+	+	+	+
RS357	+	C	-	+	R	-	-	+	-	-	+	+	-	+	-	-	+	+	+	-
RS358	+	C	+	+	R	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+

Table 2. Identification of *Bacillus* strains

Species	Number (n)	Percent (%)
B. cereus	15	36.6
B. circulans	12	29.3
B. mycoides	6	14.6
B. sphaericus	2	4.9
Bacillus sp.	6	14.6
Total	41	

Determination of bacteriocin activity

All of Bacillus strains were screened for their antibacterial activity, but only one strain RS108 was selected as the good candidate (Table 3). CFS of thr RS108 showed the antagonistic activity to the strains of all B. cereus and other Bacillus groups (Bacillus sp., B. mycoides, B. sphaericus and B. circulans).At the sime time, Bacillus sp. RS314 strain was chosen as indicator strains because of its high sensitivity.

Table 3. Antagonism of *B. cereus* RS108 against isolated *Bacillus* strains by agar spot assay

No	Indicator strain	Inhibition zone(mm)	No	Indicator strain	Inhibition zone(mm)
1	*B. mycoides* RS1	-	22	*B. circulans* RS107	-
2	*B. mycoides* RS6	-	23	*B. cereus* RS108	-
3	*B. cereus* RS8	+	24	*B. cereus* RS109	+
4	*B. circulans* RS13	-	25	*B. mycoides* RS128	++
5	*B. cereus* RS21	+	26	*B. circulans* RS159	-
6	*B. circulans* RS26	-	27	*Bacillus* sp. RS161	-
7	*B. circulans* RS35	-	28	*B. mycoides* RS164	-
8	*B. sphaericus* RS39	-	29	*Bacillus* sp. RS220	-
9	*B. cereus* RS40	+	30	*B. circulans* RS221	++
10	*B. circulans* RS41	-	31	*B. cereus* RS225	+
11	*B. circulans* RS42	-	32	*Bacillus* sp. RS261	-
12	*B. circulans* RS44	-	33	*Bacillus. sp.* RS314	+++
13	*B. mycoides* RS46	++	34	*B. cereus* RS337	+
14	*B. cereus* RS53	++	35	*B. cereus* RS340	+
15	*B. mycoides* RS65	-	36	*B. cereus* RS343	+
16	*B. sphaericus* RS69	++	37	*B. cereus* RS344	+
17	*B. circulans* RS73	-	38	*Bacillus* sp. RS355	-
18	*B. cereus* RS75	++	39	*B. cereus* RS356	+
19	*B. circulans* RS78	-	40	*B. circulans* RS357	-
20	*B. cereus* RS86	+	41	*B. cereus* RS358	-
21	*Bacillus* sp. RS105	-			

(-); Not inhibited, (+); 1-5 mm clear inhibited, (++); 5-10 mm clear inhibited, (+++); Ã 10 mm clear inhibited.

The property of bacteriocin production was investigated by using different media. Brain heart infusion was the most suitable media for the production of bacteriocin (Table 4). Different media such as BHI, LB, TSB, MHB were used in Bacillus species to produce bacteriocin[15, 16, 17], but BHI medium generally was preferred by Bacillus cereus in many studies[18, 19].

Antimicrobial activity of bacteriocin RS108

For the antimicrobial spectrum of activity, cell free supernatant of B. cereus RS108 was tested against various Gram-positive, Gram-negative bacteria and some yeasts. Table 3 and 5 indicated that bacteriocin RS108 was shown to affect of all of the tested B. cereus species and B. cereus 702 Roma. In addition, bacteriocin RS108 was effected that some other Bacillus strains, S. pyogenes ATCC 19615 and L. monocitogenes ATCC 43251.No activity was detected against Gram negative bacteria and yeast-like fungi. So, it is clear that RS108 has a significant effect on Bacillus species, this property is similar to many bacteriocin produced by Bacillus species[17, 18, 19, 20].

Although many bacteriocins have been isolated and characterized, only a few have demonstrated commercial potential in food

application. The major functional limitations for the application of bacteriocins in foods are their relatively narrow activity spectra and moderate antibacterial effects[21]. Bacteriocins produced by Gram-positive strains are mostly inhibitory to Gram-positive strains and less effective against Gram-negative strains[22]. While bacteriocin RS108 has not shown any activity against Gram negative bacteria which tested, Bacteriocin RS108 was shown activity against some Gram-positive bacteria especially two human patogenic bacteria including S. pyogenes and L. monocitogenes.

Table 4. Factors on bacteriocin activity

Treatment	Bacteriocin activity	
Enzymes		
Proteinase K	-	
Trypsine	+	
Lysozyme	+	
Lipase	+	
Amylase	+	
Catalase	+	
pH Effect		
3	+	
4	++	
5	++	
6	++	
7	+	
8	+	
9	+	
10	-	
Heat Effect		
60 ºC, 10 min	+	
70 ºC, 10 min	+	
80 ºC, 10 min	+	
90 ºC, 10 min	+	
100 ºC, 10 min	-	
Medium		
Brain Heart Infision	32 AU*	
Mueller Hinton	16 AU	
Triptic Soy Broth	16 AU	
Nutrient Broth	16 AU	
Precipitaion		
Ammonium sulfate (50%)	64 AU	
Ammonium sulfate (60%)	64 AU	
Ammonium sulfate (70%)	120 AU	
Solvent Effect	% 10	% 50
Methanol and Ethanol	+	-
Dimethyl sulfoxide	+	-
Chloroform	+	-
Aseton	+	-
Xylene	+	-
Hexane	+	-

* The bacteriocin titer was defined as the reciprocal of the highest dilution showing complete inhibition of the indicator lawn and was expressed in activity units (AU) per milliliter.
Bacteriocin activity ≤10: (+), >10: (++), No bacteriocin activity: (-).

Sensitivity to enzymes, solvent, heat, and pH

RS108 bacteriocin was comparatively stable to heat treatment. This activity was maintained during treatment up to 90°C for 10 min and disappeared at 100°C for 10 min. Bacteriocin RS108 was also pH stable in the range of 3.0- 9.0 (Table 4). Many bacteriocins have been reported to have greater bactericidal activity at low pH (pH 6.0 and below)[20, 23, 24]. At pH 7.0, many of low-molecular-weight bacteriocins are cationic, and this seems to be a unifying feature of the both the lantibiotic and non-lantibiotic-containing bacteriocins[25].

When samples were treated with proteolytic enzyme, the inhibitory activities decreased only proteinase K. No modification of activity was observed when cerein RS108 was treated with 0.5-5 mg/ml trypsine (Table 4). A study that was examined by Naclerio et al.[18] has shown that activity of a new bacteriocin, identified a Bacillus cereus, and decreased when samples were treated with trypsin, chymotrypsin and proteinase K (10mg/ml). Also, it is observed that Lysozyme, DNase, Ribonuclease A has no effect on activity.

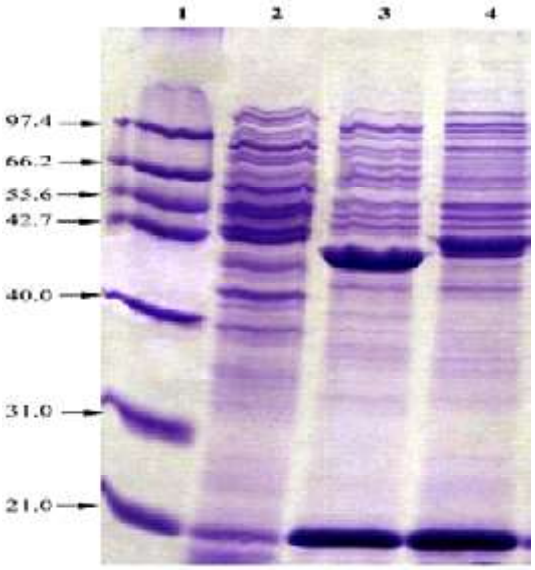
Table 5. Sensitivity of indicator microorganisms to bacteriocin RS108 by agar well diffusion test

Strain	Diameter of zone of inhibition (mm)
B. cereus RS 314	18
B. cereus 702 Roma	14
B. subtilis ATCC 1264	10
S. aureus ATCC 25923	-
S. pyogenes ATCC 19615	10
L. monocitogenes ATCC 43251	10
E. coli ATCC 25922	-
E. faecalis ATCC 29212	-
A. iwofii ATCC 19002	-
P. aeruginosa ATCC 10145	-
K. pneumoniae ATCC 13883	-
Y. pseudotuberculosis ATCC 911	-
C. albicans ATCC 60193	-
C. glabrata ATCC 66032	-
C. tropicalis ATCC 13803	-

Organic solvents were used at working concentrations of 10 and 50% (v/v). While inhibitory activity was not in effect when samples were treated with low concentration solvent, high concentration solvent was effected activity (Table 4). The results are similar to many studies[18, 19].

Production of cerein RS108

The bacteriocin RS108 activity could not be detected during the early exponential growth phase, but was suddenly detected in samples taken at the late exponential growth phase. At the same time, it was observed that B. cereus RS108 begun to sporulate (Fig 3). The results are similar to those described for cerein produced by *B. cereus*[18], turicin 7 produced by *B. thuringiensis*[12], a bacteriocin produced by *B. licheniformis*[3].



Lane 1: Molecular weight marker (Promega),
Lane 2: *B. subtilis* ATCC 6633,
Lane 3: *B. cereus* RS108 strain,
Lane 4: *B. cereus* 702 Roma strain.
Fig. 1. SDS-PAGE analysis of *B. cereus* RS108.

Partial purification of bacteriocin RS108

Partial purification of cerein RS108 by 70% ammonium sulphate precipitation was determined for producing the best bacteriocin activity. The test results of electrophoresis analyses have shown that approximately 4 kDa weight protein, estimated by calculating the different rf (relative migration) values of standard proteins, shows bacteriocin activity (Fig. 4). The low molecular masses have been reported for several Bacilli bacteriocins, such as 3162 Da thuricin 17[22], 2 KDa bacillocin 490[26] and 3.4 kDa coagulin[27].

In conclusion, it is suggested that bacteriocin RS108 is a bacteriocin produced by a newly-isolated *B. cereus* RS108. The crude bacteriocin of *B. cereus* RS108 showed inhibition on a wide range of different species of *Bacillus* strains (*B. circulans, B. mycoides* and *B. sphaericus*), and some food spoilage bacteria, such as *Bacillus cereus*, plus some human pathogenic bacteria such as *S. pyogenes*. It makes this bacteriocin a potential candidate as an antimicrobial agent and growth promoter in the

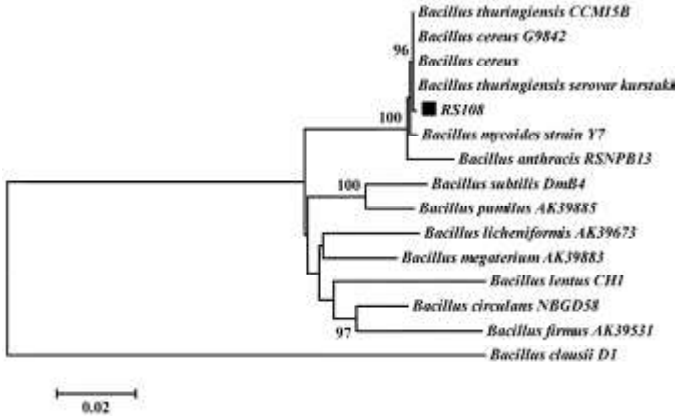


Fig. 2. Pyhlogenetic analysis of RS108 and their closely related 14 bacterial species based on the partial sequence of the 16S rRNA gene. Neighbor-joining analysis with p-distance method was used to construct the dendrogram. Bootstrap values shown next to nodes are based on 1,000 replicates. Bootstrap values C≥70% are labeled. *Bacillus cereus* RS108 isolates were indicated with black squares. The scale on the bottom of the dendrogram shows the degree of dissimilarity.

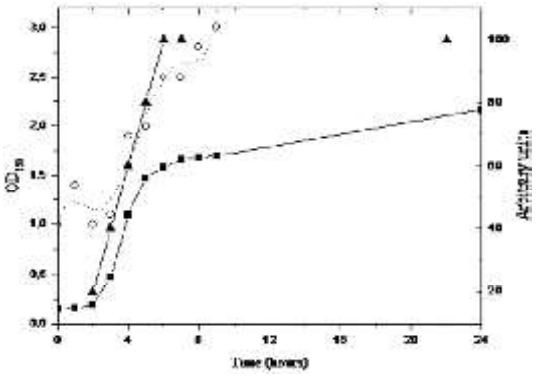
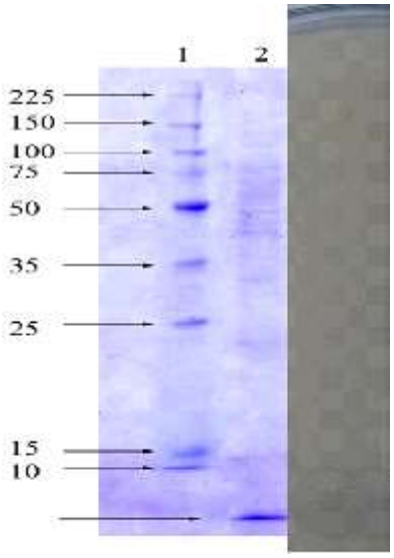


Fig. 3. Cerein RS108 production in batch cultures of *B. cereus* RS108 in BHI at 35°C. Kinetics of *B. cereus* RS108 growth (▲), spor accumulation (○) and cerein RS108 activity in samples of CFS (■)



(A) Coomassie Brillant Blue stained gel,
Lane 1: Molecular weight marker (Promega),
Lane 2: partial purified cerein RS108.
(B) Part of the gel overlaid by the soft agar with indicator strains RS314.

Fig. 4. SDS-PAGE analysis and direct detection of Bacteriocin RS108

agricultural environment. In addition, bacteriocin activity was observed at 90°C and pH 3.0-9.0. The maximum bacteriocin production was achieved at a pH between 4.0-6.0, temperature between 25-35 °C, and in BHI medium.

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