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Bacteriocin-like inhibitory substance (BLIS) activity of *Streptococcus macedonicus* MBF10-2 and its synergistic action in combination with antibiotics

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ABSTRACT

Objective: To characterize the bacteriocin-like inhibitory-substances (BLIS) activity of *Streptococcus macedonicus* MBF10-2 [named BLIS(MBF10-2)], a bacteriocinogenic strain isolated from an Indonesian tofu byproduct.

Methods: BLIS(MBF10-2) was obtained by culturing the bacterium, and standard deferred antagonism assays were used to demonstrate its activity. The antibacterial testing of fractions collected by filtration using 3–30 kDa cut-off membrane sizes were carried out by performing well diffusion method.

Results: The growth of *Micrococcus luteus*, *Streptococcus pyogenes*, *Lactococcus lactis*, *Leuconostoc mesenteroides* and *Weissella confusa* were inhibited by BLIS(MBF10-2). Interestingly, BLIS-containing fractions obtained from sequential application on ultrafiltration membranes indicated that this bacterium *Streptococcus macedonicus* MBF10-2 could produces at least two antimicrobial peptides activities, one of which is likely to be a lantibiotic peptide. Potential synergistic activity against certain Gram-positive (but not Gram-negative) species when partnered with antibiotics (ampicillin, tetracycline or kanamycin) were observed.

Conclusion: Combination of some BLIS(MBF10-2) active fractions with antibiotics (ampicillin, tetracycline or kanamycin) could demonstrate synergistic activities against certain Gram-positive species.

1. Introduction

All organisms produce antimicrobial peptides that represent part of the natural and innate immune system that protects them against invading organisms, and in the microbial world, antibiotics are an important part of the defense system of bacteria [1]. Bacteriocins are ribosomally-synthesized peptides (or proteins) produced by bacteria that show antibacterial (either bactericidal or bacteriostatic) activity against species that are closely related to the producer bacterium [1,2]. Over the past decades, many bacteriocin types have been characterized biochemically and genetically from highly diverse sources ranging from Gram-positive and Gram-negative species to archaebacteria. For example, many bacteriocins, e.g., the lantibiotic lacticin 347, are membrane-active compounds [3]. They act against their target cells by forming pores in the membrane, thus causing membrane leakage of low molecular weight substances such as protons, potassium ions, and phosphate ions, leading to disruption of membrane potential, cessation of ATP synthesis and cell death. Some peptides also act by blocking cell wall formation by interfering with the biosynthesis of peptidoglycan [4,5]. Various classification schemes have been proposed in an attempt to understand the diversity of bacteriocin structures [1,2,6–10]. Bacteriocins are therefore of great interest to the food industry as natural food

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preservatives, targeting several species of spoilage bacteria and foodborne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus* (*S. aureus*) and *Clostridium botulinum* [10–12].

The most well-known producers of bacteriocins are members of the diverse group of Gram-positive bacteria, the lactic acid bacteria (LAB) [10], with the most prolific bacteriocin-producing genera being *Streptococcus* and *Lactobacillus*. LAB have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf life characteristics [13]. Moreover, it has been shown that some strains of LAB possess interesting health-promoting properties, *i.e.*, as probiotics, with the potential to combat gastrointestinal pathogens such as *Helicobacter pylori*, *Escherichia coli,* and *Salmonella* [13]. However, some foodborne pathogens, namely *Listeria monocytogenes* and *Listeria innocua*, are already resistant to food-grade bacteriocins such as nisin, lactocin 705, and enterocin CRL35. Therefore, the development of new types of bacteriocins for use as food-grade preservatives is needed [7,13].

The emergence of multiple antibiotic resistances in pathogenic bacteria has stimulated the demand for more potent antibiotics [14]. Bacteriocins have become one of the centers of attention for potential development as alternative therapeutic agents due to their specific bactericidal or bacteriostatic action [15]. In contrast to traditional antibiotics, most bacteriocins instead exhibit a narrow spectrum mode of action, thereby minimizing collateral damage to desirable (*i.e.*, non-pathogenic) members of the normal microflora [1,5,8]. Although they often act toward species related to the producer species, bacteriocins can possess very high potency (at pico-to nanomolar concentrations) and specificity [5].

Bacteriocins also have the potential to be developed to complement traditional antibiotics as they could work synergistically against certain bacterial pathogens. This not only provides an alternative way to expand the spectrum of targeted organisms while preventing the emergence of antibiotic-resistant species, but also allows lower doses of both bacteriocin and antibiotic, hence decreasing the overall toxicity to the patient. Certain combinations have been used in clinical settings in order to combat methicillin-resistant *S. aureus* and vancomycin-resistant enterococci with promising results: (i) combination of nisin with several peptidoglycan-modulating antibiotics such as ramoplanin and bacitracin [16], (ii) combination of nisin with ampicillin, ceftriaxone and cefotaxime [17], and (iii) combination of lysostaphin with polymyxin B [18].

This study aimed to characterize the bacteriocin-like inhibitory substance(s) (BLIS) produced by *Streptococcus macedonicus* (*S. macedonicus*) MBF10-2, a bacteriocinogenic strain isolated from a tofu byproduct [19] for its BLIS activity using agar-based deferred antagonism (P-typing) assay, as well as its BLIS optimum temperature and pH. In addition, it was also aimed to obtain the most active fraction after protein fractionation using size range-specific ultrafiltration columns, and to determine BLIS-containing fractions' potential in synergistic inhibition with selected antibiotics.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The BLIS producer, *S. macedonicus* MBF10-2, was routinely cultured in MRS [20] medium [10 g/L peptone, 8 g/L LAB-Lemco (Oxoid, Hampshire, UK), 4 g/L yeast extract (Difco, New Jersey, USA), 2 g/L dipotassium hydrogen phosphate, 5 g/L sodium acetate, 2 g/L ammonium citrate, 0.2 g/L magnesium sulfate, 0.05 g/L manganese sulfate, 0.05% (v/v) Tween 80, 20 g/L dextrose]. For solid media, Bacto agar (Difco, New Jersey, USA) was added to a final concentration of 15 g/L. Incubation was carried out at 32 °C for 24 h in an anaerobic jar using Anaerogen® (Oxoid, Hampshire, UK) gas packs.

For deferred antagonism assays (DAA), strain MBF10-2 was grown on Columbia blood agar medium (Oxoid, Hampshire, UK) added with (5% sheep blood), and supplemented with calcium carbonate to minimize lactic acid-related inhibitory effects. The nine standard indicator strains [21] used in DAAs, namely *Micrococcus luteus (M. luteus)* T18 (I1), *Streptococcus pyogenes* (*S. pyogenes*) FF22 (I2), *Streptococcus constellatus* T29 (I3), *Streptococcus uberis* ATCC 27958 (I4), *S. pyogenes* 71-679 (I5), *Lactococcus lactis* (*L. lactis*) T-21 (I6), *S. pyogenes* 71-698 (I7), *S. pyogenes* W-1 (I8) and *Streptococcus dysgalactiae* subsp. *equisimillis* T148 (I9), were all routinely grown in Bacto™ Todd Hewitt Broth (THB, Oxoid, Hampshire, UK) medium which consisted of heart infusion (0.31%), neopeptone (2.0%), dextrose (0.2%), sodium chloride (0.2%), disodium phosphate (0.04%) and sodium carbonate (0.25%), at 37 °C for 24–48 h under anaerobic conditions. Additional strains used in extended spectrum DAAs, *i.e.*, *Leuconostoc mesenteroides* (*Leuc. mesenteroides*) MBF2-5, *Leuc. mesenteroides* MBF7-17 (7-17), *Leuc. mesenteroides* TISTR 120 (TISTR) and *Weissella confusa* (*W. confusa*) MBF8-1 (8-1) were all grown in MRS medium at 32 °C for 24–48 h under anaerobic conditions.

2.2. BLIS activity assay

BLIS activity of *S. macedonicus* MBF10-2 was tested by a standard deferred antagonism [also known as P-typing (producer typing)] assay as described previously [21]. *S. macedonicus* MBF10-2 was grown as a 1-cm diametric streak (the "producer streak") on blood agar medium containing calcium carbonate after which the bacterial growth is removed. The agar surface was sterilized by exposing the inverted plate to chloroform vapors for 30 min in a fume hood. After airing for 15 min, the nine standard indicator bacteria (listed in section 2.1) were applied at perpendicular angles across the original producer streak. Following anaerobic incubation at 37 °C for 24 h, the presence or absence of growth inhibition of each indicator strain was recorded. The nine indicators are divided into three triplets (*i.e.*, I1, I2, I3; I4, I5, I6; I7, I8, I9), and, depending on its position in the triplet, the growth inhibition of a particular indicator strain was given a score of 4, 2 or 1. If growth inhibition was absent, a score of 0 was given. Adding the scores within each triplet yields the three-digit P-type of the test producer strain with P-types of 000 and 777 indicating no inhibition and complete inhibition of all indicator strains, respectively. When extended spectrum indicator strains (*Leuc. mesenteroides* and *W. confusa*) were used for DAAs, only the presence or absence of inhibition is recorded. All DAAs were performed in triplicate.

2.3. Effects of temperature and pH on MBF10-2 BLIS activity

Effects of temperature on MBF10-2 BLIS activity was determined by incubating at various temperature ranging from

40 to 100 °C at an interval of 20 °C using cell-free supernatants at pH 7.0. Whereas the effect of pH on BLIS activity was adjusted by adding 1 mol/L HCl or 1 mol/L NaOH to the cell-free supernatants to obtain various pH, *i.e.*, pH 2, 4, 6, 7, 8, 10 and 12, followed by incubation at 37 °C for 60 min. All treated preparations were tested for inhibitory activity against *Leuc. mesenteroides* TISTR 120 by spot inhibitory assays [22] performed in triplicate.

2.4. Harvesting and fractionation of BLIS

For BLIS production, *S. macedonicus* MBF10-2 was grown on MRS agar supplemented with 0.5% (w/v) calcium carbonate. Cells were harvested from 10 to 12 agar plates in order to harvest as much BLIS suspension required for assays conducted in this study, with the aid of a sterile glass spreader after flooding each plate with two aliquots of 200 µL sterile 0.2 mol/L phosphate buffer (pH 7.4). An equal volume (~5 mL) of phosphate buffer was added to the cell suspension and the cells were harvested by centrifugation at 15 000×*g* for 30 min at 4 °C. BLIS-containing supernatant was collected and applied sequentially to three Amicon® Ultra-15 (Merck Millipore, Germany) centrifugal filter units with certain kDa cut-off Ultracel-3 regenerated cellulose membranes, *i.e.*, 30-kDa, 10-kDa and 3-kDa, respectively, with each flow-through being centrifuged at 5 000×*g* for 15 min at 4 °C. Each fractions retained (i.e., retentate) after each centrifugation spin was freeze-dried and re-suspended in 0.2 mol/L phosphate buffer (pH 7.4) to a concentration of 8 mg protein per milliliter. These samples were subsequently used for well diffusion assays as well as synergism experiments with antibiotics.

2.5. Measurement of protein concentration

Protein concentration of each BLIS fraction was estimated using the BCA Protein Assay Kit (Novagen, USA) according to the manufacturer's protocol. A total of 1 mL of freshly-prepared BCA reagent mix (reagent A and reagent B) from was added to a 50 µL aliquot of each BLIS fraction. Mixtures were incubated at 37 °C for 30 min, cooled to room temperature (25–27 °C), and measured at 562 nm using a GeneQuant™ 100 spectrophotometer (GE Healthcare, UK). A standard curve was derived using various concentrations (range 0.05–0.5 mg/mL) of bovine serum albumin (BSA) in dH_2O as the protein reference standard.

2.6. Well diffusion assay and synergistic activity test

Bacteriocin activities of each fraction were determined by performing a well diffusion assay [22]. *Leuc. mesenteroides* TISTR 120 or *M. luteus* T18 as indicator was harvested after overnight incubation, re-suspended in sterile saline and compared with McFarland III standard to obtain approximately 1×10^9 colony-forming units/mL (CFU/mL). *Leuc. mesenteroides* TISTR 120 diluted to 1×10^6 CFU/mL and *M. luteus* T18 diluted to 1×10^7 CFU/mL. They were also confirmed by optical density measurement at 600 nm (OD_{600} of between 0.024 and 0.036).

Each BLIS-containing fraction of *S. macedonicus* MBF10-2 prepared as described above (section 2.4) was used. Various numbers of holes were punched out of the agar medium using a cork borer of 10 mm diameter. An aliquot of each BLIS fraction (20 µL) was applied into the well, and then leaves for a while to let the fluid diffused into the agar effectively. Indicator bacteria (*Leuc. mesenteroides* TISTR 120 or *M. luteus* T18) was swabbed onto the agar surface and plated for examining the growth inhibition after 24-h incubation at 37 °C.

Screening of synergistic antimicrobial activity of the most active BLIS fraction was carried out in combination, i.e., 200 µL aliquot of BLIS preparation (10 µg/mL protein stock in 0.2 mol/L phosphate buffer pH 7.4) was mixed with an equal volume of each various antibiotic (50 µg/mL of ampicillin, 50 µg/mL of tetracycline, and 250 µg/mL of kanamycin). Twenty microliters of each BLIS containing antibiotic solution was added into the well, and then the certain indicator strain was applied on to the agar surface. Agar plates were examined for growth inhibition after 24-h incubation under anaerobic conditions at 37 °C, or in aerobic condition at 32 °C according to the strain used.

3. Results

3.1. S. macedonicus MBF10-2 produces broad-spectrum BLIS activity

When *S. macedonicus* strain MBF10-2 was tested by the standard deferred antagonism (P-typing) assay, a P-type of 636 was obtained, *i.e.*, the BLIS inhibited the growth of *M. luteus*, all four *S. pyogenes* strains tested, and *L. lactis*. In addition, the growth of all four tested *Leuc. mesenteroides* and *W. confusa* strains were also inhibited. These data indicated that strain MBF10-2 produces a single broad-spectrum bacteriocin or multiple bacteriocin activities [referred to BLIS(MBF10-2)].

3.2. Heat stability and pH tolerance of BLIS(MBF10-2)

As shown in Figure 1A, the inhibitory activity of BLIS(MBF10-2) was stable when heated up to 60 °C for 30 min. However, it was found that the activity was lost when the BLIS preparation heated up to 80 °C. BLIS(MBF10-2) activity also appeared to be unaffected by acidic pH values, performing the best activity between pH 6 and 7, but inhibition of the indicator strain *Leuc. mesenteroides* TISTR 120 was abolished when the pH was adjusted to above pH 8 (Figure 1B). Taken together, BLIS(MBF10-2) was a fairly heat-stable and acid-tolerant BLIS.

3.3. Preliminary characterization of BLIS(MBF10-2) activity by protein fractionation

In order to determine whether the activity of BLIS(MBF10-2) is due to a peptide (<10 kDa) or larger protein, the activity from cultures grown on solid media was extracted and fractionated (and concentrated) sequentially through Amicon® ultrafiltration membranes with 30, 10 and 3 kDa cut-off membranes. Various degrees of inhibition were observed with the BLIS fractions. Whereas inhibitory activity against *Leuc. mesenteroides* TISTR 120 was observed with all fractions, zones of inhibition were only observed with fractions ≥30 kDa, 10–30 kDa and 3–10 kDa but not with the fractions <3 kDa when *M. luteus* T18 was used as the indicator (Table 1). In contrast, *L. lactis* T-21 was inhibited only by the ≥30 kDa fraction (Table 1). Interestingly, three *S. pyogenes* strains (FF22, 71-698 and W-1) that were inhibited by the standard DAA were not inhibited by any of the BLIS fractions (Table 1). Collectively, the results indicate

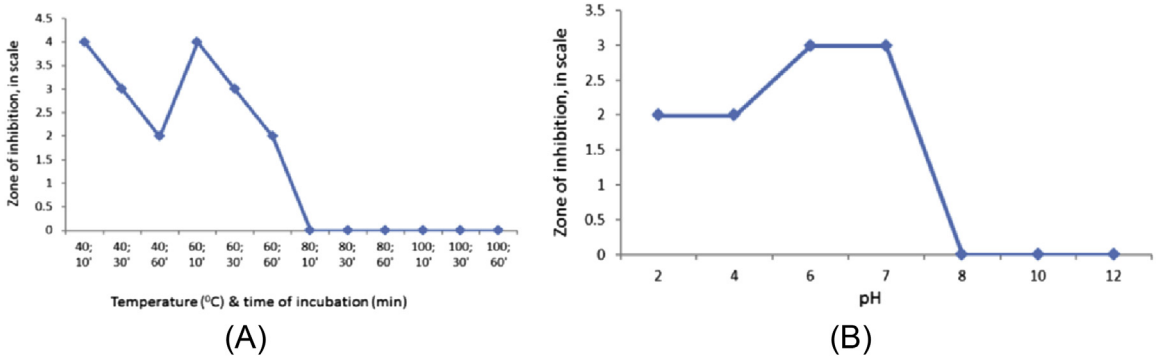

Figure 1. Biochemical behavior of *S. macedonicus* MBF10-2 BLIS activity.
(A) effect of temperature and (B) effects of pH. Treated BLIS preparations were tested by the well diffusion assay using *Leuc. mesenteroides* TISTR 120 as the indicator strain.

Table 1
Inhibitory activity of *S. macedonicus* MBF10-2 BLIS fractions against selected indicator species by well diffusion assay.

Indicator bacteria	Inhibitory activity of BLIS fraction				
	Supernatant	≥30 kDa	10–30 kDa	3–10 kDa	<3 kDa
Leuc. mesenteroides TISTR 120	+	+	+	+	+
M. luteus T18	+	+	+	+	–
L. lactis T-21	+	+	–	–	–
S. pyogenes FF22	–	–	–	–	–
S. pyogenes 71-698	–	–	–	–	–
S. pyogenes W-1	–	–	–	–	–

+ = zone of inhibition (>10 mm) observed; – = no zone of inhibition.

that *S. macedonicus* MBF10-2 may be producing multiple bacteriocins ranging from <3 kDa to ≥30 kDa.

3.4. Synergistic inhibitory activity of BLIS(MBF10-2) with antibiotics

As shown in Table 2, there was a variability in the potential synergistic inhibition activity of BLIS(MBF10-2) fraction >30 kDa in combination with three antibiotics (ampicillin, tetracycline and kanamycin, with concentration, *i.e.*, 50, 50 and 250 μg/mL, respectively), with the most consistent effect was against *M. luteus* and *L. lactis*. On the other hand, the addition of BLIS(MBF10-2) appeared to enhance the killing action of tetracycline towards *S. aureus*, the BLIS preparation also desensitized certain indicator strains to the tested antibiotic, e.g., the antibacterial action of tetracycline and kanamycin on *Leuc. mesenteroides* TISTR 120 and *Bacillus subtilis* ATCC 6633, respectively (Table 2). No synergistic activity was observed between BLIS(MBF10-2) and antibiotic against Gram-negative strains, *i.e.*, *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC19430 (Table 2). The results indicate that the use of BLIS(MBF10-2) as a complementary therapeutic agent with antibiotics would depend on the selected antibiotic and the bacterial pathogen to be targeted.

Table 2
Inhibition of selected bacteria by BLIS(MBF10-2) in combination with ampicillin, tetracycline or kanamycin.

Indicator bacteria	Diameter of inhibition zone increase (+)/decrease (–) (mm)		
	A + B	T + B	K + B
Micrococcus luteus T18	+14.0	+24.5	+13.0
Lactococcus lactis T-21	+42.0	+17.5	+28.5
Leuconostoc mesenteroides TISTR 120	+12.5	−33.0	+8.5
Escherichia coli ATCC 25922	–	–	+2.5
Bacillus subtilis ATCC 6633	–	+19.5	−25.0
Staphylococcus aureus ATCC 25923	+2.0	+35.0	−0.5
Salmonella typhi ATCC19430	+8.7	–	−14.2

Well diffusion assays were carried out and zones of inhibition were measured (in mm) using vernier calipers. All assays and measurements were performed in triplicate. A = ampicillin (50 μg/mL); B = BLIS; T = tetracycline (50 μg/mL); K = kanamycin (250 μg/mL).

4. Discussion

Bacteriocin production of several different classes by members of the genus *Streptococcus* is a well-documented phenomenon [2,23], with the most well-characterized of streptococcal bacteriocins being the salivaricins and the streptococcin/streptin peptides produced by *Streptococcus salivarius* and *S. pyogenes*, respectively [23]. The physicochemical properties of salivaricins themselves are quite diverse, ranging from small <5-kDa cationic peptides (e.g., salivaricins A, B, and 9) to large >60-kDa anionic non-lytic proteins such as salivaricin MPS [24]. The food-grade bacterium *S. macedonicus*, first isolated from Greek cheese, is no exception and produces macedocin [25], a peptide bacteriocin which is identical to streptococcin A-FF22 [26].

The present investigation aimed to further characterize the antibacterial (BLIS) activity previously observed with the Indonesian tofu isolate *S. macedonicus* strain MBF10-2 [27] but using a more specific agar-based deferred antagonism assay (DAA or "P-typing") against nine standard and four additional indicator bacteria. It was reported previously that this BLIS(MBF10-2) was positively active as bacteriocin after proteolytic enzymes assay [27].

The standard DAA [21], despite only having a limited number of indicator strains, can give valuable clues to the possible type of bacteriocin present as several of the standard indicators are bacteriocin producers themselves. For example, *Streptococcus constellatus* I3 produces the heat-sensitive lytic bacteriocin stellalysin [28], *Streptococcus uberis* I4 produces nisin U [29] and *S. pyogenes* indicators I2 and I7 produce streptococcin A-FF22 and dysgalacticin, respectively [26,30]. Therefore, if the bacteriocin producer of interest is producing any of the

bacteriocins listed above, the indicators would not be inhibited due to immunity to their respective bacteriocin. Furthermore, *M. luteus* I1 is exquisitely sensitive to lanthionine-containing (lantibiotic) peptides [23]. Modifications of the DAA, e.g., heating and pH adjustment will provide additional information as to the inhibitory agent [23]. Genetic techniques such as PCR experiments using primers targeting specific bacteriocin genes (e.g., *lan* loci) could also be used.

In this study, *S. macedonicus* MBF10-2 exhibited a P-type score 636, *i.e.*, growth inhibition of *M. luteus* (I1), *S. pyogenes* FF22 (I2), *S. pyogenes* 71-679 (I5), *L. lactis* T-21 (I6), *S. pyogenes* 71-698 (I7), and *S. pyogenes* W-1 (I8). This P-type is very similar to that of the streptococcin A-FF22 (macedocin) producer *S. pyogenes* FF22 (I2) which is 436. As strain FF22 was inhibited by *S. macedonicus* MBF10-2, this indicates that an additional heat-stable and acid-tolerant (potentially lantibiotic) BLIS activity is produced by MBF10-2 which targets *S. pyogenes*. Multiple bacteriocin production by streptococci is quite common, especially by *S. salivarius* [23]. In addition, all *Leuc. mesenteroides* and *W. confusa* indicator strains were also inhibited by BLIS(MBF10-2), a finding previously unreported for streptococcal bacteriocins.

In order to further characterize BLIS(MBF10-2) biochemically, BLIS preparations obtained from agar-grown cultures were size-fractionated using ultrafiltration membranes ranging from 3 to 30 kDa. *M. luteus* T18 and *Leuc. mesenteroides* TISTR 120 were the most sensitive of the indicator strains, being inhibited by virtually all BLIS fractions. Although TISTR 120 was inhibited by the <3-kDa BLIS fraction, the relative sensitivity of *M. luteus* and *Leuc. mesenteroides* to bacteriocins is unknown. Similarly, although the >30 kDa fraction inhibited three tested indicators (*M. luteus*, *Leuc. mesenteroides* and *L. lactis* T-21), it is possible that this activity is due to aggregation of small peptides. It was reported that the streptocin streptocins STH_1 produced by *Streptococcus gordonii* was estimated to be >200 kDa but it was later found that the size discrepancy was probably due to two small peptides aggregating and binding to serum-based components [31,32].

An unexpected and contradictory result was the lack of antibacterial activity in all liquid BLIS preparations against *S. pyogenes* when these same strains were inhibited by DAA. A possible explanation for this result may be the shift from using broth cultures to agar-based cultures for BLIS production. Previously, culture supernatants were collected from liquid culture, pH-adjusted with 1 mol/L NaOH, and sterilized by filtration before conducting well diffusion assays. In an attempt to minimize interference of substances in the medium that might affect the production of the inhibitory agent(s), protocol by harvesting cells from agar plates was modified. It can be speculated that either (i) the *S. pyogenes*-targeting bacteriocin is highly diffusable and could not be harvested from agar or (ii) the level of BLIS isolated was too low to affect *S. pyogenes*, *i.e.*, the sensitivity of *S. pyogenes* to the BLIS is much lower than that of *M. luteus* or *Leuc. mesenteroides*.

The potential of BLIS from *S. macedonicus* MBF10-2 to be developed as a synergistic complement for antibiotics as reported in this study were largely variable and target strain-dependent, and the BLIS displayed little, if any, enhancing effect against Gram-negative pathogens. In some cases, e.g., BLIS combined with kanamycin (250 μg/mL), appeared to desensitize *Bacillus subtilis* to the antibiotic. Although BLIS(MBF10-2) did exhibit some synergism when mixed with tetracycline (50 μg/mL) against *S. aureus*, this promising finding requires expansion to include a variety of pathogenic *S. aureus* strains (*e.g.*, methicillin-resistant *S. aureus*).

The results of this present study could indicate that BLIS(MBF10-2) as a complementary therapeutic agent with antibiotics would depend on the selected antibiotic and the bacterial pathogen to be targeted.

S. macedonicus MBF10-2 with multiple BLIS activities need to be further examined. From a protein perspective, more sophisticated techniques such as Amberlite XAD column [33] or ion exchange chromatography can be employed followed by N-terminal amino acid sequencing of bioactive fractions. Alternatively, genetic techniques such as PCR experiments targeting all known streptococcal bacteriocin classes could be carried out. Another approach that may ultimately be more cost-effective is to sequence the entire *S. macedonicus* MBF10-2 genome using next-generation DNA sequencing technologies as this will reveal not only the complete bacteriocin repertoire of strain MBF10-2 but all its metabolic capabilities. Once the BLIS have been identified, more focused synergistic combinations with antibiotics will be able to be implemented that will specifically target individual (or groups of) pathogenic species.

Conflicts of interest statement

We declare that we have no conflict of interest.

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