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SALIVARICIN MMAYE1 PRODUCTION IS ENHANCED IN A NEW MEDIUM AND ACTS SYNERGISTICALLY WITH PENTOCIN MQ1

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ABSTRACT
Resistance to conventional antimicrobials is burgeoning. Consequently, the world health has mandated researchers to develop novel antimicrobial agents as replacement for conventional antimicrobials. Of the potential alternatives, bacteriocins are widely considered lead compounds. Salivaricin mmaye1 and pentocin MQ1 are bacteriocins produced by *Lactobacillus salivarius* SPW1 and *Lactobacillus pentosus* CS2 respectively. These bacteriocins have been reported to possess high antimicrobial activity against human pathogens. The overall objective of this study is to optimize production of salivaricin mmaye1 and evaluate its potential synergistic action with pentocin MQ1. Results revealed that *Lactobacillus salivarius* SPW1 is non-hemolytic and sensitive to most antibiotics screened but resistant to gentamicin, streptomycin and vancomycin. Resistance to these antibiotics is not mediated by plasmids. Genes encoding salivaricin mmaye1 production are not plasmid-borne. A new medium for high biomass accumulation was developed. Salivaricin mmaye1 production is optimum at pH values of 6 and 7. Salivaricin mmaye1 and pentocin MQ1 exhibited synergism against *Micrococcus luteus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. These findings provide a glimpse into future therapeutic applications of salivaricin mmaye1.

Keywords: Antibiotic resistance, Bacteriocin, Salivaricin mmaye1, *Lactobacillus salivarius*, Synergistic action, Pentocin MQ1

INTRODUCTION

Infectious diseases have been a major health and economic conundrum since the existence of humanity (W.H.O, 2020; Wang *et al*., 2020). During the pre-antimicrobial era, infectious diseases were responsible for up to one-third of total mortality (Hocking *et al*., 2021). The discovery of antimicrobials revolutionized modern medicine and ushered in the antimicrobial era. Since the uncovering of antimicrobials, they have been used for prevention and treatment of various infectious diseases resulting in a significant decline in mortality rate and increase in life expectancy (Shlaes and Bradford, 2018). Antimicrobials are also important in the treatment of non-infectious diseases such as cancers and immuno-compromized health conditions. These agents are also used during surgery to prevent post-operative infections (Hocking *et al*., 2021).

Overuse and misuse of antimicrobials resulted in the appearance of resistance among pathogens (Sulaiman *et al*., 2020). This situation has escalated as evidenced by inundation of reports on antimicrobial resistance across the globe (Crysler and Streu, 2022). Consequently, diseases that were once treatable have become recalcitrant. As of 2020, annual global death due to antimicrobial-resistant diseases is 500 0000. If the world continues on this trajectory, global annual mortality will rise to over 10 million by 2050 (Hasan *et al*., 2020). Additionally, global economic loss due to antimicrobial-resistant infections will rise to 100 trillion USD (Denku *et al*., 2022). This fact shows that the world is gradually returning to the pre-antimicrobial era. Consequently, the world health organization has declared that alternative antimicrobials are urgently needed.

Bacteriocins are widely considered as viable replacement for conventional antibiotics. These ribosomally synthesized antimicrobial peptides are of bacterial origin. Bacteriocins are a component of bacterial innate immunity and foster niche competitiveness. They are initially synthesized in inactive forms as a self-protective mechanism preventing the producer from the impact of its bacteriocin. They become activated through proteolytic cleavage of the N-terminal leader sequence prior to release into the environment. Additionally, bacteriocin producers synthesize self-immunity proteins that confer immunity against their own bacteriocin. Bacteriocins have narrow or broad spectrum of antimicrobial activity. Inhibitory activity of bacteriocins against their microbial targets could be bactericidal or bacteriostatic (Soltani *et al*., 2021). Bacteriocins kill their targets through novel mechanism which include inhibition of; peptidoglycan biosynthesis, RNA polymerase, DNA gyrase, Asp-tRNA synthase and pore formation (Negash and Tsehai, 2020). Interestingly, bacteriocins are generally regarded as safe, very potent against their targets at micro to nanomolar concentrations and possess stupendous pH, thermal and chemical stability. These characteristics favor the use of bacteriocins as replacement for conventional antibiotics. Various studies have reported the *in vivo* potential of bacteriocins but none have scaled through clinical trials.

In our previous work, we described two novel bacteriocins namely, salivaricin mmaye1 and pentocin MQ1. Salivaricin mmaye1 is produced by *Lactobacillus salivarius* SPW1, an isolate from the human gut (Wayah and Philip, 2018a) while pentocin MQ1 was purified from *Lactobacillus pentosus* CS2 isolated from coconut shake (Wayah and Philip, 2018b). Salivaricin mmaye1 and pentocin MQ1 have molecular weights of 1221.074 Da and 2110.672 Da as reckoned by MALDI-TOF mass spectrometry. Both displayed high antimicrobial activity against human pathogens which include *Micrococcus luteus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Wayah and Philip, 2018a; Wayah and Philip, 2018b).

MATERIALS AND METHODS

Bacterial strains and culture media

Salivaricin mmaye1 and pentocin MQ1 are bacteriocins isolated from *Lactobacillus salivarius* SPW1 of human gut origin and *Lactobacillus pentosus* CS2 of coconut shake origin respectively as described in our previous study (Wayah and Philip, 2018a; Wayah and Philip, 2018b). *Lactobacillus salivarius* SPW1 and *Lactobacillus pentosus* CS2 were maintained on De man Rogose and Sharpe (MRS) agar (Merck, Darmstadt, Germany) while *Listeria monocytogenes* NCTC 10890 was maintained on Brain heart infusion agar (Merck, Darmstadt, Germany). *Micrococcus luteus* ATCC 10240, *Staphylococcus aureus* RF122, *Pseudomonas aeruginosa* PA7 and *Escherichia coli* UT181 were maintained on Mueller-Hinton agar (Merck, Darmstadt, Germany).

Safety assessment of bacteriocin producer

Hemolysis test

In order to ascertain the hemolytic potential of *Lactobacillus salivarius* SPW1, it was grown aerobically overnight on MRS agar (Merck, Darmstadt, Germany) at 37 ^{0}C and subcultured on Columbia blood agar base (Difco, Le Ponte de Claix, France) supplemented with 5% (v/v) defibrinated sheep blood (Liofilchem, Roseto d.A, Italy) at the same condition. The hemolysis results was interpreted as α-hemolysis (if agar under the colony become dark and greenish), β-hemolysis (if there is clear zone around the colonies) or γ-hemolysis (if agar remains unchanged).

Antibiotic susceptibility test

This investigation was done to unravel the antibiotic sensitivity profile of *Lactobacillus salivarius* SPW1. Antibiotic sensitivity test was carried out as described by(Zhou *et al*., 2005) with slight modifications. *Lactobacillus salivarius* SPW1 was grown in MRS broth overnight at 37 ^{0}C. The culture was centrifuged (500 rpm for 10 minutes) to collect the cell pellet which was subsequently re-suspended in phosphate-buffered saline (EMD Millipore Corp., Billerica, USA) and washed twice. The optical density (OD) (600 nm) was adjusted to 0.1. MRS agar was autoclaved and cooled to 50 ^{0}C thereafter, 200 µl of cell suspension was added, mixed properly and equal volume poured into petri dishes. After solidifying, antibiotic discs diffusion assay was performed. Antibiotic discs (Oxoid, Basingstoke, UK) used were as follows: clindamycin (2 µg), ofloxacin (5 µg), tetracycline (30 µg), erythromycin (15µg), penicillin V (10 µg), penicillin G (10 µg), streptomycin (10 µg), amoxycillin (10 µg), gentamicin (10 µg) and vancomycin (30 µg). Zone of inhibition was interpreted as: susceptible (>20 mm), moderately susceptible (15-20 mm) and resistant (<15 mm).

Plasmid profile

In order to investigate the presence of plasmids in *Lactobacillus salivarius* SPW1, plasmid isolation was carried out using easy pure® plasmid miniprep kit (TransGen Biotech, Beijing) according to manufacturer's instruction.

Optimization of growth and bacteriocin production

Development of new media and growth kinetics

This investigation was done to develop a new medium for high biomass and bacteriocin production. To achieve this, 3 growth media named, Samson-Grace-Sophia-Limitless (SGSL), Tryptone-Peptone-Yeast extract-Glucose-Morpholinoethanesulphonic Acid (TPYGMA) and Tryptone-Peptone-Yeast extract-Glucose-$CaCO_3$-Tween 80 (TPYGCaT80) were produced as described in our previous study (Wayah and Philip, 2018c). Components of SGSL medium are as follows: 1 % tryptone (Difco, Le Ponte de Claix, France), 1 % peptone (Difco, Le Ponte de Claix, France), 1 % yeast extract (Difco, Le Ponte de Claix, France), 5 % glucose (Merck, Darmstadt, Germany), 0.05 % ascorbic acid (R & M Chemicals, Essex, UK), 0.2 % sodium citrate (Peking Chemical works, Peking, China), 0.005 % manganese (II) sulphate (BDH Chemicals Ltd, Poole, England), 0.025 % magnesium sulphate (Halewood Chemical Ltd, Middlesex, England), 0.02 % sodium chloride (John Kollin Corporation, USA) and 0.1 % Tween 80 (Sigma-Aldrich, Missouri, USA). Composition of TPYGMA medium is as follows: 1 % tryptone, 1 % peptone, 1 % yeast extract, 1.5 % glucose, 1% 2-morpholinoethanesulphonic acid, MESA (Merck, Darmstadt, Germany) and 0.05 % ascorbic acid. TPYGCaT80 medium is composed of the following: 1 % tryptone, 1 % peptone, 1 % yeast extract, 1.5 % glucose, 0.1 % $CaCO_3$ and 0.1 % Tween 80. In order to investigate kinetics of growth in different growth medium, single colonies from a 24-hour old culture of *Lactobacillus salivarius* SPW1 on MRS agar were added to MRS broth and grown for 20 hours at 37 ^{0}C. This culture was added to different growth media at 2 % (v/v) and incubated at the aforementioned condition. The media used were as follows: MRS broth, SGSL, TPYGMA, TPYGCaT80, Brain-heart infusion (Merck, Darmstadt, Germany), Tryptic soy broth (Merck, Darmstadt, Germany), Todd-Hewitt broth (Difco, Le Pont de Claix, France), M17 broth (Merck, Darmstadt, Germany), M17 supplemented with 5 % glucose (GM17), M17 supplemented with 5 % sucrose (SM17), M17 supplemented with 5 % galactose (GalM17). This was followed by two successive 12 hours subculture at 37 ^{0}C. The third subculture was centrifuged at 5000 rpm for 10 minutes and the cell pellet was re-suspended and washed twice in 0.85 % saline solution at the same centrifugation condition. The cell suspension was adjusted to an optical density of 0.1 at 600 nm and 180 µl of each growth medium in a 96 well sterile plate was inoculated with 20 µl of each bacterial suspension. Optical density (OD) at 600 nm was monitored over a period of 24 hours using a multiskan spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). OD measurements were done in triplicates.

Effect of pH on growth and bacteriocin production

In order to investigate the influence of pH on salivaricin mmaye1 production, SGSL was adjusted to different pH values (3, 4, 5, 6, 7, and 8) prior to autoclaving. After autoclaving and cooling to room temperature, each medium was inoculated with 1 % (v/v) of a 24-hour old *Lactobacillus salivarius* SPW1 culture. This was followed by incubation at 37 ^{0}C for 18 hours. OD at 600 nm was measured. Bacteriocin antibacterial activity was determined by well diffusion assay. *Micrococcus luteus* ATCC 10240 was used as the indicator.

Kinetics of growth and bacteriocin production

This investigation was done to ascertain the kinetics of salivaricin mmaye1 production in the newly developed medium, SGSL. The medium was inoculated with 1 % (v/v) of a 24-hour old *L. salivarius* SPW1 culture and incubated at 37 ^{0}C for 20 hours. Two successive 12-hour subcultures were done in the same medium and the third subculture was centrifuged (5000 rpm for 10 minutes), re-suspended in 0.85 % saline solution and washed twice at the same condition. The OD (600 nm) of the cell suspension was adjusted to 0.1 and 5 % (v/v) of this cell suspension was added to SGSL. The culture was incubated at 37 ^{0}C. OD (600 nm) and bacteriocin

production were monitored over a period for 24 hours. *Micrococcus luteus* ATCC 10240 was used as the indicator.

Synergistic studies

In order to investigate if there is synergism between salivaricin mmaye1 and pentocin MQ1, the two bacteriocin preparations (400 AU/ml each) were combined in a 1: 1 ratio. Forty microliters (40 µl) of the mixture was used in well diffusion assay against selected bacterial targets. Each bacteriocin preparation was used as positive control.

Data analysis

Data generated from this study was subjected to one–way analysis of variance using SPSS software (version 22). Results were expressed as means of three (3) replications. Least significant difference was used for mean separation. Significantly different mean values were assigned different alphabets, "a" being the highest.

RESULTS AND DISCUSSION

Safety assessment of bacteriocin producer and plasmid profile

These investigations were done to assess the safety of *Lactobacillus salivarius* SPW1. Results revealed that *Lactobacillus salivarius* SPW1 exhibited γ-hemolysis (**Plate 1**). *Lactobacillus salivarius* SPW1 was susceptible to ofloxacin, clindamycin, tetracycline, erythromycin, penicillin V, penicillin G and amoxicillin but resistant to gentamicin, streptomycin and vancomycin**.** Bacteriocinogenic *Lactobacillus salivarius* is one of the major components of the beneficial human gut microbiota which offers several health benefits including keeping the population of pathogenic bacteria low and more recently conferring resistance to cancers (Bhatt *et al*., 2017; Lynch and Pedersen, 2016). Exhibition of γ-hemolysis by *Lactobacillus salivarius* SPW1 shows its non-hemolytic nature and suggests its future probiotic application.

Plate 1: Hemolysis test for *Lactobacillus salivarius* SPW1.

L. salivarius SPW1 was subcultured on Columbia blood agar base supplemented with 5% defibrinated sheep blood to observe its effect on red blood cells.

In order to investigate if genes encoding antibiotic resistance and salivaricin mmaye1 production are plasmid-borne, profiling of plasmid was done. Agarose gel electrophoresis revealed clear bands for the 1 kb molecular ladder but no band was seen for *Lactobacillus salivarius* SPW1 **(Figure 1).** This shows the absence of plasmids in *Lactobacillus salivarius* SPW1.

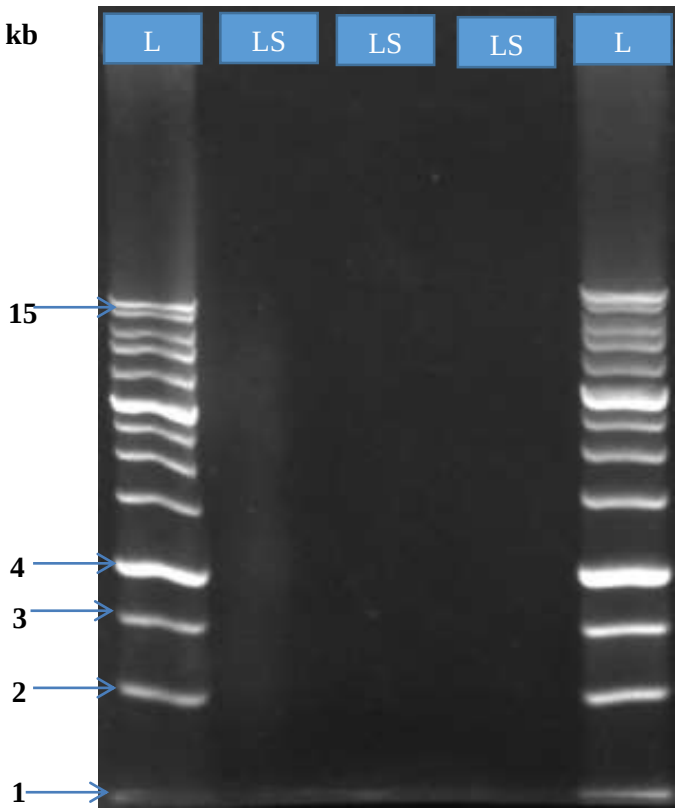

Figure 1: Gel image after plasmid isolation.
L, 1 kb molecular ladder; LS, extract from *Lactobacillus pentosus* SPW1.

Although it was resistant to gentamycin, streptomycin and vancomycin the genetic determinants of these antibiotics are not plasmid-borne as revealed by the absence of plasmids in *Lactobacillus salivarius* SPW1. Considering the fact that plasmid transfer is the major means of bacterial horizontal gene transfer (Salto *et al*., 2018), it can be deduced that genes conferring resistance to gentamycin, streptomycin and vancomycin are not transmissible to pathogenic bacterial strains. Moreover, vancomycin resistance is a widespread, inherent, chromosomally-located characteristic of many strains of LAB (Gueimonde *et al*., 2013). The mechanism of resistance to gentamicin, an aminoglycoside, involves mutational changes in genes encoding ribosomes, changes in membrane permeability, action of aminoglycoside-modifying enzymes, proteolytic cleavage and inactivation of the antibiotic (Kumar *et al*., 2018; Liasi *et al*., 2009). It is pertinent to mention that resistance of *Lactobacillus salivarius* SPW1 to gentamycin, streptomycin and vancomycin could be advantageous regarding its future probiotic application because its population would not be decreased in the gastrointestinal tract of patients undergoing antibiotic therapy.

Absence of plasmids in *Lactobacillus salivarius* SPW1 also shows that genes encoding salivaricin mmaye1 production are not plasmid-borne. This suggests that they are located on chromosomes. Chromosome-borne bacteriocin genes are more stable than plasmid-encoded bacteriocin genes because plasmids, being small mobile genetic elements can leak out of bacterial cells (Lowy, 2011; Mathers *et al*., 2018; Salto *et al*., 2018). From this perspective, bacteriocinogenic LAB strains with chromosome-borne bacteriocin genes are more desirable than those with plasmid-borne bacteriocin genes. This finding shows the genetic stability of *Lactobacillus salivarius* SPW1. A few chromosome-borne bacteriocins have been reported. These include penisin (Baindara *et al*., 2016), gessericin S and gessericin T (Kasuga *et al*., 2019).

Optimization of growth and bacteriocin production

Growth kinetics of Lactobacillus salivarius SPW1 in different media

Growth kinetics of *Lactobacillus salivarius* SPW1 in 11 different growth medium was studied **(Figure 2)**. It turned out that SGSL produced the highest Optical density (OD) reaching a maximum of 1.793 after 24 hours of incubation. This was followed by MRS broth (1.637), TPYGCaT80 (1.033), M17 supplemented with sucrose (SM17) (0.326), M17 supplemented with glucose (GM17) (0.282), brain heart infusion (BHI) (0.211), M17 (0.201), M17 supplemented with galactose (GalM17) (0.185), TPYGMA (0.184), Todd-Hewitt broth (THB) (0.127) and finally, tryptic soy broth (TSB) (0.056).

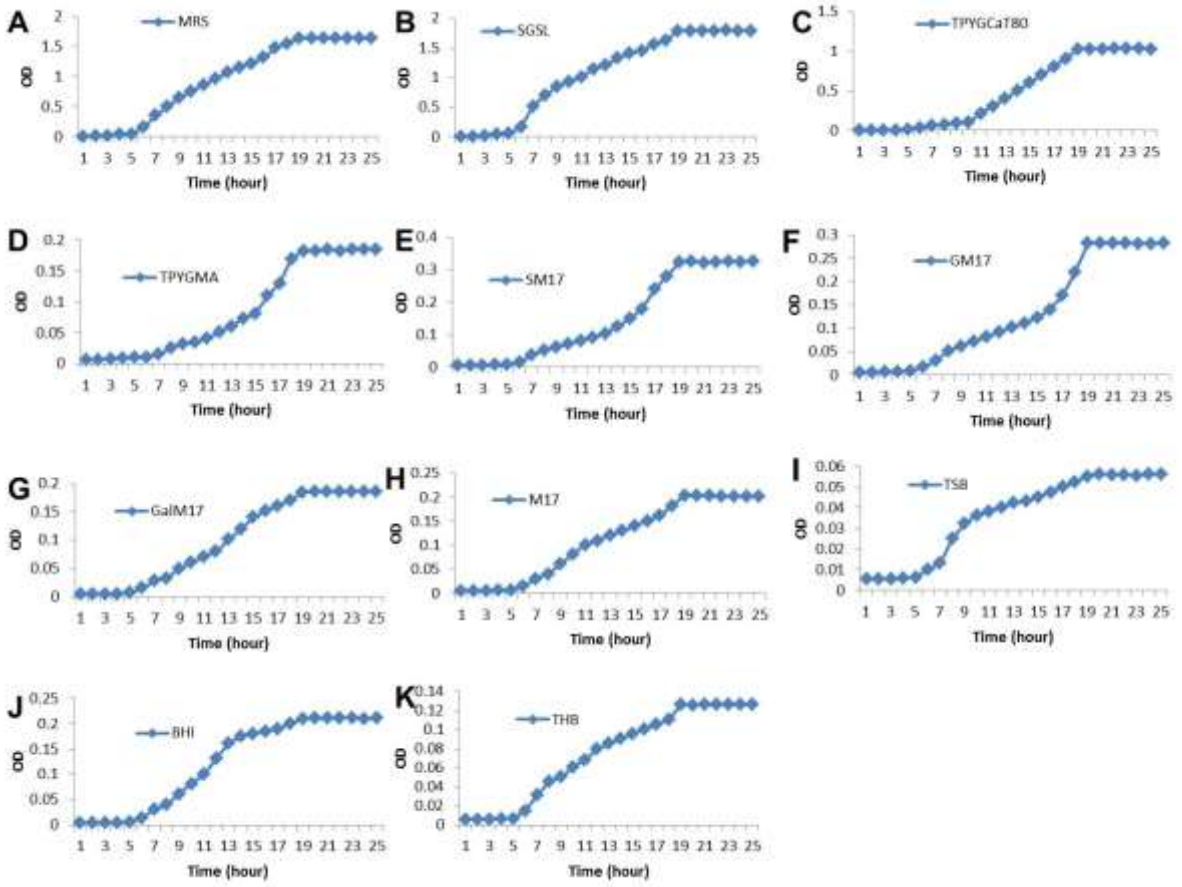

Figure 2: Growth kinetics of *Lactobacillus salivarius* SPW1 in different media.

L. salivarius SPW1 was subcultured in **(A)** MRS**, (B)** SGSL **(C)** TPYGCaT80 **(D)** TPYGMA **(E)** SM17 **(**M17 supplemented with 5 % sucrose) **(F)** GM17 **(**M17 supplemented with 5 % glucose) **(G)** GalM17 **(**M17 supplemented with 5 % galactose) **(H)** M17 **(I)** TSB **(**tryptic soy broth) **(J)** BHI **(**brain-heart infusion) **(K)** THB **(**Todd-Hewitt broth).

In all the media tested, growth of *Lactobacillus salivarius* SPW1 was in 3 stages; lag, exponential and stationary phase. Stationary phase was attained after 18 hours of incubation for

all culture media. High growth in SGSL and MRS is due to the fact that they contain more nutrients that meet the nutritional needs of the bacteriocin producer than the remaining media. Moreover, SGSL and MRS contain magnesium sulphate and manganese (II) sulphate, two important sources of mineral elements which are lacking in other media except M17 and its derivatives. Furthermore, both SGSL and MRS contain Tween 80 which have been shown to enhance growth of LAB according to previous studies (Wang *et al*., 2018; Wayah and Philip, 2018).

The observed higher growth in SGSL over MRS was due to differences in their compositions. The components of SGSL supports higher growth of *Lactobacillus salivarius* SPW1. *Lactobacillus salivarius* SPW1 preferred sodium chloride (in SGSL) over sodium acetate (in MRS). Media composition influences growth of LAB (Abbasiliasi *et al*., 2017). The growth of *Enterococcus faecalis* (Bautista-Gallego *et al*., 2008) was influenced by sodium chloride. It is important to mention that of the three carbon sources, sucrose had the highest influence on growth while galactose had the least. This observation indicates the preference of *Lactobacillus salivarius* SPW1 for sucrose over glucose and galactose. It is imperative to recognize the significant growth of *Lactobacillus salivarius* SPW1 in TPYGCaT80, a newly developed, 6-component growth medium. This paves the way for its future industrial use for biomass accumulation considering the fact that it may be cost-effective compared to MRS and SGSL. The huge growth difference observed between TPYGCaT80 and TPYGMA is attributed to the inclusion of $CaCO_3$ and Tween 80 in the former.

Effect of pH on growth and bacteriocin production

Influence of pH on salivaricin mmaye1 production in SGSL was investigated. Maximum level of bacteriocin activity (activity = 640 AU/ml) was obtained at pH value of 6.00 (OD = 1.631) and 7.00 (OD = 1.670) and for the control (pH 5.57, OD = 1.612) while bacteriocin production was lower (activity = 320 AU/ml) at pH 5.00 (OD = 1.57) and 8.00 (OD = 1.701). There was no bacteriocin production at pH values of 3.00 and 4.00 **(Figure 3)**.

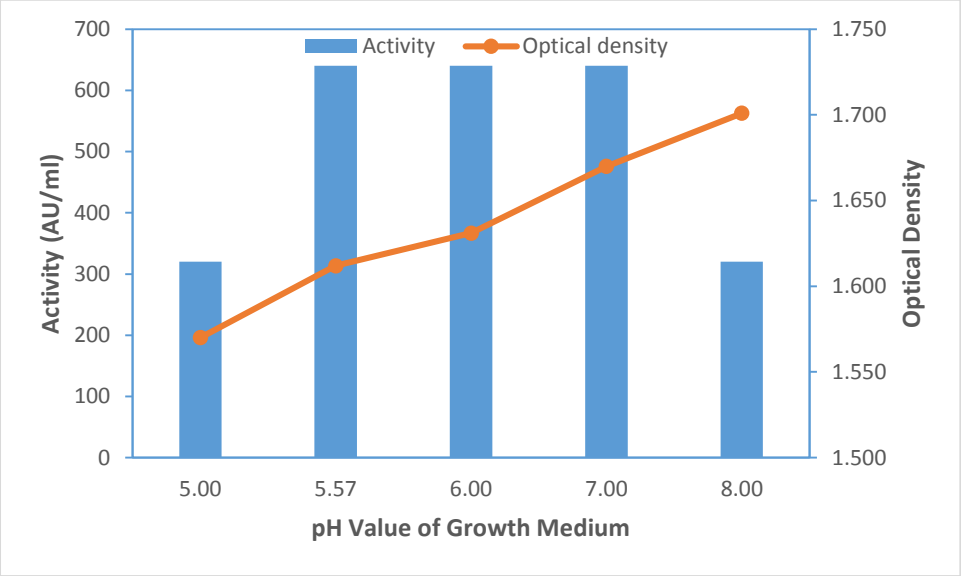

Figure 3: Effect of pH on growth and salivaricin mmaye1 production.

Bacteriocin producer was inoculated into SGSL of varying pH values and bacteriocin titer was measured after 18 hours of incubation. pH = 5.57 is the control with unadjusted pH.

Initial pH of SGSL influenced salivaricin mmaye1 production. Although growth was optimum at a pH value of 8, maximum level of salivaricin mmaye1 production was obtained at pH values of 6 and 7. This shows that optimum growth condition did not coincide with optimum bacteriocin production. This observation is due to the fact that pH value of 6 and 7 enhanced the production and solubility of salivaricin mmaye1 (Mataragas *et al*., 2003). Similar results was reported for sakacin P (Møretrø *et al*., 2000). Salivaricin mmaye1production was lowest when pH was adjusted to 5 due to poor growth. There was no bacteriocin production at pH 3 and 4 because growth was severely inhibited.

Kinetics of growth and bacteriocin production in a newly developed medium

Kinetics of growth and production of salivaricin mmaye1 in SGSL was investigated. Bacteriocin production was detected after 3 hours of incubation **(Figure 4)** and peaked after 18 hours in the early stationary phase of growth. Similar results was described in *Lactobacillus pentosus* B231 (Guerreiro *et al*., 2014). Growth of the producer was in three phases: lag phase, log phase and stationary phase **(Figure 4A).** pH variation during growth and bacteriocin production is shown in **Figure 4B**. There was decline in pH with increase in OD over a period of 24 hours indicating the inverse relationship between the two parameters. This can be explored in the manipulation of pH changes during salivaricin mmaye1 production for the purpose of yield optimization in industrial applications.

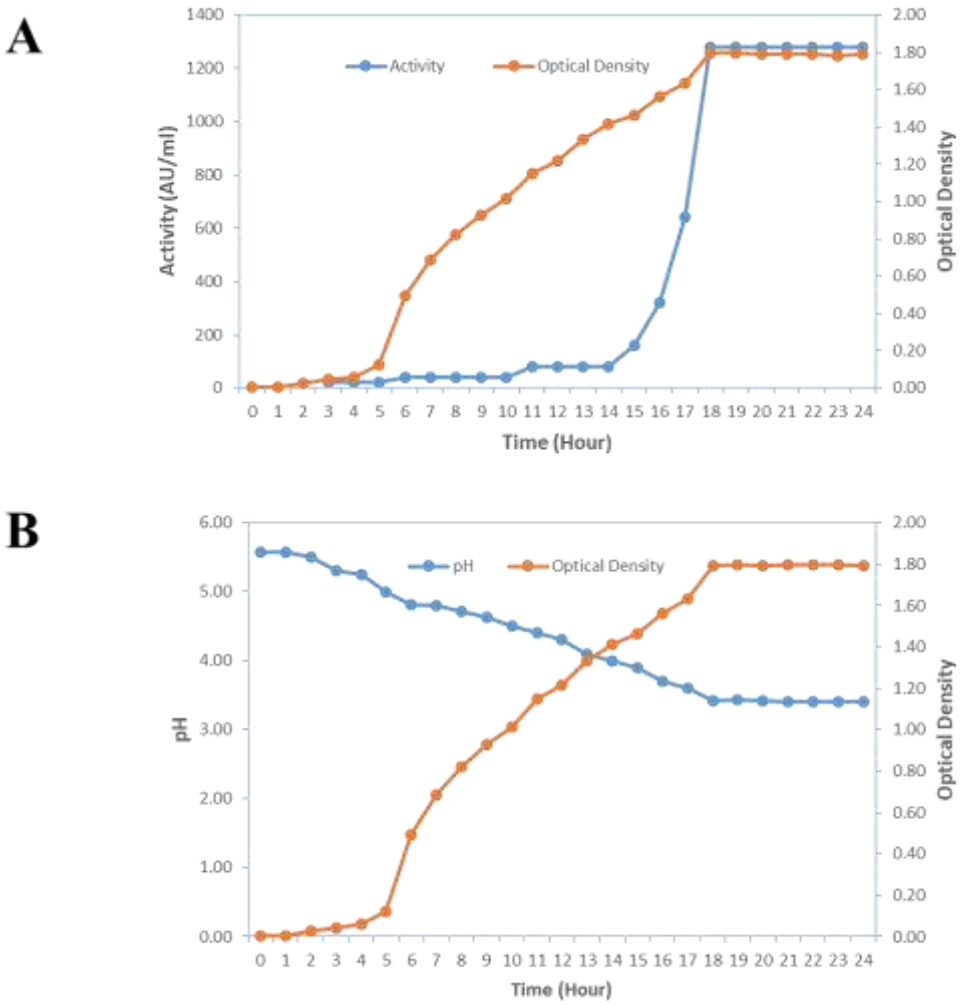

Figure 4: Kinetics of growth and production of salivaricin mmaye1 in SGSL.

Lactobacillus salivarius SPW1 was subcultured in SGSL and bacteriocin production during growth was monitored for 24 hours. (A) Time-course of bacteriocin production (B) pH variation during time-course of bacteriocin production.

Synergistic studies

Zones of inhibition (ZOI) obtained for *Micrococcus luteus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* after combined use of pentocin MQ1 and salivaricin mmaye1 was significantly higher ($p < 0.05$) than that obtained for the individual bacteriocins. In the cases of *Staphylococcus aureus* and *Escherichia coli*, combined application of pentocin MQ1 and salivaricin mmaye1 did not produce significantly higher ZOI ($p > 0.05$) **(Table 1)**.

Although occurrence of resistance towards bacteriocins is a rarity (Ghosh *et al*., 2019) which is due to their high potency and novel modes of action however, a few cases of resistance have been reported. Resistance to nisin has been observed in a strain of *Micrococcus flavus* and *Lactococcus lactis*. A strain of *Listeria monocytogenes* has developed resistance towards mesentericin Y105. Although it is suggested that resistance development is as a result of cell wall and or cell membrane modifications (Draper *et al*., 2015), application of high amount of bacteriocin could generate the pressure required for development of resistance as observed with several antibiotics (Adam, 2018; Huang *et al*., 2018; MacNair *et al*., 2018).

Table 1: Synergistic action of pentocin MQ1 and salivaricin mmaye1.

Bacterial target	ZOI (mm)		
	P	S	P + S
Micrococcus luteus ATCC10240	23.20 ± 0.458^{b}	20.30 ± 0.700^{c}	25.18 ± 0.470^{a}
Staphylococcus aureus RF122	18.68 ± 0.557^{b}	16.92 ± 0.614^{a}	19.48 ± 0.365^{a}
Listeria monocytogenes NCTC 10890	21.90 ± 0.780^{b}	21.48 ± 0.507^{b}	23.92 ± 0.411^{a}
Pseudomonas aeruginosa PA7	17.28 ± 0.139^{c}	18.08 ± 0.352^{b}	19.30 ± 0.383^{a}
Escherichia coli UT181	19.68 ± 0.538^{a}	19.98 ± 0.626^{a}	20.00 ± 0.280^{a}

[3]P: Pentocin MQ1, S: Salivaricin mmaye1, P + S: Pentocin MQ1 and Salivaricin mmaye1, ZOI: Zone of inhibition

Combined application of bacteriocins could reduce the minimum inhibitory or bactericidal concentration thereby reducing the amount of bacteriocin needed for therapeutic applications. Moreover, decreased use of bacteriocin through identifying highly effective synergistic bacteriocin combinations could help in reducing the cost of employing bacteriocins in medical applications and broaden their antimicrobial spectrum facilitating more effective control of food-borne pathogenic and spoilage bacteria. Nisin V in combination with penicillin produced enhanced antibacterial activity against *Staphylococcus aureus* SA113 (Field *et al*., 2016). Bacteriocin AS-48 acted synergistically with lysozyme against *Propionibacterium acnes* (Cebrián *et al*., 2018). Several combinations of antimicrobial peptides resulted in increased inhibitory activity against *Escherichia coli* MG1655 (Yu *et al*., 2016). In this study, salivaricin mmaye1 and pentocin MQ1 acted synergistically against *Micrococcus luteus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* resulting in enhanced antibacterial activity as evident by significantly increased ZOI **(Table 1)**. This finding would be useful in their future medical applications.

CONCLUSIONS

Lactobacillus salivarius SPW1 is non-hemolytic and susceptible to most of the antibiotics tested, suggesting its safety for *in situ* therapeutic applications. Absence of plasmid in *Lactobacillus salivarius* SPW1 suggests that salivaricin mmaye1 is not plasmid-borne. A new medium for high biomass accumulation was developed paving the way for future commercialization. Optimum salivaricin mmaye1 production occurred at pH values of 6 and 7. Salivaricin mmaye1 production started after 3 hours of incubation and peaked after 18 hours. Pentocin MQ1 and salivaricin mmaye1 displayed synergistic antibacterial activity against *Micrococcus luteus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* which can be explored for future medical applications.

Conflict of interest

The authors declare that they have no competing interest.

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