THE COMBINED ANTIMICROBIAL ACTIVITY OF CAYENNE CHILI PEPPER (*Capsicum frutescens*) EXTRACT AND *Bifidobacterium breve* BS2-PB3 AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus*

[*AKTIVITAS ANTIMIKROBA GABUNGAN EKSTRAK CABAI RAWIT* (*Capsicum frutescens*) *DAN Bifidobacterium breve BS2-PB3 TERHADAP METHICILLIN-RESISTANT Staphylococcus aureus*]

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ABSTRACT

Antibiotic resistance is increasingly becoming a global health threat, prompting the search for alternative treatments from natural compounds. Capsaicin is a bioactive component of Capsicum species, exhibiting antimicrobial, antifungal, anticancer, and anti-inflammatory properties. Probiotics such as *Bifidobacterium breve* are also known for their immunomodulatory and antimicrobial effects. This study explored the synergistic potential of capsaicin and *B. breve* combination. Capsaicin was extracted from *Capsicum frutescens* using grinding and solvent methods, and its concentration was measured by spectrophotometry at 650 nm. The capsaicin extract was added to *B. breve* BS2-PB3 cultures and tested against Methicillin-resistant *Staphylococcus aureus* (MRSA). The results showed a significant increase in clear zone diameter with capsaicin supplementation compared to individual controls, indicating synergistic potential in enhancing the antimicrobial activity.

Keywords: antibacterial activity; *Bifidobacterium breve*; capsaicin; *methicillin-resistant Staphylococcus aureus*

ABSTRAK

Resistensi antibiotik semakin menjadi ancaman kesehatan global, sehingga mendorong pencarian alternatif pengobatan dari senyawa alami. Kapsaisin merupakan komponen bioaktif dari spesies Capsicum yang menunjukkan sifat antimikroba, antifungal, antikanker dan anti-inflamasi. Probiotik seperti Bifidobacterium breve juga dikenal karena efek imunomodulator dan antimikrobanya. Penelitian ini mengeksplorasi potensi sinergis kombinasi capsaicin dari ekstrak cabai rawit dan B. breve strain BS2-PB3. Capsaisin diekstraksi dari Capsicum frutescens menggunakan metode penggilingan dan pelarut, dimana konsentrasinya diukur dengan spektrofotometri pada 650 nm. Ekstrak cabai yang berisikan capsaicin ditambahkan ke kultur B. breve BS2-PB3 dan diuji terhadap Methicillin-resistant Staphylococcus aureus (MRSA). Hasil menunjukkan peningkatan signifikan pada diameter zona bening ketika MRSA dipaparkan terhadap kombinasi B. breve dengan suplementasi ekstrak cabai dibandingkan perlakuan-perlakuan individual. Hal tersebut menunjukkan potensi sinergis antara kultur probiotik dengan suplementasi kapsaisin dalam meningkatkan aktivitas antimikroba.

Kata kunci: *aktivitas antibakteri; Bifidobacterium breve; kapsaisin; methicillin-resistant Staphylococcus aureus*

INTRODUCTION

Antibiotic resistance poses a serious threat to modern medicine, as microorganisms can develop genetic mutations that reduce antibiotic effectiveness (Chen *et al*., 2021; Yang & Yang, 2019). This adaptability is continually stimulated by the introduction of new antibiotics, leading to the emergence of more resistant variants. To address this growing issue, researchers are exploring alternative natural compounds, particularly from diverse plant species, to enhance antimicrobial activity (Khare *et al*., 2021).

In Southeast Asia, the *Solanaceae* plant family, particularly chili peppers (*Capsicum annuum*) and cayenne peppers (*Capsicum frutescens*), are key sources of capsaicinoids, especially capsaicin. Capsaicin is known for its role in increasing taste and pungency in dishes and its various bioactive properties (Othman *et al*., 2011). Recent studies highlight capsaicin's pharmaceutical potential, including its analgesic, antioxidant, anticancer and antimicrobial effects (Menezes *et al*., 2022; Romero-Luna *et al*., 2022). Given its known antimicrobial properties, capsaicin is considered a promising candidate for combating antibiotic-resistant pathogens, either as a standalone agent or in combination with existing pharmaceuticals (Oyedemi *et al*., 2019).

In Indonesia, cayenne chili peppers are consumed in large quantities, reaching 490,830 tons as per the 2021 National Socioeconomic Survey. This widespread consumption of capsaicin, the main bioactive compound in chili, provides health benefits such as enhanced immunity and potential resistance to antibiotic-resistant pathogens due to its natural antimicrobial properties (Qiu *et al*., 2012). However, many individuals avoid chili peppers because of their intense spiciness. Moreover, excessive capsaicin intake can lead to side effects like gastric inflammation, diarrhoea and gastrointestinal discomfort from irregular bowel movements (Ao *et al*., 2022; Xiang *et al*., 2022). These side effects hinder the full utilization of capsaicin's health benefits (Fuchtbauer *et al*., 2021; Lu *et al*., 2019; Othman *et al*., 2011).

To harness capsaicin's antimicrobial benefits without the drawbacks of overconsumption or adverse effects, combining it with probiotics like *Bifidobacterium breve* that is known for its anti-inflammatory and immune-boosting properties, offers a promising solution (Marini *et al*., 2015; Othman *et al*., 2011). This study aimed to explore the synergy between capsaicin extracted from cayenne chili peppers (*Capsicum frutescens*) and *Bifidobacterium breve* strain BS2-PB3 to

enhance antimicrobial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA). The methods included (i) estimating capsaicin content through spectrophotometric analysis, (ii) testing the antimicrobial activity of capsaicin and *Bifidobacterium breve* individually against MRSA, as well as (iii) assessing their combined effect using the well-diffusion method. This research seeked to provide an effective approach to combat antibiotic-resistant pathogens through probiotic synergy.

MATERIALS AND METHOD

Materials and Tools

The materials used in this experiment are acetone, phosphomolybdic acid (Merck, Germany), NaOH (Merck, Germany), TPY medium (Tryptic Soy Broth, Peptone, Yeast, Glucose, K2HPO4, & Tween 60%), Nutrient Broth medium (Merck, Germany), pure capsaicin standard solution ≥50% (HPLC) (Merck Sigma-Aldrich, Germany), Anaerogen 2.5L sachets (ThermoFisher), Clindamycin 300 mg tablet (Novell Pharmaceutical Laboratories, Indonesia), and Nutrient Agar (Merck, Germany).

The tools used in this experiment were incubator (LabTech, Korea), mortar and pestle (TOHO, China), BioDrop DUO UV/Vis spectrophotometer (Biochrom, UK), microtube centrifuge (TOMY, Japan), water bath (Laboratory Thermostatic Devices, China), autoclave, laminar air flow (ABL Equipment, Indonesia) and oven incubator (Anytester, China).

The samples used in this experiment were capsaicin solution (extracted from red cayenne peppers; *Capsicum frutescens*), *Bifidobacterium breve* BS2-PB3 strain that was isolated from human breast milk by Department of Biology UPH as well as Methicillin-resistant *Staphylococcus aureus* (MRSA) supplied from Department of Medical Laboratory Technology UPH.

Material Collection

One hundred grams of red cayenne peppers (*Capsicum frutescens*) were bought from a local or online market in Tangerang, Indonesia. The collected materials were brought into the laboratory at UPH for further processing. Prior to capsaicin extraction, the materials were thoroughly washed with water to remove any potential debris and surface microorganisms that might tamper with the results.

Capsaicin Sample Extraction

Capsaicin sample extraction was carried out according to Peeyananjarassri *et al.* (2022) method of extraction with modification. Prior to extraction, the cayenne chili peppers were first weighted to account for their dry weight, which was used to calculate the change in the moisture

content: [Final Weight (W2) - Initial Weight (W1)]/(W1)]. The peppers were subsequently dried in an incubator oven at 67.5°C for 48 hours. The dried sample was taken and ground using a mortar and pestle. The capsaicin was extracted from the grinded sample by using the reflux method using acetone (1 g/10 mL) as the solvent for 15 minutes to get the crude extract. Afterwards, the crude extract was taken and transferred into a 50 mL Falcon tubes and then centrifuged at 11,200 x g for 10 minutes at 4°C. The resulting supernatant was transferred to a petri dish for evaporation on a water bath at 57.5°C. In calculating the capsaicin concentration, the dry weight after evaporation was measured using the formula [W2 (Final Weight) – W1 (Petri dish Weight)] to determine the mass of capsaicin after acetone was evaporated.

For quantification of the capsaicin using spectrophotometry, the dried sample was mixed with phosphomolybdic acid (3 mL) and 0.4 M NaOH (0.4 mL). This reaction will turn the solution to be of a clear-blue color which intensity can be measured through spectrophotometry at 650 nm. For the treatment of *B. breve*, a certain amount of the dried capsaicin was dissolved with TPY broth medium. The TPY broth supplemented with capsaicin was then used as the growth medium of *B. breve*.

Bifidobacterium breve BS2-PB3 Liquid Culture

B. breve BS2-PB3 was cultured in a glass vial filled with 5 mL TPY medium and grown in an anaerobic condition at 37ºC for 48 hours. The OD_{600} of the culture was measured, subsequently serially diluted, then spread to TPY agar to determine the cell number. Each capsaicin sample media (five samples; serially diluted) was inoculated with an appropriate cell number (~10^9 CFU/mL) of *B. breve*. The culture was incubated at 37°C for 48 hours in anaerobic condition. Then cell number of each treatment was measured to analyse the effect of capsaicin on the viability of the bacteria.

Combined _Bifidobacterium breve_ Culture Supplemented with Capsaicin

The extracted capsaicin sample was taken and mixed with 10 mL of TPY medium to dissolve and form the stock solution. The stock formed would be used to create subsequent samples used in the experiment such as the five serial diluted samples of supplemented *B. breve* culture with capsaicin (CAPBb sample series), capsaicin control sample (CAPc; 5x103 mg/L) and as an additional experiment involving separately cultured *B. breve* without capsaicin supplement in the mixture (CAPBb-S sample series). Standard dilution formula (c1 x V1 = c2 x

V2) was used to determine the required concentration for the first dilution sample for each series and control (A is the starting sample code; CAPBb-A, CAPBb-SA and CAPc). Five mL of the mixture were serial diluted five times into five glass vials filled with 5 mL of TPY medium, resulting in five different concentrations (1/2 X [$5x10^3$ mg/L], 1/4 X [$2.5x10^3$ mg/L], 1/8 X [$1.25x10^3$ mg/L], 1/16 X [$6.25x10^2$ mg/L], and 1/32 X [$3.125x10^2$ mg/L]). Each of the different concentrated capsaicin samples were subsequently added with 1 mL of *B. breve* ($1x10^9$ CFU/mL) culture sample. The combined culture was incubated for 48 hours in an anaerobic condition at 37 ºC. The control sample (CAPc) was done the same way using the stock solution, with a final concentration of ($5x10^3$ mg/L).

The Antimicrobial Activity of *B. breve* Culture Grown in Capsaicin-Supplemented Media Against MRSA

Prior to the cultivation, 5 mL of Nutrient Broth (NB) were prepared for *S. aureus* culture and 90 mL of NA medium was prepared for the six petri dishes (15 mL for each). The *B. breve* was grown in TPY broth medium supplemented with capsaicin extract while the Methicillin-resistant *Staphylococcus aureus* was grown in NB. All cultures were incubated at 37°C for 24 hours (anaerobic condition for *B. breve* and aerobic condition for *S. aureus*). *B. breve* culture was centrifuged at 10,000 x g for 10 minutes, then the supernatant was taken. Fifteen millilitres of NA medium were poured into a sterile petri dish then left to set. Afterwards, 50 µL of *S. aureus* culture was inoculated on the agar using spread plate method. Three wells were made on the NA medium and filled with approximately 80 µL of supernatant from *B. breve* grown in the presence of capsaicin, positive control (clindamycin), negative control (sterile TPY), capsaicin control (CAPc), and B. breve control (Bbc). Samples were incubated at 37ºC for 24 hours. The formed clear zone was observed and its diameter was measured. Total petri dishes used for the experiment were seven, as to accommodate the six sample trials (One petri for the mixed samples and one control, and another for solely control samples) and an additional petri to test the antimicrobial profile of separately cultured *B. breve* added with capsaicin.

RESULTS AND DISCUSSION

Capsaicin's Extraction and Concentration

Capsaicin extracted from cayenne chili peppers (*Capsicum frutescens*) was weighted for its capsaicin paste, in which the concentration was quantified using an equation derived from a standard curve formed using pure capsaicin to accurately

determine its concentration. Cayenne chili peppers (*Capsicum frutescens*) were dried prior to extraction to fully eliminate the moisture content of the fruit as to allow a selective extraction of the sole capsaicin compound. Results of total moisture content, capsaicin's paste weight, as well as the standard curve data can be seen in Table 1, 2 and 3, respectively.

Table 1 shows that the cayenne chili peppers (*Capsicum frutescens*) used for capsaicin extraction had 81.59% ± 0.05 moisture content loss during drying. Removing moisture was crucial to prevent dilution of capsaicin and allowed extraction from stems and seeds. Additionally, it helped eliminate potential microbial contamination in the extract (Magied *et al*., 2014).

The moisture content of the sample in this research (81.25% ± 0.025) was higher compared to other studies (66.97% ± 0.05) (Mercy *et al*., 2016). This difference may be due to variations in the type or harvest age of the cayenne chili peppers used (Mueller-Seitz *et al*., 2008).

In this experiment, commercially sourced cayenne chili peppers were used, making it difficult to control growth conditions and the harvest age. The peppers' maturity, indicated by color (green, orange, red), affects their moisture content (Choi *et al*., 2022). Although only ripe red peppers were selected, it was not possible to control the harvest age. Therefore, capsaicin content may vary depending on the peppers' maturity at harvest (Choi *et al*., 2022; Mo *et al*., 2011).

Table 1. Cayenne chili pepper (*Capsicum frutescens*) moisture content

Mass (g)			Moisture Content (%)
Before	**After**	**Loss**	
554.60 ± 4.08	105.38 ± 0.78	449.28 ± 3.32	81.25 ± 0.025

Note: Before (initial weight of capsaicin prior to drying); After (final weight of capsaicin after drying). Final data was written as mean (n=3) ± standard deviation.

Table 2 and 3 show the paste concentration of the extracted capsaicin after evaporating the acetone and the capsaicin concentration as determined through spectrophotometry, respectively. The weight of the chili's extract was ~5 mg, while the spectrophotometry method indicated ~6.58 mg capsaicin. The spectrophotometry measurement was more accurate as it measured the wavelength absorption of capsaicin through the reaction with phosphomolybdic acid, creating a clear-blue solution which intensity can be measured with a wavelength of 650 nm (Nagoth *et al*., 2014; Orobiyi, 2018). Effectively, this method measures the true amount of capsaicin rather than through a miniscule difference in the weight

calculation, which relies on the accuracy of the scale used. However, due to only 1.5 mg of difference, the data were shown to be very close and indicated a good range of capsaicin concentration that was acceptable to be used for testing.

Table 2. Capsaicin extract paste weight and concentration

Mass (g)			Concentration based on m/v (mg/L)
Before	**After**	**CAP Paste Weight**	
70.16 ± 0.77	37.02 ± 0.02	0.053 ± 0.02	$5x10^2$

Note: Before = the weight of 100 mL of capsaicin extract after centrifuge, excluding the weight of Petri dish; After = the weight after evaporation procedure. CAP = Capsaicin. Paste Weight: (Petri weight – After result). Final data was written as mean (n=3) ± standard deviation.

Table 3. Pure capsaicin standard curve data measured at 650 nm wavelength

Capsaicin Standard Curve	
Concentration (mg/ml)	**Absorbance**
2	1.12
4	2.21
8	3.86
10	4.27
Capsaicin Concentration based on standard curve	
Concentration (mg/ml)	**Absorbance**
6.58	0.31

Note: The results were derived from one experiment. The absorbance value shown in the table has been accounted for with 10x dilution factor (DF) to reach 0.1 – 1.0 range for the spectrophotometry reading.

Antimicrobial Profile of Capsaicin and *Bifidobacterium breve* Mixture Against Methicillin-Resistant *Staphylococcus aureus*.

Bifidobacterium breve BS2-PB3 was successfully cultured in the growth media supplemented with five different concentrations of the extracted capsaicin (50 mg – 3.125 mg). The supernatant of *B. breve* cultures was then taken as samples, and their antimicrobial activity against MRSA was measured through the well diffusion method. Clindamycin (500 g/L) and sterile TPY broth were used as positive and negative control, respectively. An additional sample of supernatant from *B. breve* grown in TPY media without capsaicin supplementation was added as another control to the samples, as well as a capsaicin extract sample to account the antimicrobial property of *B. breve* metabolite as well capsaicin. The result of the clear zone diameter (Table 4).

Table 4. Antimicrobial profile of capsaicin and *Bifidobacterium breve* BS2-PB3 against methicillin-resistant *Staphylococcus aureus*

Trials	Clear zone diameter (mm)								
	Controls				Samples (*CAPBb*)				
	(+)	(-)	(x)						
	CD	TPY	CAPc	Bbc	A	B	C	D	E
1	34	6	18	6	28	19	17	16	13
2	33	6	30	6	26	15	13	11	6
3	30	6	23	8	29	15	6	6	6
Final Total	32.33 ± 2.08	6.00 ± 0.00	23.67 ± 6.03	6.67 ± 1.15	27.67 ± 1.53	16.33 ± 2.31	12.00 ± 5.57	11.00 ± 5.00	8.33 ± 4.04

Note: CAPc = Capsaicin control sample ($5x10^3$ mg/L); CD = Clindamycin (500g/L), TPY = Trypticase peptone yeast medium, Bbc = *Bifidobacterium breve* supernatant; *CAPBb* = Capsaicin and *Bifidobacterium breve* culture sample. *CAPBb*-A = $5x10^3$ mg/L; *CAPBb*-B = $2.5x10^3$ mg/L; *CAPBb-C* = $1.25x10^3$ mg/L; *CAPBb-D* = $6.25x10^2$ mg/L; *CAPBb*-E = $3.125x10^2$ mg/L of capsaicin within the culture of *B. breve*. Each *CAPBb* sample had 10^9 CFU/mL of *B. breve* (value derived after measuring at OD_{600}). The incubation time was 48 hours. Final data were shown as mean and standard deviation.

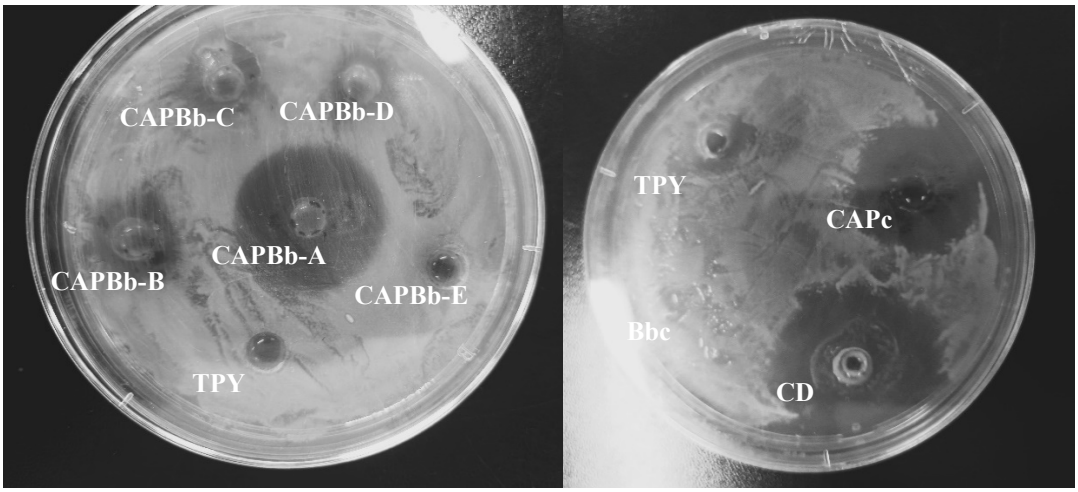

Figure 1. Representative image of the well-diffusion result of *CAPBb* sample series against MRSA.
Note: A: *CAPBb* Sample series (*CAPBb-A*, *CAPBb-B*, *CAPBb-C*, *CAPBb-D*, *CAPBb-E*, and TPY); B: Sample controls (CD [Positive], TPY [Negative], CAPc, and Bbc).

Table 4 shows that capsaicin exhibited moderate antimicrobial activity against MRSA in the well-diffusion experiment. Both the control capsaicin sample (CAPc) and diluted capsaicin samples (CAPBb-A to CAPBb-E, $5x10^3$ mg/L – $3.125x10^2$ mg/L) produced clear zones exceeding the well diameter (6 mm), comparable to the susceptibility zone for clindamycin (24–30 mm) based on CLSI and EUCAST standards. The antimicrobial effect decreased with dilution; CAPBb-E's clear zone (8 ± 3.8 mm) was about 30% of CAPBb-A (28 ± 1.5 mm). Samples with concentrations below $5x10^3$ mg/L showed lower activity than clindamycin, suggesting that capsaicin concentrations below this level were less effective (Wang *et al*.,

2019). The study aligned with Fuchtbauer *et al*. (2021), who reported capsaicin's inhibitory concentration range for *S. aureus* as 60–250 mg/L, supporting the antimicrobial potential of even the lowest concentration tested (Fuchtbauer *et al*., 2021; Wang *et al*., 2019).

The reduced antimicrobial activity observed in this study may be attributed to the capsaicin's lower purity, leading to a lower actual concentration than initially measured. The phosphomolybdic acid reaction, which typically produces a clear-blue solution upon detecting capsaicin, resulted in a greenish color, suggesting impurities or a lower capsaicin concentration (Nagoth *et al*., 2014). Previous study reported that capsaicin purity and concentration significantly affect the reaction's color, with lower purity yielding a green hue. The absence of advanced analytical techniques, such as high-performance liquid chromatography (HPLC) or simulated moving bed chromatography, limited the assessment of capsaicin purity in this study, as these methods are costly and require specialized equipment (Lu & Cui, 2019). Despite these limitations, it was plausible that with more precise measurements and known capsaicin purity, the antimicrobial activity observed—measured by the clear zone diameter—could be larger and more consistent with established findings, reinforcing capsaicin's efficacy as a potent antimicrobial agent (Fuchtbauer *et al*., 2021; Wang *et al*., 2019).

The results revealed that the antimicrobial profile of the CAPBb-A culture was notably higher than that of the CAPc control sample, with the same concentration. Specifically, the average antimicrobial activity of CAPBb-A samples showed an-18% increase, rising from a mean value of 23 ± 6.3 mm in the CAPc control to 28 ± 1.5 mm in the CAPBb-A culture. This suggested a synergistic effect between the antimicrobial properties of capsaicin and the culture supernatant of *Bifidobacterium breve*. Despite its probiotic properties, which typically include producing bacteriocins, altering pH levels and increasing nutrient adhesion to outcompete other bacteria (Kusharyati *et al*., 2020; Javvadi *et al*., 2022; Martinez *et al*., 2013), *B. breve* seems to lack a capability to produce bacteriocin. *In-silico* and comparative genomics analyses of *B. breve* strain 110^{1A} confirmed the absence of any bacteriocin-producing genes (Valdez-Baez *et al*., 2022) and whole genoma análisis of the *B. breve* BS2-PB3 strain used in this study similarly revealed no such gene (data not shown).

The study by Valdez-Baez et al. (2022) had attributed the inhibitory effect of

B. breve to potential changes in the pH environment and its adhesive capability, which prevented pathogen colonization. The adhesive capability is more effective in the gut microbiota than in agar media, but the pH change is significant in this context. During fermentation, *B. breve* could lower the pH of the culture medium, from approximately pH 7 to 5.5, due to the production of short-chain fatty acids (SCFA). Another research also suggests that metabolites, e.g., lactic acid and hydrogen peroxide, produced by *B. breve* could contribute to lowering pH and combating MRSA (Besten *et al*., 2013). These pH changes and metabolites likely account for the increased antimicrobial activity observed in the capsaicin-supplemented culture sample, enhancing the clear-zone diameter against MRSA (Besten *et al*., 2013; Valdez-Baez *et al*., 2022).

The lack of bacteriocins in *Bifidobacterium breve* makes it hard to explain the increased antimicrobial activity in capsaicin-supplemented cultures. Efthimiou *et al*. (2019) found that MRSA survives across a wide pH range, while Lemaire *et al*. (2007) showed increased MRSA susceptibility to β-lactam antibiotics at pH ≤ 5.5. This suggests pH changes affect MRSA susceptibility, but it remains unclear if the enhanced activity is due to pH changes or metabolites from *B. breve* strain BS2-PB3. An additional control experiment was subsequently performed to compare the antimicrobial effects of *B. breve* cultured alone and mixed with capsaicin at the end of bacterial culture (CAPBb-S) versus *B. breve* culture supplemented with capsaicin (CAPBb). The CAPBb-S-A sample showed a 23 mm clear zone, significantly lower than the 28 ± 1.5 mm zone in CAPBb-A, indicating that the culturing pase (i.e., supplementing capsaicin into culture médium of *B. breve*) may influence the results.

Table 5. Antimicrobial profile of separately cultured *Bifidobacterium breve* sample without capsaicin supplement against MRSA.

Trials	**Clear zone diameter (mm)**				
	Samples (*CAPBb-S*)				
	A	B	C	D	E
1	23	6	6	6	6

Note: *CAPBb-S* = Capsaicin mixed with separately cultured *Bifidobacterium breve* sample. *CAPBb*-S_A = $5x10^3$ mg/L; *CAPBb*-S_B = $2.5x10^3$ mg/L; *CAPBb*-S_C = $1.25x10^3$ mg/L; *CAPBb*-S_D = $6.25x10^2$ mg/L; *CAPBb*-S_E = $3.125x10^2$ mg/L. Each *CAPBb-S* sample has 10^9 CFU/mL of *B. breve* (value derived after measuring at OD_{600}). The incubation time was 48 hrs.

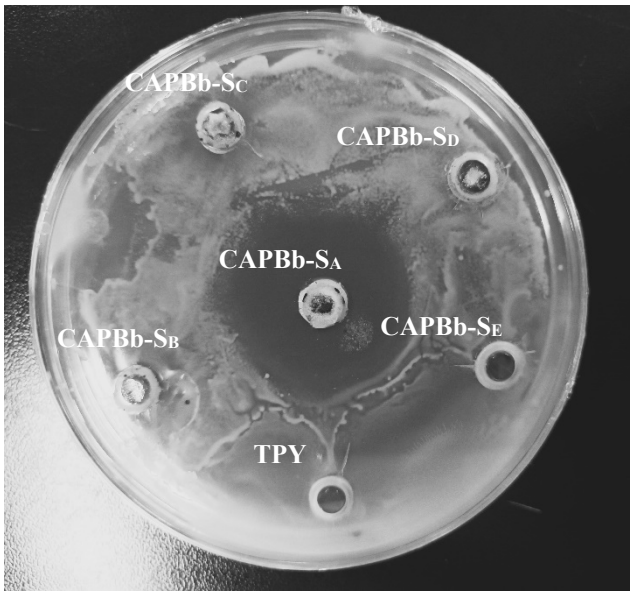

Figure 2. Image of the well-diffusion result of *CAPBb-S* sample series against MRSA
Note: CAPBb-S sample series (CAPBb-S_A, CAPBb-S_B, CAPBb-S_C, CAPBb-S_D, CAPBb-S_E, and TPY).

Capsaicin has been shown to enhance intestinal flora growth, such as increasing *Faecalibacterium prausnitzii* in mice (Wang *et al*., 2020) and balancing gut flora by reducing *Bacteroides* and *Parabacteroides* (Song *et al*., 2017). Despite this, the exact mechanism through which capsaicin enhances the antimicrobial activity of *B. breve* remains unclear. It is possible that capsaicin acts as a potent augmenter for *B. breve*. Oyedemi *et al*. (2019) suggest that capsaicinoids, known for their antimicrobial properties, hold potential for developing new antimicrobial drugs and enhancing the efficacy of existing antibiotics.

CONCLUSION

In conclusion, capsaicin from cayenne chili peppers (*Capsicum frutescens*) can be effectively supplemented into the culture of *B. breve* strain BS2-PB3, even at higher concentrations, to produce a strong antimicrobial profile against MRSA. This enhanced activity is likely due to capsaicin's potent antimicrobial properties boosting the limited antibacterial capacity of *B. breve* BS2-PB3. This novel approach of using capsaicin to improve probiotics offers potential solutions to antibiotic resistance, potentially leading to new pharmaceutical or natural products to combat this issue.

SUGGESTIONS

Future experiments should include selection of particular chili and better methods to extract chilli to obtain higher yield of capsaicin. Using another method to quantify the capsaicin concentration from the chili's extract, such as High-Performance Liquid Chromatography

(HPLC), would allow a more accurate identification and purification of capsaicin. Furthermore, utilize a better species and strain of probiotics might provide higher antibacterial capability.

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