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RESEARCH ARTICLE

Antibacterial Activity of Probiotics Cell-Free Fermentation Filtrate from *Passiflora edulis* Sims. againts Pathogen bacteria

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ABSTRACT:

Fermentation of probiotics (MM1, MM2, and MM3) isolated from red passion fruit (Passiflora edulis Sims.) in De Man Rogose Sharpe broth medium has been performed. Determination of antibacterial activity of probiotics cell free fermentation filtrate (PCFFF) against *Mycobacterium tuberculosis H37Rv* has been carried out. The Minimum Inhibitory Concentration (MIC) and Minimum Bacteriosidal Concentration (MBC) of the PCFFF against *Eschericia coli* Extended Spectrum Beta Lactamase (ESBL) and Methycillin Resistant *Staphylococcus aureus* (MRSA) has also been done. Determination of the MM1 and MM2 PCFFF potential ratio to vancomycin against MRSA were also reported. It was found that, the PCFFF was unable to inhibit the growth of Mycobacterium tuberculosis, but the MM1 and MM2 PCFFF were able to inhibit the growth of ESBL and MRSA with MIC values of 25% and MBC 50% respectively, while the MM3 PCFFF was unable to inhibit ESBL at 100% concentration. The MM3 PCFFF showed inhibitory activity against MRSA at a concentration of 100%. The potential ratio of the MM1 and MM2 PCFFF to vancomycin standard solution (10, 5, and 2.5 ppm) against MRSA were 92.70% and 82.77% respectively.

KEYWORDS: ESBL, MRSA, *Mycobacterium tuberculosis H37Rv*, Probiotics, *Passiflora edulis* Sims.

INTRODUCTION:

Infectious diseases are still a major health problem in developing tropical countries such as Indonesia. Along with the COVID-19 pandemic, it gives a dark record for infectious diseases. In this study, the infectious diseases that will be discussed are infections by Mycobacterium tuberculosis and infections by resistant bacteria such *Escherichia coli* Extended Spectrum Beta-Lactamase and Methycillin Resistant *Staphylococcus aureus*. Irrational use of antibacterial agents will make the situation worse and increase bacterial resistance.

Many bacteria are resistant to some antibiotics, such as Extended Spectrum Beta Lactamase (ESBL) and Methicillin Resistant *Staphylococcus aureus* (MRSA), so that the use of antibiotics is irrational in this condition. Bacterial resistance to antibiotics has become a serious global problem. 440,000 new cases of MDR-TB (Tuberculosis-Multi-Drug Resistance) are reported every year, causing 150,000 deaths worldwide[1].

Oral administration of probiotics will enhance the immune system by interacting with intestinal epithelial cells (IECs) or immune cells associated with the lamina propria, through Toll like receptors (TLRs), and inducing the production of various cytokines or chemokines. Macrophage chemoattractant protein 1, produced by IECs, sends signals to other immune cells leading to activation of the mucosal immune system (MIS), characterized by an increase in immunoglobulin

A+ cells in the gut, bronchi and breast. glands, and T cell activation. In particular, probiotics activate regulatory T cells that release IL-10. probiotics strengthen the intestinal barrier with increased mucin and Paneth Cells. So that it can modulate the good microbiota in the gut by suppressing the growth of potential pathogenic bacteria in the gut. Therefore, the interaction of probiotics with IECs, macrophages and dendritic cells (DC) plays an important role in this immune response without causing an inflammatory pattern[2].

Passion fruit (Passiflora edulis), a member of the family Passifloraceae, has more than 500 species (Paull and Duarte, 2012; Reis et al., 2018). This plant comes from Brazil and has spread to other countries in Asia, Australia, Africa, India, South America, and the Caribbean. Passion fruit has other variants that can be identified from the color of the fruit, such as yellow (P. edulis var. flavicarpa), purple (P. edulis var.edulis), and orange (P. edulis var. Caerulea)[3].

In a previous study, the filtrate of fermented red passion fruit pulp (Passiflora edulis Sims.) in de man rogosa sharpe broth (MRS-broth) media was able to inhibit methicillin resistant Staphylococcus aureus (MRSA) and Eschericia coli extended spectrum beta-lactamase (E.coli ESBL) with a minimum inhibitory concentration (MIC) of 25%, while the minimum value of bactericidal concentration (MBC) is 50%. Potential probiotic candidate have been isolated from red passion fruit pulp in de man rogosa sharpe agar (MRS-agar) media, which have been tested for resistance to acid pH (pH 2.5 and pH 3), NaCl salt (1%, 4%), and 8%), 5% phenol solution. The 10 isolates obtained, three isolates (MM 1, MM 2 and MM 3 were potential as probiotics and had been tested for compatibility and resistance to erythromycin and vancomycin antibiotics[4].

The purpose of this study was to determine the antibacterial activity of PCFFF (MM1, MM2, and MM3) against *Mycobacterium tuberculosis*, determine the Minimum Inhibitory Concentration (MIC) and Minimum Bacteriosidal Concentration (MBC) of PCFFF (MM1, MM2, and MM3) against *Eschericia coli* Extended Spectrum Beta Lactamase (ESBL) and Methycillin Resistant *Staphylococcus aureus* (MRSA), determine the ratio potencial of probiotic isolates from red passion fruit to MRSA.

MATERIALS AND METHODS:

Materials:

Nutrien agar (Merck®), Nutrien broth (Merck®), de Man Rogosa Sharpe broth (Merck®), Middlebrook Media 7H9 ((Merck®), *Mycobacterium tuberculosis H37Rv, Eschericia coli* Extended Spectrum Beta Lactamase (ESBL) isolate, Methycillin Resistant *Staphylococcus aureus* (MRSA) isolate, and probiotics cell-free fermentation filtrate from red passion fruit (*Passiflora edulis* Sims.) in de Man Rogosa Sharpe broth medium.

Methods:

Rejuvinating Probiotics:

To rejuvenate probiotic cells, each probiotic isolate MM1, MM2, and MM3 was inoculated into de Man Rogosa and Sharpe broth (MRS-broth) media, then incubated for 24 hours at 37^0C temperature**.**

Probiotic Cell Free Fermentation Preparation:

The suspensions of each probiotic isolate MM1, MM2, and MM3 aged 24 were diluted until the number of colonies was approximately 10^6 to 10^9CFU/mL (equivalent to 25%T). Each of the probiotic suspensions was pipetted 5mL, suspended and fermented into 45 mL of MRS-broth media at room temperature. Then filtered using a millipore sieve of 0.2µm. The cell free fermentation filtrate obtained is then stored and carried out for the next testing.

Antibacterial Activity Test againts Mycobacterium tuberculosis H37Rv:

Media preparation:

Media MRS-broth, weighed 52.2grams of MRS-broth base suspended in 1 L of distilled water. Then sterilized by autoclaving at 121^0C for 30 minutes. Middlebrook Media 7H9, weighed 1 gram of Middlebrook 7H9 Broth Base suspended in 1 L of tween 80. Then sterilized by autoclaving at 121^0C for 30 minutes.

Preparation of Mycobacterium tuberculosis H37Rv:

25mL of middlebrook7H9 liquid medium was taken, and 2.5mL of OADC was added, 0.5mL of PANTA + 4 OADC, and homogenized. Then 1mL of Mycobacterium tuberculosis strain H37Rv was added (aged 3-4 weeks), and suspended in a sterile tube containing 25mL of Middlebrook 7H9 media and homogenized.

Mycobacterium tuberculosis Antibacterial Activity Test:

Micro well plate-24 was prepared under sterile conditions, the work was carried out aseptically in a Biosafety Cabinet (BSC). A total of 50L Middlebrook 7H9 medium was added to the plate (each in duplicate) as a negative control. A total of 50L Middlebrook 7H9 media with the addition of bacteria to the plate (each in duplicate) as a positive control. Then 50 L of the probiotic suspension was pipetted into the wells (each in duplicate). After that, 950L of Mtb bacterial suspension was added to all wells on the plate and then homogenized. Then it was incubated in an incubator for 7 days at 37^0C. Colonies were observed using a fluorescence microscope.

Determining Procedure for the minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) The PCFFF (MM1, MM2, MM3):

The each PCFFFs were made in series with dilutions of 100%, 50%, 25%, and 12.5% in Nutrien Broth (NB) media. Each was replicated in duplicate. Each of the test solutions (PCFFFs) in various concentrations was pipetted 5mL and put into a test tube and added test bacteria (50µL) then incubated at 37^0C for 24 hours, then 1mL pipetted and inoculated on Nutrien Agar (NA) media and incubated at temperature 37^0C for 24 hours. Then observed and counted the number of test bacterial colonies that grew (total plate count). The minimum inhibition concentration value is determined based on the lowest concentration of PCFFFs that can still grow the test bacteria, while the minimum bacteriocidal concentration value is determined based on the lowest concentration of PCFFFs which is able to inhibit the growth of the test bacteria.

Potency Ratio Test Procedure:

The PCFFF were made in series with dilutions of 100%, 50%, and 25% Nutrien Broth (NB) media. Vancomycin standard antibiotics with concentrations of 10ppm, 5 ppm, and 2.5ppm. Prepare test bacteria (ESBL and MRSA) with a concentration of 10^6 - 10^8CFU/mL (25%T). The test bacteria were inoculated on nutrient agar media with the pour plate method. Antibacterial activity test by cylindrical plate (well) method.

RESULT:

Antibacterial Activity against *Mycobacterium tuberculosis H37RV*

The results of the PCFFFs against *Mycobacterium tuberculosis H37Rv* can be seen in table 1 below;

Table 1. Test Results of Antibacterial Activity of PCFF MRS broth medium against *Mycobacetrium tuberculosis H37Rv*

Probiotic Isolate	The Growth of *Mycobacetrium tuberculosis H37RV* in each Week				Additional Information
	1	2	3	4	
MM1	+	+	+	+	Resistant
MM2	+	+	+	+	Resistant
MM3	+	+	+	+	Resistant
Mix (MM1, MM2, and MM3)	+	+	+	+	Resistant

The probiotics cell-free fermentation filtrates in MRS-broth media was not able to inhibit the growth of *Mycobacetrium tuberculosis H37Rv*. This is indicated by the continued growth of these bacteria during the four-week observation period can be seen in figure 1 below;

Figure 1. *Mycobacterium tuberculosis H37Rv* wich Given PCFF in MRS-broth Media Still Grow During Four Weeks Observation.

Minimum Inhibitory Concentration (MIC) and Minimum Bacteriosidal Concentration (MBC) of PCFFF in MRS-broth Media againts *Esccericia coli* Extended Spectrum Beta-Lactamase.

The results of MIC dan MBC of PCFFF against *Esccericia coli* Extended Spectrum Beta-Lactamase can be seen in table 2 , table 3 and table 4 below;

Table 2. Minimum Inhibitory Concentration and Minimum Bacteriosidal Concentration of MM1 PCFFF against *Eschericia coli* Extended Spectrum Beta-Lactamase

Test filtrate concentration	Total Plate Count (CFU/mL)		
	1	2	Average
100%	0	0	0
50% (MBC)	0	0	0
25% (MIC)	98	87	93
12,5%	>300	>300	>300

Table 3. Minimum Inhibitory Concentration and Minimum Bacteriosidal Concentration of MM2 PCFFF against against *Eschericia coli* Extended Spectrum Beta-Lactamase

Test filtrate concentration	Total Plate Count (CFU/mL)		
	1	2	Average
100%	0	0	0
50% (MBC)	0	0	0
25% (MIC)	117	123	120
12,5%	>300	>300	>300

Table 4. Minimum Inhibitory Concentration and Minimum Bacteriosidal Concentration of MM3 PCFFF against against *Eschericia coli* Extended Spectrum Beta-Lactamase

Test filtrate concentration	Total Plate Count (CFU/mL)		
	1	2	Average
100%	>300	>300	>300
50%	>300	>300	>300
25%	>300	>300	>300
12,5%	>300	>300	>300

Minimum Inhibitory Concentration (MIC) and Minimum Bacteriosidal Concentration (MBC) of PCFFF againts Methycillin Resistant *Staphylococcus aureus* (MRSA).

The results of determining MIC and MBC of MM1 PCFFF against Methycillin Resistant *Staphylococcus aureus* (MRSA) can be seen in table 5 , table 6 and table 7 below;

Table 5. Minimum Inhibitory Concentration and Minimum Bacteriosidal Concentration of MM1 PCFFF against against Methycillin Resistant *Staphylococcus aureus* (MRSA).

Test filtrate concentration	Total Plate Count (CFU/mL)		
	1	2	Average
100%	0	0	0
50% (MBC)	0	0	0
25% (MIC)	13	23	18
12,5%	37	85	61

Table 6. Minimum Inhibitory Concentration and Minimum Bacteriosidal Concentration of MM2 PCFFF against against Methycillin Resistant *Staphylococcus aureus* (MRSA).

Test filtrate concentration	Total Plate Count (CFU/mL)		
	1	2	Average
100%	0	0	0
50% (MIC)	0	0	0
25% (MBC)	0	6	3
12,5%	71	23	47

Table 7. Minimum Inhibitory Concentration and Minimum Bacteriosidal Concentration of MM3 PCFFF against against Methycillin Resistant *Staphylococcus aureus* (MRSA).

Test filtrate concentration	Total Plate Count (CFU/mL)		
	1	2	Average
100% (MBC)	0	0	0
50%	>300	>300	>300
25%	>300	>300	>300
12,5%	>300	>300	>300

Ratio Potency of PCFFF againts Methycillin Resistant *Staphylococcus aureus* (MRSA):

The potential ratio of MM1 probiotic isolate to vancomycin can be seen in table 8 and figure 2, and the potential ratio of MM2 PCFFF to vancomycin can be seen in table 9 and figure 3 below;

Tabel 8. Result of Ratio Potency Test of MM1 PCFFF and Vancomycin againt MRSA

Replication	Diameter of Inhibition Zone (mm)					
	U3	U2	U1	S3	S2	S1
1	21	21,5	15	21	18	13,5
2	25	17,5	15	20	16	14
3	18	19,5	17,5	18	17,5	14
Σ	64	58,5	47,5	59	51,5	41,5
Mean	21,3333	19,5	15,8333	19,6667	17,1667	13,8333
Linear Contrast	16,5			17,5		
Quantity of the Preparations	(64+58,5+47,5)=170			(59+51,5+41,5)=152		

*Additional Information : U1= MM1 25%; U2= MM1 50%; U3= MM1 100%; S1= Vancomycin 2,5%; S2= Vancomycin 5%; S3= Vancomycin 10%; Lu= ΣU_3- ΣU_1 = 16,5; L_S= ΣT_3- ΣT_1 = 17,5.

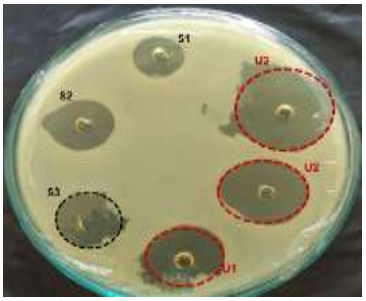

Figure 2. Result of sRatio Potency Test of MM1 PCFFF and Vancomycin againt MRSA

Concentration Log Interval Side by Side:

i = log S_1 – log S_2 = log S_2 – log S_3
= log 41,5 – log 51,5 = log 51,5 – log 59
= 1,6180– 1,7118 = 1,7118– 1,7708
= -0,094 = -0,059
i = -0,059

Concentration Log Zone Regression Slope All Preparations:

b = Lu + Ls/ (d-1). i. n. h = (16,5 + 17,5)/(3-1)x (-0,059) x 3 x 2 = -60,7345
Yu = S/ n . d = 170/ 3x3 = 18,889
Ys = U/ n. d = 152/ 9 = 16,889
Mu = Yu-Ys/b = 18,889 – 16,889 / -60,7345 = -0,0329
Antilog = 0,9270
Ratio Potency = 92,7

Tabel 9. Result of Ratio Potency Test of MM2 PCFFF and Vancomycin againt MRSA

Replication	Diameter of Inhibition Zone (mm)					
	U3	U2	U1	S3	S2	S1
1	22,5	16,5	16	22	16	15
2	20,5	16,5	14,5	16	17,5	15,5
3	18,5	15,5	13,5	16,5	18,5	15,5
Σ	61,5	48,5	44	54,5	52	46
Mean	20,5	16,1667	14,6667	18,1667	17,3333	15,3333
Linear Contrast	17,5			8,5		
Quantity of the Preparations	(61,5+48,5+44=154)			(54,5+52+46 = 152,5)		

*Additional Information :U1= MM2 25%; U2= MM2 50%; U3= MM2 100%; S1= Vancomycin 2,5%; S2= Vancomycin 5%
S3= Vancomycin 10%; Lu= ΣU_3- ΣU_1 = 16,5; L_S= ΣT_3- ΣT_1 = 17,5

Figure 3. Result of sRatio Potency Test of MM2 PCFFF and Vancomycin againt MRSA

Concentration Log Interval Side by Side:
$i = \log S_1 - \log S_2 = \log S_2 - \log S_3$
$= \log 46 - \log 52 = \log 52 - \log 54{,}5$
$= 1{,}6627 - 1{,}7160 = 1{,}7160 - 1{,}736$
$= -0{,}0533 \qquad = -0{,}0204$
i = -0,0204

Concentration Log Zone Regression Slope All Preparations:
b = Lu + Ls/ (d-1). i. n. h = (17,5+8,5)/(3-1)x (-0,0204) x 3 x 2 = -106,2091
Yu = S/ n . d = 154/ 3x3 = 25,6667
Ys = U/ n. d = 152,5/ 9 = 16,9444
Mu = Yu-Ys/b= 25,6667-16,9444 / -106,2091= -0,0821
Antilog = 0,8277
Ratio Potency = 82,77%

DISCUSSION:

Red passion fruit (*Passiflora edulis* Sims.) is classified as sour passion fruit. Passion fruit itself has many species and varieties. It is estimated that there are about 500 species of *Passiflora* in the family Passifloraceae. The most common types of sour passion fruit in Indonesia are purple passion fruit that grows in the highlands, red and yellow passion fruit that grows in the lowlands. The sour passion fruit (100g) contains 69-80 g of water, 2.3g of protein, 2.0g of fat (in seeds), 16 g of carbohydrates, 3.5g of fiber, 10mg of Ca, 1, 0mg Fe, 20 SI vitamin A, very little thiamine, 0.1 mg riboflavin, 1.5 mg niacin, and 20-80mg vitamin C[5]. Due to the complete nutritional content of passion fruit, passion fruit is a natural habitat for a source of probiotic bacteria. Purple passion fruit (*Passiflora edulis Sims. var edulis*) has been isolated containing probiotics *Lactobacillus bulgaricus* and *L. Heterohiochii* (Zahro, 2007). Based on previous research, Three isolates MM1, MM2, and MM3 from red passion fruit (*Passiflora edulis* Sims.) have been isolated which are potential as probiotics because they are resistant to acidic pH, phenolic compounds, and NaCl salts, all three are resistant to erythromycin and vancomycin antibiotics and are compatible with all three[4]. In this study, the antibacterial activity of probiotic isolates from red passion fruit was tested against the pathogenic bacteria such as *Mycobacterium tuberculosis*, *Escherichia coli* Extended Spectrum Beta-Lactamase and Methycillin Resistant *Staphylococcus aureus*.

In this study, PCFFF from red passion fruit were not able to inhibit growth of *Mycobacetrium tuberculosis H37RV,* but the fermentation filtrate of red passion fruit pulp werw able to inhibit the growth of *Mycobacetrium tuberculosis H37RV.* This may be due to that the fermentation filtrate red passion fruit still contained the complate compounds from the secondary metabolite content of passion fruit which give antibacterial activity and also metabolites from probiotics in the red passion fruit, so can give strong inhibition against *Mycobacetrium tuberculosis H37RV.* Passion fruit contains phytochemical compounds that may give antibacterial activity including cyanogenic glycosides passibiflorin, epipassibiflorin, passicapsin, passicoriacin, epipassicoriacin, cyanogenic-b-rutinoside., epitetraphilin B, amygdalin, prunacin, triterpenoid glycosides and salicylate glycosides. Other chemical compounds such as b-carbolin alkaloids harman, harmine, harmaline and harmalol, phenols, carotenes and g-lactones are also found in passion[7].

Mycobacetrium tuberculosis H37RV is a virulent *Mycobacetrium tuberculosis* strain and the most commonly found in tuberculosis cases. *Mycobacterium tuberculosis* is a unique Gram positive with cell walls that contain thick lipids and mycolic acids and do not contain phospholipids so they cannot retain color during Gram staining. *Mycobacterium tuberculosis* contains glycolipids that are rich in mycolic acid, peptidoglycan, lipo arabino manan (LAM), this high lipid content makes these bacteria resistant to many antimicrobial compounds. Phospatidyl isnocytosil mannosides (PIM), phthiocerol dimycocerate, umbilical factor, sulfolipids and wax D are able to interfere with host defenses so that they remain alive in phagosomes so that Mtb bacteria can escape the phagostosis process[8].

The infection caused by Extended spectrum beta-lactamase (ESBL) continue to increase throughout the world[9], therefore in this study the discovery of a new alternative as an antibacterial against Extended Spectrum β-Lactamase. The antibiotic- resistant Enterobacteriaceae family is a major cause of hospital admission and associated morbidity and mortality in children[10]. Enterobacteriaceae, especially Escherichia coli are many opportunistic pathogens that cause infections in hospitals and are a major cause of urinary tract infections, gastrointestinal infections, bloodstream infections, and meningitis in humans[11]. *Escherichia coli* is a major reservoir of genes encoding Extended spectrum beta-lactamases. There are 350 ESBL variants in the world and divided into nine separate families based on their amino acid sequence namely; TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, OXA. The main variants are TEM, SHV, CTX-M and OXA. The blaCTX-M gene caused the most infections and was increasing rapidly. The gene is found in clinical isolates of *Escherichia coli* bacteria worldwide[9].In this study, the MIC and MBC values of the cell-free fermentation filtrat probiotic isolated from red passion fruit were determined against *E.coli* ESBL bacteria. Based on this study, the MIC value of the probiotic isolates MM1 and MM2 was at a concentration of 25%, while the MM3 isolate at a concentration of 25% was still able to grow

large numbers of *E.coli* ESBL bacteria. The minimum bacteriocidal concentration values of the probiotic isolates MM1 and MM2 was at a concentration of 50%, while the MM3 isolate at a concentration of 50% was still able to grow large numbers of *E.coli* ESBL bacteria. The ability of the antibacterial activity of Bacillus species depends on the type of strain.

The probiotics isolated from red passion fruit are the *Bacillus* group, were identified as *Bacillus subtilis* (MM1), *Bacillus wiedmannii* (MM2), and *Bacillus cereus* (MM3)[4]. *Bacillus* is widely used as a probiotic because of its good stability to heat, gastric pH, and moisture. It has been proven in previous studies that the probiotic *Bacillus subtillis* is able to produce bacteriocins and has strong inhibition and is resistant to acidic pH[12]. The genus Bacillus has about 377 species and most of them contain antimicrobial compounds. Antimicrobial compounds produced by Bacillus spp. including volatile compounds, ribosomal peptides/ RPs (bacteriocins and enzymes (lactonase, decarboxylase, acylase, deaminase)), polyketides (PKs), and non-ribosomal peptides/ NRPs (lipopeptides/LPs, fatty acid chains, and siderophores)[13].

Staphylococcus aureus is a commensal and pathogenic bacterium. The virulence factor of *S. aureus* is caused by the compound proteins it secretes and proteins present on the surface of these bacteria known as microbial surface components that recognize adhesive matrix molecules (MSCRAMMs). Different strains of *S. aureus* have different constellations of MSCRAMMs and cause specific infections. Factors that cause *S. aureus* to become resistant to antimicrobial compounds are its ability to form biofilms and small-colony variance (SCVs) forms[14].

Methicillin was first introduced in 1959, and within one year it was reported that methicillin-resistant isolates were reported. Resistance to methicillin was reported by the MecA gene that encodes Penicillin-binding protein (PBP2A) thereby reducing affinity for beta-lactam antibiotics[14]. In this study, the MIC and MBC of PCFFF were determined against MRSA. Based on this study, the MIC of MM1 and MM2 PCFFF was at a concentration of 25%, while the MM3 PCFFF at a concentration of 25% was still able to grow large numbers of MRSA. The minimum inhibition concebtration of MM3 is 100%. The minimum bacteriocidal concentration of MM1 and MM2 PCFFF was at a concentration of 50%, and MM3 does not have bactericidal ability.

Bacillus spp. is in great demand in the functional food sector due to its benefits for human health and its ability to survive in the pH of the digestive tract and is more stable to heat during processing and storage[15]. Although the use of *Bacillus* as a probiotic is still controversial regarding its pathogenic properties and great benefits, there are several groups of bacillus that are excluded from the pathogen group, and have antimicrobial, antioxidant, and immunomodulatory activities[16]. Several species of Bacillus have also been used for the production of additional nutraceuticals including vitamins (eg, riboflavin, cobalamin, inositol) and carotenoids for the synthesis of several health supplements for human consumption[17].

The potential of *Bacillus* as a probiotic, this study will test the ratio potency of MM1 and MM2 PCFFF againt MRSA with a comparison standard of vancomycin antibiotic. The ratio potency was not carried out on MM3 PCFFF because based on its MIC and MBC showed that MM3 PCFFF was less sensitive to MRSA. In addition, MM3 PCFFF were *Bacillus cereus*, a group of pathogenic Bacillus bacteria. *Bacillus cereus* produces enterotoxins (cereulide) which can cause food poisoning[18]. The test results showed that the ratio of MM1 PCFFF to MM2 PCFFF was 26%, MM1 PCFFF to standard antibiotics (Vancomycin) was 92.70%, MM2 PCFFF to vancomycin were 82.77%.

Inappropriate use of antibiotics can cause bacterial resistance. Probiotics are an alternative to antibiotics that are currently being researched to treat bacterial infections[19]. Probiotics are able to produce antibacterial compounds such as organic acids and bacteriocins. Bacteriocins are biomolecules produced by microorganisms and have narrow and wide spaces spectrum activity. Bacteriocins have various sizes of proteins, microbial targets, and their mechanism of action in inhibiting pathogenic bacteria. Bacteriocins are very important in medicine because it is made by non-pathogenic bacteria usually colonize the human body. Various probiotics bacteria are used as potential therapeutic agents[20]. Probiotics will provide health benefits including inhibiting pathogenic bacteria if consumed in sufficient quantities amount that helps in prevention and treatment[21]. Probiotics have also been shown to be able to prevent gastrointestinal infections and strengthen the immune system and protect the intestinal flora in humans. Probiotics can be given to patients who are taking long-term antibiotics to improve the balance of microflora in the digestive tract if consumed in sufficient quantities[22].

CONCLUSION:

Based on the research that has been done, the PCFFF unable to inhibit the growth of *Mycobacterium tuberculosis*, but the MM1 and MM2 PCFFF were able to inhibit the growth of ESBL and MRSA with MIC values of 25% and MBC 50%. MM3 PCFFF was unable

to inhibited ESBL but was able to inhibit MRSA at a concentration of 100%. The ratio potency of the MM1 PCFFF to the vancomycin antibiotic was 92.70%, while the MM2 PCFFF was 82.77%.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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