JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTIMICROBIAL ACTIVITY OF METABOLITES PRODUCED BY NOVEL COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS) ISOLATED FROM FERMENTED DAIRY PRODUCTS IN MALANG, INDONESIA

Alif Firman Firdausy, Ziyana Walidah, Kurnia Mufidah, Azizah Nurul Rahmadhany, Nofita Diyah Ningrum, Afnan Adila*

Address(es):
Universitas Islam Negeri Maulana Malik Ibrahim Malang, Faculty of Medicine and Health Science, Department of Pharmaceutical Science, Locari Street, Tlekung-65151, Batu City, Indonesia, Phone number: +6281336713332.

*Corresponding author: aliffirman.firdausy@uin-malang.ac.id

https://doi.org/10.55251/jmbfs.9200

ARTICLE INFO

Received 14. 7. 2022
Revised 15. 1. 2023
Accepted 17. 1. 2023
Published 1. 4. 2023

Regular article

OPEN ACCESS

ABSTRACT

Yogurt and kefir are well-known fermented dairy products that provide many benefits to human health. Microorganisms contained in yogurt and kefir play an important role to produce bioactive metabolites, including eradicating pathogens. This study presents the antimicrobial activity of 12 strains isolated from kefir and yogurt samples obtained in Malang, Indonesia. Strain K3 from kefir shows the highest antimicrobial activity with the diameter of the inhibitory zone was 8.11±0.07 mm against *Escherichia coli* and 8.20±0.17 mm against *Staphylococcus aureus*. Phenotypic characterization through Gram-staining, endospore-forming, motility, catalase, oxidase, and carbohydrate fermentation test, and genotypic identification through the 16S rRNA gene sequencing and phylogenetic analysis was performed to identify the species of isolate. Based on the result, K3 was characterized as non-motile Gram-negative bacilli bacteria, tested negative in endospore formation, catalase, and oxidase. According to the nucleotide sequence of 16S rRNA gene, the isolate was classified as *Staphylococcus saprophyticus* strain UTI-045 with 100% of similarity. Furthermore, the GC-MS analysis of the strain's cell-free supernatant (CFS) reveals 3,6-Dioxa-2, 7-disilaoctane, 2,2,4,7,7-pentamethyl- (Rt=9.449 min); and Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (Rt=11.328 min) as the active metabolites.

Keywords: fermentation, dairy product, coagulase-negative staphylococci, antimicrobial

INTRODUCTION

Fermented dairy products are well-known as beneficial food/beverages for the human gut. There are various types of fermented dairy products spread worldwide and each of them has different characteristics based on its production method and composition. Yogurt and kefir are two popular fermented dairy products that can be obtained all over the world. The difference between yogurt and kefir is the presence of yeast in the kefir starter besides the special bacteria that conduct the fermentation process, called lactic acid bacteria (LAB) **(Gul *et al.*, 2015)**. LAB is beneficial bacteria that are known as probiotics, which are living in the human digestive system to provide some health benefits, such as preventing the growth of pathogens by producing antimicrobial compounds. Malang is a highland district located in East Java and is known to be one of Indonesia's biggest producers of milk and its derivatives, including yogurt and kefir **(Saati *et al.*, 2013)**. Microbial content of yogurt and kefir products in Malang are predicted to be active against several pathogens. In accordance with that, **Raras *et al.*, (2019)** screened strains isolated from the starter and the product of goat milk kefir originating from Malang for its antibiofilm ability against *Klebsiella pneumoniae.*
The characteristic of LAB and the metabolites they produce could be varying based on composition, medium, condition of fermentation, and where the starter was obtained **(Plessas *et al.*, 2017)**. **Kim et al., (2019)** demonstrated the antimicrobial activity of four different isolated LAB and found that their antimicrobial characteristic differed in terms of inhibiting some types of pathogens. The study of LAB and its potential, especially to fight against colonised pathogens by producing active metabolites, could be useful in reducing the chance of infections in the human gastrointestinal tract **(Harnentis *et al.*, 2020)**.
In this study, LABs were isolated from commercial kefir and yogurt products in Malang and then screened based on their ability to produce active metabolites against representatives of Gram-positive and negative pathogens, respectively *Staphylococcus aureus* and *Escherichia coli*. A GC-MS procedure was performed to investigate which metabolites were produced by LAB and predicted as responsible for its antimicrobial activity. This study also aimed to explore novel LAB strains that conduct the anti-pathogenic activity isolated from fermented dairy products from Malang, Indonesia.

MATERIAL AND METHODS

Isolation and characterization of LAB

Yogurt (Yumoo® produced by Az-Syauqi, Pujon, Malang, East Java, Indonesia) and kefir (TalithaFata® produced by CV. Tiga Nada Wisa Perkasa, Sukun, Malang, East Java, Indonesia) were diluted four times (10^{-1} to 10^{-4}) and inoculated on Petri dishes containing DeMan, Rogosa, Sharpe (MRS) agar. The dishes were incubated at 37°C for 48 hours under microaerophilic (5% CO_2) conditions. Isolated colonies were purified and inoculated by streaking on a fresh MRS agar medium. The purified strains were then inoculated into milk yeast extract (10% skim milk, 15% glucose, 0.5% yeast extract with 5% glycerol) medium and stored under -20°C as a stock culture for the next procedure **(Deshmukh & Thorat, 2013; Maldonado *et al.*, 2007)**.
Selected LAB isolates were characterized phenotypically by morphology (Gram-staining, cell and colony shape, and endospore-forming test), physiology (motility test), and biochemistry (catalase test, oxidase test, and carbohydrate fermentation test) assays **(Lani *et al.*, 2021; Sanam *et al.*, 2022)**.

Screening for the antimicrobial activity

Cell-free supernatants (CFS) of isolated LAB were extracted by precipitation via centrifugation of sub-cultured LAB isolate in MRS broth (0.5 McFarland standard). The centrifugation was performed at 10000 rpm for 10 min and the supernatant obtained was filtered through a sterile 0.22 µm millipore membrane (Biostellar®) **(Egbe & Lennox, 2019)**.
The antimicrobial activity of CFSs against *S. aureus* and *E. coli* (obtained from Laboratory of Microbiology, Faculty of Medicine, Universitas Brawijaya, Indonesia) was subsequently analyzed by the Kirby-Bauer test. Diffusion disks of 6 mm diameter (Oxoid®) containing CFSs and 30µg of chloramphenicol (as positive control) were appropriately overlaid on Mueller Hinton Agar (MHA) medium. The plates were then incubated at 37°C for 24 hours, and the diameter of each inhibition zone was calculated.

The analysis of 16S rRNA gene sequence alignment

DNA of isolated LAB was extracted using Quick-DNA Bacterial Miniprep Kit (Zymo Research®). Each DNA was subsequently amplified by PCR method using

27F (5'-AGAGTTTGATCCTGGCTCAG-3') forward and 1492R (5'-GGTTACCTTGTTACGACTT-3') reverse primers (Frank et al., 2008). The total volume of mixture for PCR reaction is 50 µL. It is contained 100 ng of template DNA, 25 µL of 2× AmpMaster®Taq, and 10pmol/µL each of primers. The mixture was heated at 95°C for 2 min and subjected to 35 rounds of thermal cycling at 95°C for 20 s, 49°C for 10 s, and 72°C for 5 min.

The gene sequencing was conducted in a bidirectional method and the sequence of nucleotides was then aligned with the NCBI GenBank DNA database using BLASTn (http://www.ncbi.nlm.nih.gov.blast) **(Liu *et al*., 2012; Osborne, 2017)**. The phylogenetic tree was constructed using the neighbor-joining method by NCBI BLAST tree according to the nucleotide sequences of LAB obtained from the previous step **(dos Santos Leandro *et al*., 2021)**.

GC-MS procedure

Metabolite profiling through GC-MS analysis procedure was adopted from **Chaudhary *et al*., (2020)**. CFSs were pre-treated by derivatization using BSTFA (N,O-Bis(trimethylsilyl) trifluoroacetamide) with 1% chlorotrimethylsilane (CTMS). Gas chromatography-mass spectroscopy (GC-MS) analysis of the derived sample was conducted by injecting 6µL of aliquot into TG 5MS (30 m×0.25mm, 0.25µm thickness) capillary column in a splitless mode. The column was made of 5% diphenyl: 95% dimethyl polysiloxane, with the initial temperature of the oven being 50°C for 3 minutes. Hereafter, the temperature was increased gradually by 15°C/min until 220°C. The MS was operated in the scanning mode with a mass range between 30 to 500 m/z. The quadrupole MS parameters were set as follows: ion source 230°C; split-flow 40 mL/min; carrier flow 1.5 mL/min; injector temperature 250°C; 70 eV of electronic impact. Each target compounds (indicated from peaks in chromatogram) were identified by its fragment spectrum using database library provided by National Institute of Standards and Technology (NIST).

RESULTS AND DISCUSSION

Phenotypic characterization of LAB isolated from yogurt and kefir

Six colonies were isolated from yogurt (Y1, Y2, Y3, Y4, Y5, Y6) and kefir (K1, K2, K3, K4, K5, and K6). Y1-6 colonies exhibited the characteristic of LAB: non-motile Gram-positive bacilli, catalase-negative, oxidase-negative, non-endospore-forming, and capable of fermenting carbohydrates (Figure 1 & Table 1). K1-6 colonies exhibited the same characteristics as K1-6 colonies except for the result of Gram staining. K1-6 colonies showed a red-magenta color under the microscope, hence indicated as Gram-negative bacteria (Figure 2).

Table 1 Characteristics of colonies isolated from the sample of yogurt and kefir in Malang, Indonesia

Characteristics	Isolated strain	
	Y 1-6	**K 1-6**
Colony morphology		
- Pigmentation	white	white
- Colony shape	circular	circular
- Margin	entire	entire
- Elevation	convex	convex
- Optical properties	opaque	opaque
- Texture	dry	dry
Cell morphology		
- Gram	positive	negative
- Cell shape	bacilli	Bacilli
Cell physiology		
- Motility	non-motile	non-motile
- Endospore forming	negative	negative
Biochemistry properties		
- Catalase	negative	negative
- Oxidase	negative	negative
- Carbohydrate fermentation	positive	positive

Antimicrobial activity of the isolated strains

The ability of the strains to produce active metabolites within their CFSs against *S. aureus* and *E. coli* was evaluated by the disc diffusion method. The diameter (mm) of the inhibition zone observed in this study was measured in triplicates (horizontal, vertical, and diagonal). The data obtained were then statistically analyzed using Kruskal-Wallis. According to the test, there was a significant difference ($p<0.05$) between the inhibition zone's diameter of positive control (chloramphenicol), negative control (aquadest), and CFSs against *S. aureus* and *E. coli* (Table 2). Chloramphenicol elicited the highest inhibitory zone against *S. aureus* and *E. coli* with an average of diameter more than 18 mm (Figure 3).

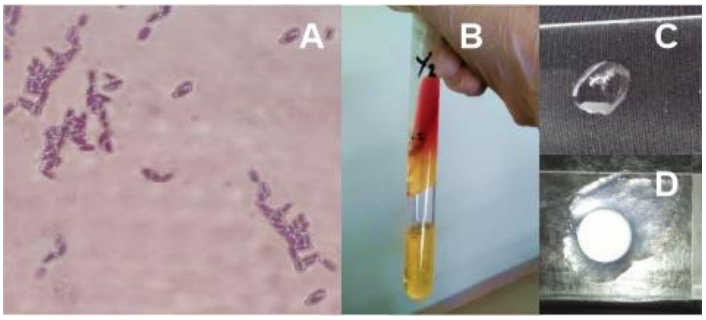

Figure 1 Phenotypic characterisation of LABs: endospore staining shows negative result (1000× magnification)(A), positive carbohydrate fermentation test showed by yellow-turning medium with CO_2 gas formation (B), negative result of catalase test (C), and oxidase disc remain white shows negative result of oxidase test

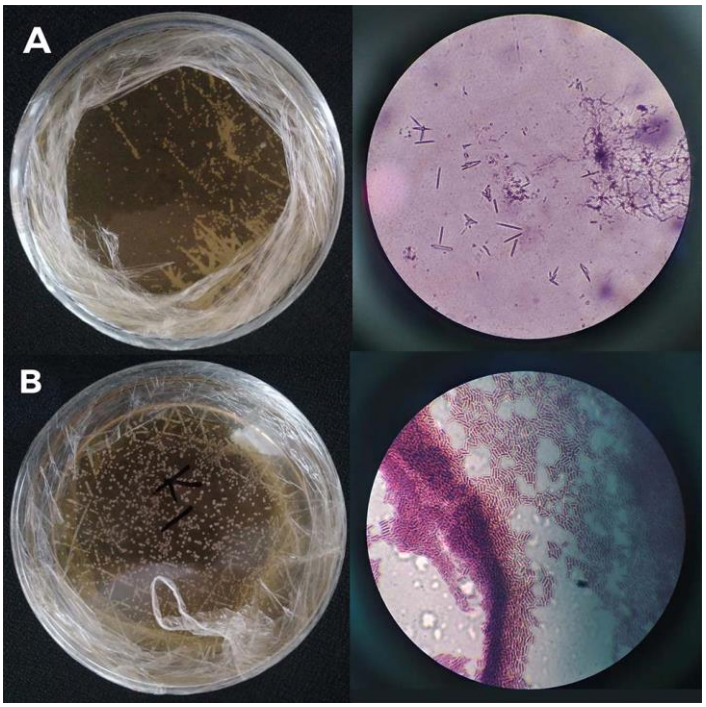

Figure 2 Bacterial colonies isolated from the sample product of yogurt (A) and kefir (B) in Malang, Indonesia (left). Result of Gram staining under light-microscope (1000× magnification)

Table 2 Antimicrobial activity of CFS of the strains isolated from kefir and yogurt against *S. aureus* and *E. coli*

Origin	Sample	Inhibitory zone±SD (mm)	
		Escherichia coli	***Staphylococcus aureus***
Yogurt	Positive c.	18.76± 0.65	24.8 ± 0.26
	Negative c.	8.18 ± 0.12	6.70 ± 0.00
	Y1	8.55 ± 0.08	7.03 ± 0.05
	Y2	8.26 ± 0.15	7.00 ± 0.00
	Y3	9.11 ± 1.89	7.06 ± 0.20
	Y4	8.70 ± 0.17	6.78 ± 0.20
	Y5	8.93 ± 0.15	6.95 ± 0.10
	Y6	9.46 ± 0.16	7.30 ± 0.05
	P-value	0.003*	0.010*
Kefir	Positive c.	22.33±1.15	20.65±0.67
	Negative c.	7.16±0.15	7.10±0.17
	K1	7.31±0.14	7.43±0.12
	K2	7.53±0.12	7.35±0.18
	K3	8.11±0.07	8.20±0.17
	K4	7.93±0.20	7.13±0.12
	K5	7.70±0.26	7.33±0.12
	K6	7.75±0.27	7.11±0.28
	P-value	0.005*	0.013*

Note: Positive-control (30µg chloramphenicol); negative control (aquadest); *P-significance of Kruskal-Wallis test <0.05

The strains isolated from yogurt (Y3 and Y6) also exhibited relatively stronger antibacterial activity against *E. coli* than other isolates with a diameter of 9.11 ± 1.89 mm and 9.46 ± 0.16 mm respectively. On the other hand, only strain K3 isolated from kefir, shows the higher inhibitory zone (8.11±0.07 mm against *E. coli* and 8.20±0.17 mm against *S. aureus*), while other isolates from kefir were

below. Moreover, the result of the Mann-Whitney U-test post hoc analysis proves that there was a significant difference ($p<0.05$) between the diameter of the inhibitory zone of Y6 and K3 against the negative control (Table 3). Hence, it could be proven that Y6 and K3 presented antimicrobial activity against *S. aureus* and *E. coli*.

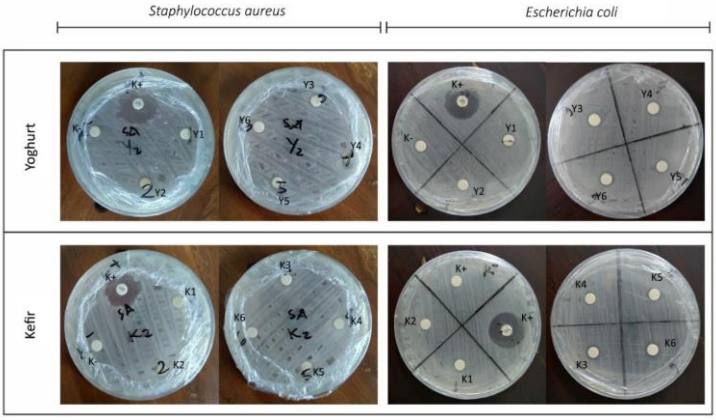

Figure 3 Microbial activity test of the strain isolated from yogurt and kefir against *S. aureus* (left-side) and *E. coli* (right-side)

Table 3 Mann-Whitney U-test post hoc analysis result of Y6, K3, and negative control

Escherichia coli				*Staphylococcus aureus*			
Strains	**K-**	**Y6**	**K3**	**Strains**	**K-**	**Y6**	**K3**
K-		0.050	0.043*	**K-**		0.037*	0.050
Y6	0.050		0.046	**Y6**	0.037*		0.077
K3	0.043*	0.046		**K3**	0.050	0.077	

Note: Negative control (K-); *P-significance of Mann-Whitney post hoc (<0.05)

Strain identification and phylogenetic analysis

The identification of potential isolates (K3 and Y6) was carried out by the 16S rRNA gene sequencing. The PCR product of gene amplification using 27F/1492R primers was an amplicon of about ~1400 bp **(Wu *et al.*, 2014)** (Figure 4). Two 16S rRNA nucleotide sequences obtained from the gene sequencing method were analyzed using NCBI BLASTn. Top-10 hit BLAST results against NCBI GenBank database of each sample are shown in Table 4. Samples of K3 and Y6 showed a high similarity percentage (98 to 100%) with the 16S rRNA gene of other species deposited in GenBank. Y6 isolated from yogurt shows the highest similarity with *Pluralibacter pyrinus* DSM12410T strain (99.86% similarity), while K3 isolated from kefir shows the highest similarity with *Staphylococcus saprophyticus* UTI-045 strain (100% similarity).

The phylogenetic tree constructed by the neighbor-joining method reveals the relationship between Y6 and K3 with the top-10 similar species obtained from GenBank with the BLASTn method. From the branch of phylogenetic tree, we can assume that the Y6 isolate most likely is *Pluralibacter pyrinus* and in a cluster with strain 533.12.1 (Figure 5) Whereas, the K3 isolate belongs to *Staphylococcus saprophyticus* strain UTI-045 based on its closest cluster (Figure 6).

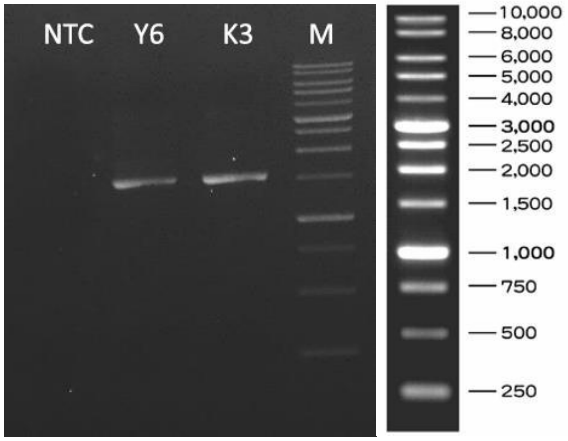

Figure 4 The result of gel agarose (0.8%) electrophoresis, showing amplificated DNA fragments of 16S rRNA gene. NTC: non-template control; Y6; K3; M: marker 1 Kb DNA ladder (2.5 µL)

Table 4 Top-10 BLAST nucleotide of Y6 and K3 isolates against GenBank database

Strain	**Sample**	***Species***	***Strain***	***Identity* (%)**	**GenBank *Accession number***
Y6	Yogurt	*Pluralibacter pyrinus*	DSM12410T	99.86	LN875036.1
		Pluralibacter pyrinus	E872	99.86	EF059884.1
		Enterobacter sp.	NAB3a	99.43	AY395008.1
		Pluralibacter pyrinus	533.12.1	99.71	MG859616.1
		Enterobacter sp.	NAB3	98.94	AY395007.1
		Enterobacter hormaechei	CPO 4.200	98.93	MN733028.1
		Enterobacter hormaechei	40a	98.93	MN294585.1
		Enterobacter hormaechei	15a1	98.93	MN294583.1
		Enterobacter hormaechei	VITJS3A	98.93	MN258703.1
		Enterobacter ludwigii	SNSK 244	98.93	MG576153.1
K3	Kefir	*Staphylococcus saprophyticus*	UTI-045	100	CPO54831.1
		Staphylococcus saprophyticus	Cqsm h2	100	MN826552.1
		Staphylococcus saprophyticus	UTI-042y	100	CP054438.1
		Staphylococcus edaphicus	1328	100	MT573756.1
		Staphylococcus edaphicus	Uyi 20	100	MT507214.1
		Staphylococcus sp.	Atelim2C	100	MT386290.1
		Staphylococcus edaphicus	BPs2	100	MT269536.1
		Staphylococcus arlettae	115607.1	100	MN851079.1
		Staphylococcus arlettae	156818.1	100	MN851078.1
		Staphylococcus saprophyticus	100	100	MF457583.1

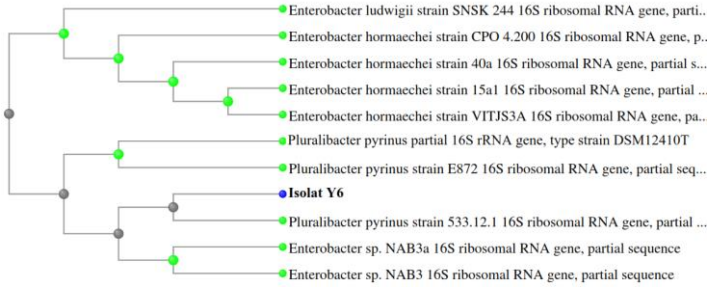

Figure 5 Phylogenetic tree showing relationship between Y6 strain isolated from yogurt and top-10 BLASTn result of NCBI

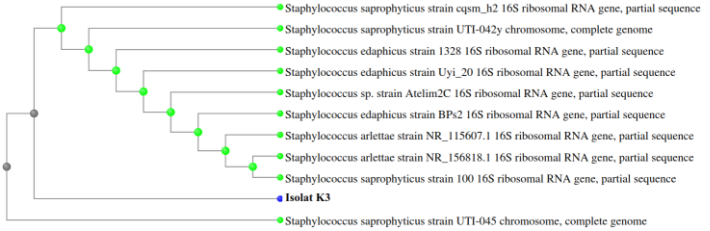

Figure 6 Phylogenetic tree showing the relationship between K3 isolated from kefir and top-10 BLASTn result of NCBI

According to the taxonomy and classification system, *Pluralibacter pyrinus* belongs to *Enterobacteriaceae* family and was recently re-classified into a genus of *Pluralibacter* (previously *Enterobacter*) (Brady et al., 2013). *P. pyrinus* was firstly identified by **Young R C *et al.*, (1993)** from brown leaf spot lesions of pear trees. *Enterobacteriaceae*, including *P. pyrinus*, are widespread in nature and known as food contaminating bacteria. A previous study by **Sobeih *et al.*, (2020)** concludes that *Enterobacteria sp.* is mostly detected in raw milk and dairy product samples. *P. pyrinus* detected in the sample of this study could be the presence of contamination during the handle of material before sequencing. In accordance with that, although the dairy product has been already sterilized by pasteurization or ultra-heat exposure, the contaminating microbes still could present through improper handling.

Staphylococcus saprophyticus is classified under the family of *Staphylococceae* and the genus of *Staphylococcus* **(Schoch *et al.*, 2020)**. *Staphylococcus sp*. is ubiquitous and found mostly on animal/human skin and mucosal membranes. However, *Staphylococcus sp*. sometimes could be isolated from processed foods and beneficially used in the making of fermented meat and various dairy products **(Soares *et al.*, 2011)**. *S. saprophyticus* is included in coagulase-negative staphylococci (CNS) which are relatively safe compared to coagulase-positive staphylococci (CPS), such as *S. schleiferi* and *S. aureus* **(Fontana & Favaro, 2018)**. The unconventional use of CNS in the food fermentation process can be an alternative way to provide more quality in fermented products. *S. carnosus, S. xylosus, S. equorum,* and *S. vitulinus* are also known as members of CNS that are widely used as microbial starters for the fermentation process of meat, cheese, and fish. Their versatile metabolism and robustness against various growth conditions are two of the other advantages besides their ability to provide better sensory quality (color, smells, and taste) of the products **(Stavropoulou *et al*., 2018)**. CNSs were also known to have antibacterial activity against pathogens. *Staphylococcus chromogenes* L217, a CNS from bovine teat apex skin**,** was reported to have bactericidal activity against *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Staphylococcus aureus* by producing bacteriocins named nukacin L217 **(Braem *et al*., 2014)**. Another finding by **Lee et al., (2018)** presented the potential of *Staphylococcus equorum* KS1039 (CNS used in fermented food) to produce lactococcin 972 known to be active against some pathogens including *S. aureus.* Despite the use of CNS in fermented products is generally considered prospering, their safety aspects remain uncertain and must be investigated more **(Heo et al., 2020)**.

Profile of metabolite produced by strain isolated from yogurt and kefir

Metabolite profiling by GC-MS analysis was used in this study to provide information on any metabolites produced by selected strains isolated from yogurt and kefir. In total, 6 compounds were obtained from the CFS of Y6 strain and 8 compounds from K3 strain. The GC-MS chromatograms are shown in Figure 7, while the compounds identified, their retention time, peak area, area percentage and their molecular formula are represented in Table 5.

Table 5 Compounds identified in CFS of Y6 strain and K3 strain

Strain	Rt (min)	Peak area	Area (%)	Compound name	Molecular formula	CAS number
Y6	2.204	3.549e+7	20.95	Disiloxane, hexamethyl-	$C_6H_{18}OSi_2$	107-46-0
	3.149	5.338e+7	31.51	Acetamide, 2, 2, 2-trifluoro-N-(trimethylsilyl)-	$C_5H_{10}F_3NOSi$	55982-15-5
	5.303	7.969e+7	47.04	Bis(trimethylsilyl)trifluoroacetamide	$C_8H_{18}F_3NOSi_2$	25561-30-2
	5.711	533025	0.31	Trisiloxane, octamethyl-	$C_8H_{24}O_2Si_3$	107-51-7
	7.528	140819	0.08	Trifluoromethyl-bis-(trimethylsilyl)methyl ketone	$C_9H_{19}F_3OSi_2$	None
	9.451	181721	0.11	3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl-	$C_9H_{24}O_2Si_2$	17887-27-3*
K3	2.201	4.192e+7	23.43	Disiloxane, hexamethyl-	$C_6H_{18}OSi_2$	107-46-0
	3.160	6.440e+7	35.99	Acetamide, 2, 2, 2-trifluoro-N-(trimethylsilyl)-	$C_5H_{10}F_3NOSi$	55982-15-5
	5.339	7.075e+7	39.54	Bis(trimethylsilyl)trifluoroacetamide	$C_8H_{18}F_3NOSi_2$	25561-30-2
	5.715	576110	0.32	Trisiloxane, octamethyl-	$C_8H_{24}O_2Si_3$	107-51-7
	7.531	205894	0.12	Trifluoromethyl-bis-(trimethylsilyl)methyl ketone	$C_9H_{19}F_3OSi_2$	None
	9.449	234077	0.13	3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl	$C_9H_{24}O_2Si_2$	17887-27-3*
	10.709	399285	0.22	Butane, 2,3-bis(trimthylsiloxy)-	$C_{10}H_{26}O_2Si_2$	53274-85-4
	11.328	441784	0.25	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_9H_{22}O_3Si_2$	17596-96-2*

Note: Rt=retention time; *compounds considered as metabolites

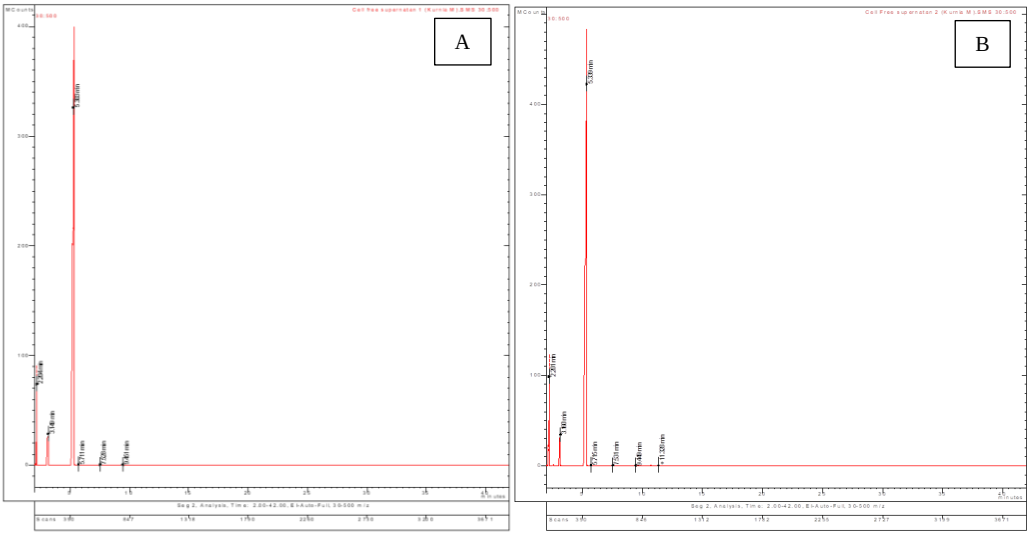

Figure 7 GC-MS chromatogram of compounds detected in CFS of (A) Y6 strain and (B) K3 strain

There is an identical pattern of peaks that appeared in the chromatogram of Y6 and K3 strains. Peak 1 to 6 in each chromatogram shows almost the same in intensity and retention time. The maximum peak area and percentage belong to Bis(trimethylsilyl)trifluoroacetamide (BSTFA), with 47.04% in CFS of Y6 strain and 39.54% in CFS of K3 strain. BSTFA is the reagent used in this research for the purpose of sample derivatization. BSTFA and trimethylchlorosylane (TMCS) were added to conduct a silylation reaction by 'capping' labile functional groups with the more stable trimethylsilyl group for GC-MS analysis **(Schummer *et al.*, 2009)**. In accordance with that, other peaks obtained from GC analysis also indicated as the derivative compounds of BSTFA, namely Disiloxane, hexamethyl-; Acetamide, 2, 2, 2-trifluoro-N-(trimethylsilyl)-; Trisiloxane, octamethyl-; Trifluoromethyl-bis-(trimethylsilyl)methyl ketone; and Butane, 2,3-bis(trimthylsiloxy)-. Nevertheless, two compounds viz. 3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl-; and Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester, considered as metabolites of the strains. 3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl- or 1,2-propanediol is a common metabolite produced by bacteria and yeasts. *Lactobacillus brevis, L. buchneri, Clostridium thermobutyricum, Thermoanaerobactericum thermosaccharolyticum,* and *Saccharomyces cerevisae* are reported to produce 1,2-propanediol as their product of sugars and lactic acid metabolisms **(Saxena *et al.*, 2010)**. 1,2-propanediol was also reported as the metabolite of some lactic acid bacteria, namely *L. plantarum, Pediococcus acidilactici* and *Pediococcus pentosaceus* **(Chaudhary *et al.*, 2020)**. 1,2-propanediol, namely propylene glycol, is known as antimicrobial and preservatives agent due to its bactericidal activity against pathogens such as *S. mutans, E. faecalis,* and *E. coli* **(Kinnunen & Koskela, 1991; Nalawade *et al.*, 2015)**. Propylene glycol, as one of the metabolites produced by LAB during fermentation could inhibit the growth of pathogenic bacteria. Propanoic acid, 2-[(trimethylsilyl)oxy], trimethylsilyl ester, is a derivate of lactic acid. Lactic acid is mainly found in fermented dairy products as the primary metabolite of microorganisms. Lactic acid exhibits some antimicrobial properties against pathogens through physiological and morphological alteration of pathogens by disrupting the structure of their cell membranes **(Wang *et al*., 2015)**.

CONCLUSION

The screening of the 6 strains isolated from yogurt and 6 strains isolated from kefir showed their ability to provide antibacterial activity against *E. coli* and *S. aureus*. K3 strain isolated from kefir produced inhibitory zones of 8.11±0.07 mm against *E. coli* and 8.20±0.17 mm against *S. aureus*, while Y6 strain from yogurt produced inhibitory zones of 9.46 ± 0.16 mm against *E. coli* and 7.30 ± 0.05 mm against *S. aureus*. However, chloramphenicol provided the highest inhibitory zones in both pathogens by 22.33±1.15 mm against *E. coli* and 24.8 ± 0.26 mm against *S. aureus*. The 16S rRNA gene sequence revealed that Y6 was classified as *Pluralibacter pyrinus* 533.12.1 strain, while K3 as *Staphylococcus saprophyticus* UTI-045. Regarding their ability to eradicate pathogens, two active metabolites viz. 3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl-; and Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester, was successfully identified. This finding provides another evidence of the beneficial use of coagulase-negative staphylococci (CNS) for the starter of fermented dairy products.

Acknowledgments: This work was supported by LPPM Universitas Islam Negeri Maulana Malik Ibrahim Malang, Indonesia through Bantuan Operasional Perguruan Tinggi Negeri (BOPTN) Litapdimas. And supported by the Faculty of Medicine and Health Science, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Indonesia through the Lecturer and Student Collaboration Research Grant [DIPA-025.04.2.423812/2021].

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