

# Antimicrobial effect of coconut oil on *Staphylococcus aureus*: an implication of *Staphylococcus epidermidis* induced fermentation

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Received: 30 November 2021; Revised: 11 April 2022; Accepted: 18 April 2022; Published: 6 June 2022

DOI <https://doi.org/10.28916/lsm.6.1.2022.100>

## ABSTRACT

*Staphylococcus epidermidis* is a commensal bacterium of human skin. *S. epidermidis* possesses lipolytic activity to digest skin surface lipids into the smallest unit of fatty acids (FAs). Most FAs hold antimicrobial properties essential for protecting skin from invading microorganisms. In this study, we were interested in virgin coconut oil (VCO), the source of several medium-chain fatty acids (MCFAs) such as lauric acid and caprylic acid. Those MCFAs products demonstrated remarkable antibacterial activity. Our results showed that crude supernatant from the culture medium of *S. epidermidis* with VCO fermentation exhibited the growth inhibition effect on *Staphylococcus aureus*. This bacterium causes a wide range of skin diseases. A co-culture of *S. epidermidis* and *S. aureus* in a rich medium with 2.5% (v/v) VCO significantly reduced the growth of *S. aureus* compared to those without VCO (p-value <0.05). Moreover, time-kill kinetics study showed that the supernatant from the culture medium of *S. epidermidis* with VCO fermentation showed an efficient antimicrobial activity against *S. aureus* after 18 hours of incubation. Our results concluded that the culture of *S. epidermidis* with VCO plausibly induced fermentation of natural lipid sources aiming the production of MCFAs with antibacterial activity, particularly suppression of skin pathogen *S. aureus* growth. The skin commensal bacterium *S. epidermidis* might help produce MCFAs from skin products containing VCO and make more benefits for skin infection protection.

**Keywords:** Coconut oil; medium-chain fatty acids; skin commensal bacteria; *Staphylococcus aureus* and *Staphylococcus epidermidis*

## INTRODUCTION

The skin is the largest organ of the human body that comprises several microorganisms such as fungi, viruses, and bacteria. The latter is the largest kingdom on human skin, containing beneficial and pathogenic bacteria (Byrd et al., 2018). *Staphylococcus aureus* causes various skin diseases such as impetigo, folliculitis, and atopic dermatitis. Moreover, it can lead to life-threatening diseases like septicemia, endocarditis, and toxic shock syndrome (Mrochen et al., 2020).

A conventional method used to treat *S. aureus* infection is antibiotics treatment which can be disturbed other microorganisms and lead to antibiotics resistance bacteria (Byrd et al., 2018). Recently, several studies suggested the new concepts to avoid antibiotic usage for skin infections, such as using natural substances, prebiotics, probiotics, and postbiotics (Wang et al., 2016; Wang et al., 2014; Weber et al., 2019; Zolkiewicz et al., 2020).

*Staphylococcus epidermidis*, the most common bacterium colonized on human skin and mucous membrane, can act as a pathogen in indwelling device patients or opportunistic infection in a predisposed patient. However, usually *S. epidermidis* colonizes human skin as a benign resident and can produce antibiotic agents like antimicrobial peptides (Cogen et al., 2008) or antimicrobial lipids (Asada, 1968).

Fatty acids, natural substances from triglyceride digestion by lipase enzyme of cells and microbes on the skin (Asada, 1968), possess antimicrobial activities (Moore et al., 2020; Watanabe et al., 2019). Virgin Coconut Oil (VCO) is a source of fatty acids such as lauric acids, myristic acids, and caprylic acids, usually found in skin products. The previous studies reported on the increasing fatty acids and ameliorating antimicrobial effects of VCO after fermentation by microorganisms (Khoramnia et al., 2013; Widianingrum et al., 2019). Therefore, this study evaluated the antimicrobial effects of VCO against *S. aureus* by *S. epidermidis* fermentation.





## METHODOLOGY

### Bacterial cells preparation

*S. epidermidis* (ATCC 12228) and *S. aureus* (ATCC 29213) provided by the Department of Microbiology, Faculty of Pharmacy, Mahidol University, were cultured on tryptic soy agar (TSA) (BD, MD, USA) at 37 °C, overnight. Then the single colonies were selected for inoculation into tryptic soy broth (TSB) (BD, MD, USA) and incubated under the same conditions. The cell was collected by centrifugation at 4500 rpm for 15 min and measured absorbance at 600 nm (OD<sub>600</sub>) = 0.6 (10<sup>8</sup> CFU/ml), then washed with Phosphate Buffer Saline (PBS) (Apsalagen, Bangkok, Thailand) 2 times and suspended with PBS.

### Co-culture assays

Since *S. epidermidis* and *S. aureus* are colonized on human skin, co-culture assays were selected and applied based on Wang et al.'s (2016) research to demonstrate the antimicrobial effects of fermented VCO. *S. epidermidis* (10<sup>6</sup> CFU/ml) and *S. aureus* (10<sup>6</sup> CFU/ml) were inoculated into a Rich medium [yeast extract (Axil Scientific Pte Ltd, Singapore Science Park II, Singapore) 10 g/l, TSB 3 g/l, K<sub>2</sub>HPO<sub>4</sub> (CARLO ERBA Reagents S.A.S., Val de Reuil, France) 2.5 g/l, and KH<sub>2</sub>PO<sub>4</sub> (Univar, Taren Point, Australia) 1.5 g/l] containing with or without 2.5% v/v of VCO (Tropicana Oil, Nakhon Chai Si, Nakhon Pathom), incubated at 37°C with shaking at 200 rpm. After five days of incubation, the co-culture was diluted, ranging from 10<sup>-1</sup> to 10<sup>-5</sup> with PBS. The cultured suspension was spot (10 µl) on 1.5% Rich medium agar containing polymyxin B (100 µg/ml) (Pharma Nueva, Bangkok, Thailand) to enumerate the colony of *S. aureus* (Finegold & Sweeney, 1961). Overnight culturing of the single colony on countable dilution was observed and interpreted to Log<sub>10</sub> CFU/ml. The spot appearances of *S. epidermidis* and *S. aureus* on Rich medium agar and selective Rich medium + Polymyxin B agar plates were demonstrated in Figure 1.

Bacterial Strains	Agar plates	Results
<i>S. epidermidis</i> ATCC 12228	Rich medium agar	
	Rich medium agar + Polymyxin B	
<i>S. aureus</i> ATCC 29213	Rich medium agar	
	Rich medium agar + Polymyxin B	

**Figure 1:** The spot-appearances of *S. epidermidis* and *S. aureus* on Rich medium agar and selective Rich medium agar + Polymyxin B agar plates. The clustered *S. epidermidis* and *S. aureus* grew well on rich medium agar. When Polymyxin B (100 µg/ml) was added to the agar, *S. aureus* still occurred while *S. epidermidis* disappeared. Rich medium agar + Polymyxin B (100 µg/ml) was selected as *S. aureus* selective agar.

## The fermented VCO preparation

*S. epidermidis* fermenting VCO supernatant was prepared by inoculating *S. epidermidis* ( $10^6$  CFU/ml) into a rich medium-plus VCO (2.5% v/v) (RCS) and then incubated at 37°C, shaking 200 rpm for five days. Rich medium (R) and rich medium-plus VCO (2.5% v/v) (RC) were prepared with the same manners as control. Subsequently, the supernatant was collected after five days of incubation by centrifuged at 4500 rpm for 15 min and filtered with 0.22  $\mu$ m syringe filters, then kept at 4°C.

## Antimicrobial effect

Antimicrobial effect protocols according to the Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure of the American Society for Testing and Materials (ASTM, 2016) were selected by inoculation *S. aureus* ( $10^8$  CFU/ml) 0.1 ml into 10 ml of the supernatants, then observed the *S. aureus* growth on TSA plates at 6, 12, 18, and 24 hrs. The supernatant was replaced by 0.9% peptone saline (Merck, MA, USA) for *S. aureus* baseline enumeration.

## Statistical analysis






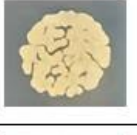
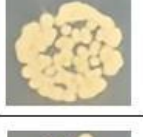

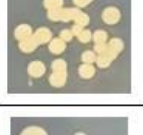



The comparison in co-culture assays was evaluated using an independent t-test. The efficiency of the supernatant in antimicrobial effect was evaluated by one-way ANOVA followed by Scheffe's post hoc test. The p value < 0.05(\*) was accepted to be significant.

## RESULTS

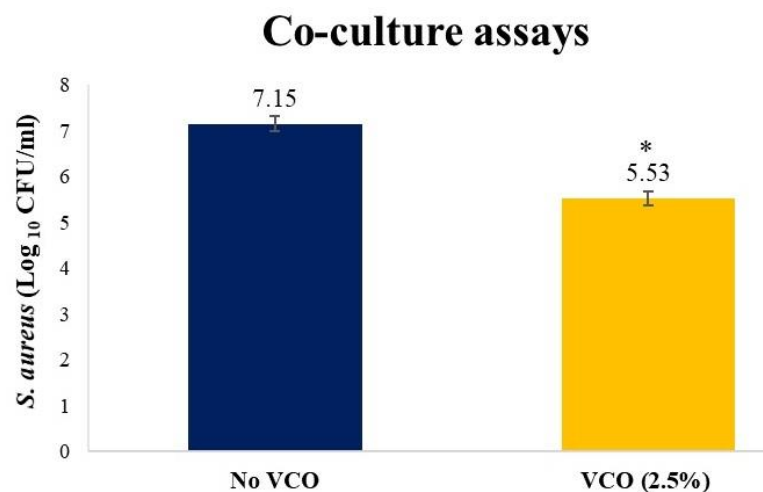
### Co-culture assays

*S. aureus* plus *S. epidermidis* were cultured in a Rich medium composed with or without 2.5% of VCO (v/v). Then the killing effect of fermented VCO on *S. aureus* was determined. The co-cultures, after five days, were diluted to  $10^{-1}$ - $10^{-5}$  then spot (10  $\mu$ l) on *S. aureus* selective agar [rich medium agar plus Polymyxin B (100  $\mu$ g/ml)] (Finegold & Sweeney, 1961). The number of *S. aureus* in co-culture plus VCO (2.5% v/v) [ $(3.5 \pm 1.0) \times 10^5$  CFU/ml] was significantly decreased when compared with the VCO-absent medium [ $(1.5 \pm 0.6) \times 10^7$  CFU/ml] (Figure 2). The result suggested that the fermented VCO can interfere with the growth of *S. aureus*.

A

Dilutions	No VCO	VCO (2.5%)
1		
$10^{-1}$		
$10^{-2}$		
$10^{-3}$		
$10^{-4}$		
$10^{-5}$		

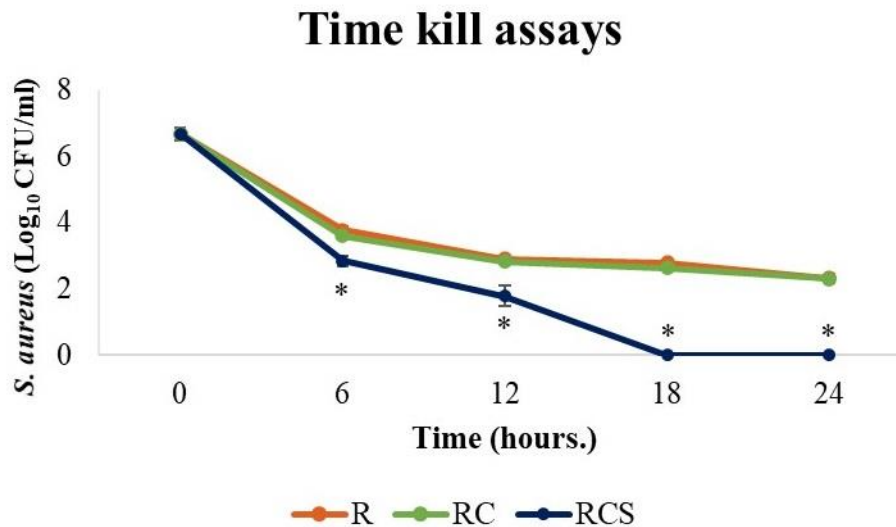
B



**Figure 2:** Effect of fermented VCO in rich medium on suppression of *S. aureus*, under the co-cultured condition of *S. epidermidis* and *S. aureus*. Overnight cultures of *S. aureus* on the selective agar plates were counted (A) and exhibited in Log<sub>10</sub> CFU/ml (B). The number of *S. aureus* in co-culture consisting of VCO (2.5% v/v) decreased compared with the VCO absent medium. Data are the mean  $\pm$  SD. Statistical analysis were evaluated by an independent t-test, and p value < 0.05(\*) showed significance.

## Antimicrobial effect

To indicate the effects of *S. epidermidis* fermented medium, *S. aureus* ( $10^8$  CFU/ml) 0.1 ml was inoculated into 10 ml of the supernatants (R, RC, and RCS) and observed the growth of *S. aureus* from 6, 12, 18, and 24 hrs. At the same time, peptone saline (0.9%) was used for enumerated *S. aureus* at initiation (0 h). The time-kill kinetics result showed the suppression of *S. aureus* with all supernatants, but RCS presented the best result with ultimately killing *S. aureus* in the medium after 18 hours of fermentation (Figure 3). The results indicated that the fermented VCO by *S. epidermidis* possessed an antimicrobial effect on *S. aureus*



**Figure 3:** The trend of *S. aureus* suppression of the supernatants during 0 to 24 hours. R: supernatant of Rich medium only, RC: supernatant of Rich medium-plus VCO (2.5% v/v), and RCS: supernatant of Rich medium-plus VCO (2.5% v/v) plus *S. epidermidis* ( $10^6$  CFU/ml). The RCS supernatant showed an efficient antimicrobial activity against *S. aureus* after 18 hours of incubation. Data are the mean  $\pm$  SD. Statistical analysis was evaluated by one-way ANOVA followed by Scheffe's post hoc test, and p value  $< 0.05$  (\*) showed significance.

## DISCUSSION

*S. aureus* is a pathogen that causes a wide range of disorders and is problematic with antibacterial resistance (Liu, 2009). Several studies revealed the alternative of *S. aureus* infection treatments with natural substances, which are sources of enormous phytochemicals (Kali, 2015). In this study, VCO was selected for bacterial fermentation; according to previous reports, antimicrobial activities were improved after fermentation by *Geotrichum candidum* (Khoramnia et al., 2013). VCO comprises several FAs, such as lauric acids, myristic acids, caprylic acids, and capric acids (Moore et al., 2020), which possess antibacterial activity against *S. aureus* (Watanabe et al., 2019). Our results demonstrated the prominent killing effect of fermented VCO by *S. epidermidis* on *S. aureus*, suggesting that *S. epidermidis* probably induced VCO fermentation to produce antimicrobial activity. The active substances and mechanisms of activity should be clarified in the future.

## CONCLUSION

The fermented VCO by *S. epidermidis* performs the suppression of *S. aureus* growth which is a probable effect of the increasing of MCFAs from by lipolytic activity of *S. epidermidis*.

## AUTHOR CONTRIBUTIONS

Jaturong Pratuangdejkul and Veena Satitpatipan were responsible for designing the experiments and approved the final manuscript. Lalita Mahaklan performed the experiments and drafted the manuscript.

## ETHICS APPROVAL

Not applicable.

## FUNDING

The direct financial support for this work was the 60<sup>th</sup> Year Supreme Reign of His Majesty King Bhumibol Adulyadej Scholarship for Academic Year 2018 from the Faculty of Graduate Studies, Mahidol University.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work.

## ACKNOWLEDGEMENTS

The direct financial support of this work was the 60<sup>th</sup> Year Supreme Reign of His Majesty King Bhumibol Adulyadej Scholarship for Academic Year 2018 from the Faculty of Graduate Studies, Mahidol University. We would like to thank all our colleagues for consulting and providing all facilities, including Assoc. Prof. Waree Limwikrant and Assist. Prof. Nattawut Charoenthai from the Department of Manufacturing Pharmacy, Mahidol University, provided VCO and polymyxin B powder.

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**Citation:**

Mahaklan, L., Pratuangdejkul, J., & Satitpatipan, V. (2022). Antimicrobial effect of coconut oil on *Staphylococcus aureus*: an implication of *Staphylococcus epidermidis* induced fermentation. *Life Sciences, Medicine and Biomedicine*, 6(1).  
<https://doi.org/10.28916/lsm6.1.2022.100>



**Life Sciences, Medicine and Biomedicine**  
ISSN: 2600-7207

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