The Study of Reducing The Amount of Diacetyl Production by Skin Bacteria; *Staphylococcus aureus* with the Metabolite Substance Obtained from *Bacillus subtilis.*

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Introduction

Body odor is an unpleasant odor caused by the metabolism of *Staphylococcus aureus* on the body's skin and sweat, resulting in the production of diacetyl, a key chemical compound of this odor. Currently, there are two primary types of odor control product; deodorant which contain antibiotic substance and antiperspirant which has ability to clogged skin's pores. These products might caused an irritation from clogged the sweat pore, an increase in antibiotic resistance, and a disruption of microbiome balance. This study aims to use *Bacillus subtilis* metabolites to reduce the diacetyl produced by *Staphylococcus aureus*, a bacterial flora on the skin, to create a product that reduces odor without relying on antimicrobial agents or clogging pores materials.

Objectives

- To study the differences in density and types of bacteria on the skin between daily routines of volunteers
- To study ability of reducing diacetyl by *Bacillus subtilis* metabolite

Methodology

1 Odor Producing Factors
- Bacteria Isolation
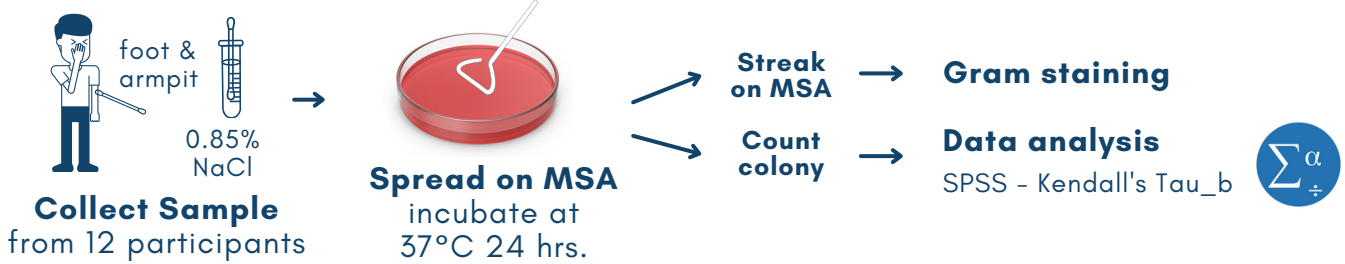
1. IRB Submission
– Exemption Review

2. Data Collection - google form

- gender
- BMI
- exercise
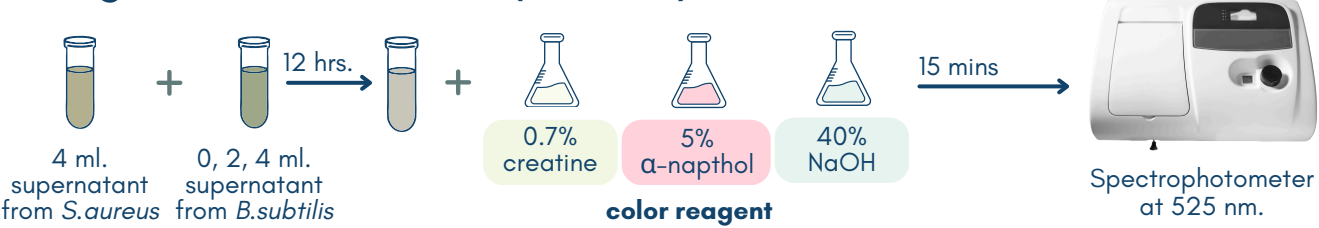- shower frequency
- deodorant usage

3. Bacteria Isolation

foot & armpit → 0.85% NaCl → Spread on MSA incubate at 37°C 24 hrs. →
- Streak on MSA → **Gram staining**
- Count colony → **Data analysis** SPSS - Kendall's Tau_b $\sum_{s}^{\alpha}$

Collect Sample from 12 participants

2 Detect Diacetyl
- test *B. subtilis* ability to reduce Diacetyl

1. Metabolite Seperation

Incubate Bacteria
- *S. aureus* at 37°C 24 hrs.
- *B. subtilis* at 37°C 24, 48, 72 hrs.

Sonicate → **Centrifuges** → Metabolite / Bacteria

2. Voges-Proskauer test (VP test) - 3 ratio

4 ml. supernatant from *S.aureus* + 0, 2, 4 ml. supernatant from *B.subtilis* → 12 hrs. + 0.7% creatine / 5% α-napthol / 40% NaOH → 15 mins → Spectrophotometer at 525 nm.
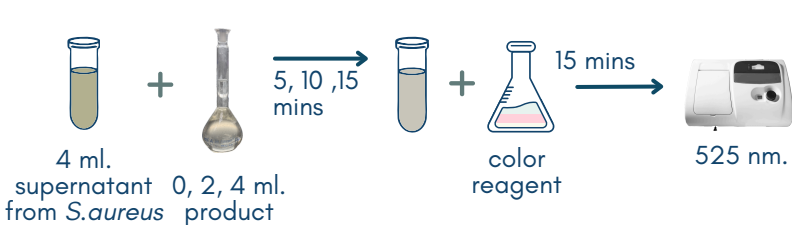
color reagent

3 Product Deve
- test product ability to reduce Diacetyl

1. Mixed Product
- Metabolite of *B.subtilis* 25%
- Organic aloe vera 15%
- Bamboo charcoal 10%
- Butylene glycol 4%
- Peppermint essential oils 0.5%
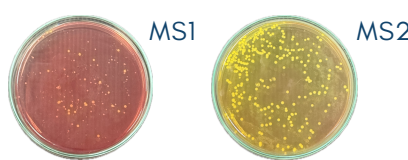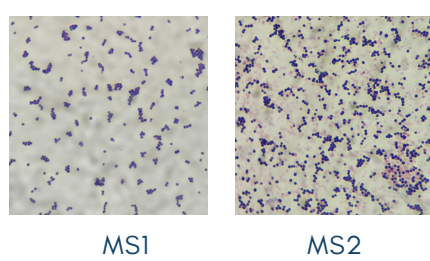- Potassium sorbate 0.5 %

2. VP test

4 ml. supernatant from *S.aureus* + 0, 2, 4 ml. product → 5, 10 ,15 mins + color reagent → 15 mins → 525 nm.

Results

1 Odor Producing Factors

Spread plate
- 99% : *S. aureus*
- 01 % : *S. epidermidis*

MS1   MS2

Gram staining

MS1   MS2

Discussion
Most bacteria found on skin was likely to be *S. aureus*

Data analysis

			Body Mass Index	Exercise frequency	Frequency of roll-on use	Exercise duration	Bathing frequency	Amount of *S.aureus* on armpit	Amount of *S.aureus* on foot
Kendall's tau_b	Bacteria isolated from armpit	Correlation Coefficient	-.194	-.276	.175	-.506*	-.336	1.000	.477*
		Sig. (2-tailed)	.447	.274	.488	.039	.192	.	.036
		N	12	12	12	12	12	12	12
	Bacteria isolated from foot	Correlation Coefficient	.041	-.061	.000	-.707**	-.069	.477*	1.000
		Sig. (2-tailed)	.868	.804	1.000	.003	.784	.036	.
		N	12	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 1 : correlation between amount of colony counted from bacterial iolated from volunteer and dialy routine

Discussion
- Exercise duration was inversely related to the amount of bacteria in armpit and foot area.
- A significant relationship was found between bacteria in the foot and armpit areas.
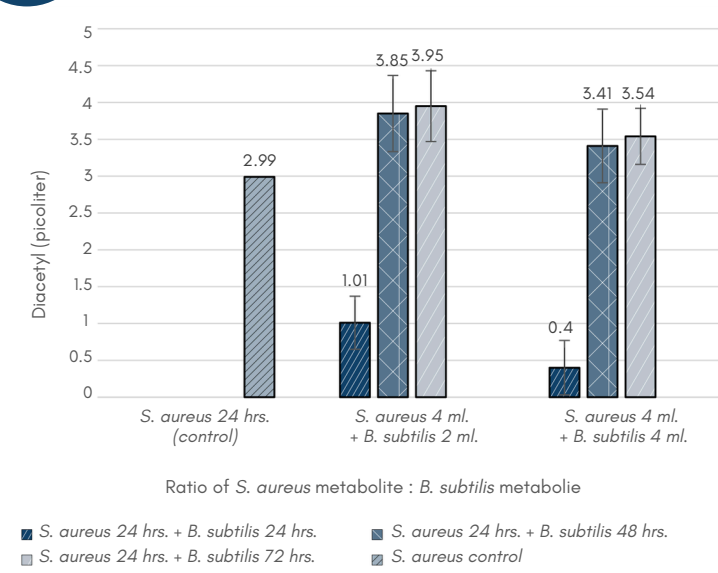
2 Diacetyl Detection

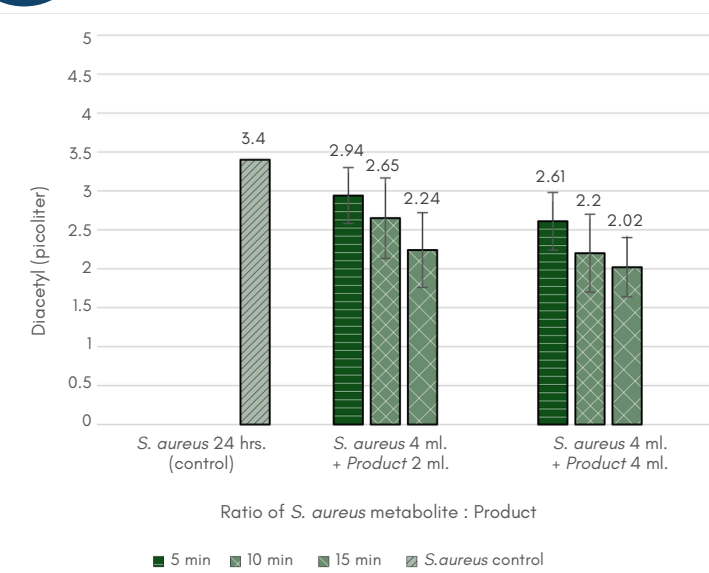Figure 1 : Diacetyl detection tested B. subtilis metabolite ability to reduce diacetyl

- *S. aureus* 24 hrs. + B. subtilis 24 hrs.
- *S. aureus* 24 hrs. + B. subtilis 72 hrs.
- *S. aureus* 24 hrs. + B. subtilis 48 hrs.
- *S. aureus* control

3 Product Development

Figure 2 : Diacetyl detection tested product ability to reduce diacetyl

- 5 min
- 10 min
- 15 min
- *S.aureus* control

Discussion
- Only *B. subtilis* metabolite incubated 24 hours had ability to reduce diacetyl.
  - highest BDH production 24-36 hrs.
  - diacetyl production continued increase
- Best ratio is metabolite from *S. aureus* 4 ml. : *B. subtilis* 2 ml.
  - limited NADH available

Conclussion

The resident skin microbiota was identified as *S. aureus*. The Exercise duration showed significant correlation with the density of *S. aureus* in both axillary and planta regions. The 24 hours incubated *B. subtilis* have the greatest ability to reduce diacetyl. The product was developed by using the 24 hours incubated *B. subtills* metabolite and was able to retain the metabolite ability.

Reference :
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3. Nicholson, W.L., 2008, "The Bacillus subtilis ydjL(bdhA) Gene Encodes Acetoin Reductase/2,3-Butanediol Dehydrogenase", Applied and Environmental Microbiology[Electronic], vol. 74, No. 22, pp. 6832-6838, Available : AMERICAN SOCIETY FOR MICROBIOLOGY, [2025, 23 February].
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