

# *Clitoria ternatea* Linn Extract as Natural pH Indicator in Mannitol Salt Agar Medium

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**Abstract-Objectives:** Mannitol Salt Agar (MSA) medium used to distinguish mannitol fermented ability among *Staphylococcus* species. The ability to ferment mannitol visualized by color changing in agar medium. Fenol red is color indicator usually used in standard MSA medium which has ability to turn from red to yellow in acid pH. This study aim is to modify the MSA medium using *Clitoria ternatea* Linn flower extract as natural pH indicator. The flower dissolved in boiled distilled water and filtrated. Modified MSA made by 10% of *C. ternatea* Linn flower extract content. *Staphylococcus aureus* and *Staphylococcus epidermidis* were used as tested bacteria. The result showed the color changing in modified MSA agar medium from blue to purple in medium which inoculated by *S. aureus*, but not showed color changing in medium which inoculated by *S. epidermidis*. This result is appropriate with the standard medium, which the *S. aureus* has ability to ferment mannitol causing the color changing in MSA medium. The conclusion of this study is the modified MSA agar medium that contain *C. ternatea* Linn flower extract has ability to distinguish mannitol fermented bacteria and can used as MSA alternative formula.

**Keywords:** *Clitoria ternatea* Linn, pH indicator, bacteria differentiation medium

## I. INTRODUCTION

Mannitol Salt Agar (MSA) is one of differential medium which differentiate between mannitol fermented and mannitol nonfermented bacteria. Bacterial fermented ability usually showed by yielding different color in the medium. That color changing is indicator of pH shift caused by acid compound as fermentation product. In several study proved that natural indicator can prepared as pH indicator. *C. ternatea* flower extract can used as natural indicator in acid-base titration [1]; as colorimetric bio-indicator [2]; and as natural dye on animal blood smear staining [3]. Intend to develop these advantages, *C. ternatea* flower extract applies as pH indicator in MSA bacterial differential medium. MSA medium composition

usually use fenol red as pH indicator which can shift the color change from red orange (in alkali pH) to yellow (in acid pH). *C. ternatea* flower extract also can shift the color change in different pH, gradually from dark blue (in alkali pH) to pink (in acid pH). In clinical laboratory MSA used to distinguish between important *Staphylococcus* species such as *S. aureus*, *S. epidermidis* and *S. saprophyticus*. The ability of mannitol fermentation of these bacteria differentiated by color changing in MSA medium. *S. aureus* has ability to ferment the mannitol and produce some acid as the fermentation product, but the other *Staphylococcus* has no ability to ferment. This study aim is to apply *C. ternatea* flower extract as natural indicator in modified MSA. *S. aureus* and *S. epidermidis* used as test bacteria to prove the stability of the pH indicator in MSA differential medium.

## II. MATERIAL AND METHOD

### A. Procedure

Dried flower (75 pieces) of *Clitoria ternatea* was dissolved in 100 mL distilled water and boiled until the color infused in the water then filtered by filter paper. Modified MSA made by added 10% of *Clitoria ternatea* extract then autoclaved at 15 lb pressure (121 °C) for 15 minutes. Anthocyanin was characterized by spectrophotometric and Thin Layer Chromatography (TLC). Tested bacteria inoculated in the medium, medium incubated at 37°C in 24 hour, the result was observed.

### B. Data Analysis

The result of this test observed by the color change of modified MSA.

### III. RESULT

Modified MSA showed different color appearance between two test bacteria. This result is appropriate with the ability of each bacteria which *S. aureus* has ability to ferment mannitol while *S. epidermidis* has no ability to ferment mannitol.

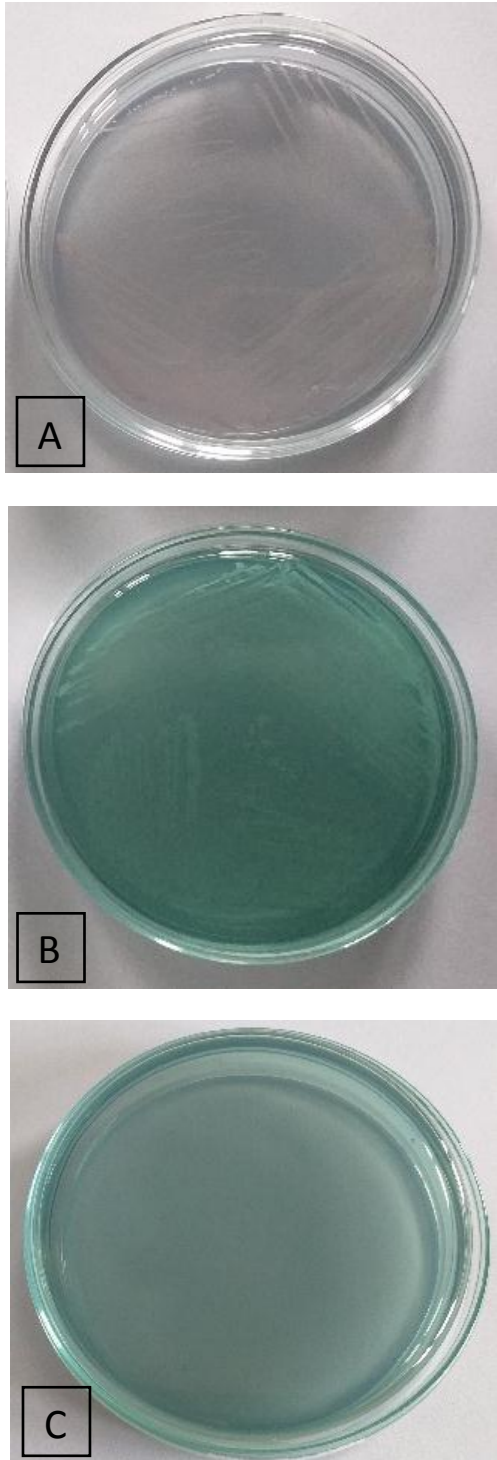


Figure 1. Mannitol fermentation ability of tested bacteria proved by different color appearance in

modified MSA medium. Which *S. aureus* has ability to ferment mannitol by showed color shift to purple [A], while *S. epidermidis* showed no color change (blue) as proved no ability to ferment mannitol [B]. Control showed no change in color showed blue color [C].

### IV. DISCUSSION

*C. ternatea* flower extract used as natural indicator in many research fields. The compound which role as pH indicator was identified and measured by TLC using BAW (4: 1: 5) eluent. Observation using visible light source showed orange red spot on silica gel plate while observation using UV showed fluorescent yellow. The RF value was 0,31 and identified as Pelargonidin 3,5 diglucoside [4]. Spectrophotometric wave length value was 285 and identified as anthocyanin (Pelargonidin 3,5-GG(+coumaric acid)) [5].

*S. aureus* key enzyme in mannitol metabolism is mannitol-1-phosphate dehydrogenase (MIPDH), but its pathophysiological roles has not been established [6]. Mannitol fermentation can produce acid in *S. aureus* and discriminate it from other members of the genus [7]. Meanwhile *S. epidermidis* has no enzyme to ferment mannitol. MSA agar medium which contain mannitol usually used to test the presence of MIPDH in microorganism. Fermentation product of the bacteria such as acids released into the medium and lower its pH. *C. ternatea* as the pH indicator which contained in the medium will change medium color from blue to purple.

### V. CONCLUSION

The conclusion is *C. ternatea* has ability and stability as pH indicator in MSA as differentiation medium between *S. aureus* and other Staphylococcus species such as *S. epidermidis*. The modified MSA which contain *C. ternatea* aqueous extract can distinguish between MIPDH produced bacteria and MIPDH non produced bacteria. The ability differentiated by medium color changing from blue to purple.

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