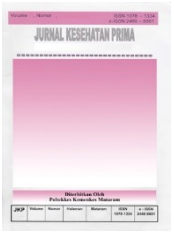
Jurnal Kesehatan Prima

http://jkp.poltekkes-mataram.ac.id/index.php/home/index

p-ISSN: 1978-1334 (Print); e-ISSN: 2460-8661 (Online



Optimal concentration of *Syzygium aromaticum* powder added to Anchovy TURLM Medium to Prevent The Degradation Shape of *Mycobacterium tuberculosis* Cells

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Received: 02 January 2022/Accepted:23 February 2022/Published Online: 23 February 2022

Abstract
Alternative clinical diagnostic media for *Mycobacterium tuberculosis* (MTB) include Anchovy Sweet Seaweed Medium (TURLM). This media reported has good sensitivity and specificity, as well as growth speed, colony count, and fertility rates for MTB examination. In this study, six different clove flower powder concentrations were added to the TURLM media (*Syzygium aromaticum*). The goal of this study was to look at the vegetative shape of MTB bacteria in six different TURLM variations. This study was conducted at Microbiology Laboratory of the Medical Laboratory Technician Department at Politeknik Kesehatan Kementerian Kesehatan in Mataram, Indonesia. These findings revealed that complete MTB vegetative cells were mostly found in TURLM media with the addition of 0.5 g clove flower powder

Keywords: Mycobacterium tuberculosis,;Alternative media; Vegetative form; TURLM

INTRODUCTION

Mycobacterium tuberculosis (MTB) caused Tuberculosis (TB). MTB has resurfaced in the 21st century as a big problem with high morbidity. Due to the difficulty in disease management and treatment, as well as the massive reservoir the pathogen maintains in human populations through latent infection, tuberculosis is a major public health concern. (D'Ambrosio et al., 2021; Matteelli et al., 2017; Sharma et al., 2012). World Health Organization (WHO) reported total TB incidence in 2020 were 824.000 cases (WHO, 2021). This disease has a significant economic impact on livestock production because of the costs of testing and slaughtering infected animals. (Cowie et al., 2015; Gortazar et al., 2015; Meiring et al., 2018; Poirier et al., 2019).

More than 140 species of *Mycobacterium* exist, which are divided into three groups: MTB *complex* (MTBC), *M. leprae,* and *mycobacteria* other than MTB and *M. leprae*, collectively known as *Non-tuberculous mycobacteria* (NTM). The most well-known member of the MTBC is *Mycobacterium tuberculosis* (MTB), an obligate human pathogen and the causative agent of tuberculosis (TB), which is still one of the world's most serious public health issues (Van Ingen, 2013).

The etiological agent of tuberculosis is MTB, a slow-growing bacterium. Agar-based and egg-based media incorporating green malachite and Middlebrook broths or solid media are the "gold standard" for isolation, culture, and definite diagnosis of MTB. (Drancourt et al., 2003). Acid-fast bacilli microscopy with Ziehl-Neelsen (ZN) staining has been evaluated as the simplest and fastest diagnostic method for MTB

laboratory diagnosis, but it has low sensitivity and specificity and requires 5,000-10,000 bacilli per milliliter. Reproduction in culture media is the "gold standard" method for identifying mycobacteria, and it can be 500 times more sensitive than ZN staining. However, it is time-consuming and difficult, and it necessitates additional biochemical tests. Alternative media have been developed in response to the need for methods that are faster, easier to use, and have a high sensitivity and specificity rate (Kurtoglu et al., 2014; Ninet et al., 1999).

Rohmi et al. (2021) reported that Anchovy Sweet Seaweed Medium (TURLM) media can be used as an alternative clinical diagnostic media for MTBC. TURLM has sensitivity and specificity, growth speed, number of colonies, and fertility rates for good MTB growth, but the weakness of this formula 3 of TURLM is that there is still fungal contamination in both control and test media, so data are needed to test the media's stability and resistance in storage, especially in the case of MTB growth. The specific problem occurs also about the shape of the MTB bacteria. Some of the MTB bacteria reported has degraded into fragment MTBC. This will cause ambiguous results for some case like MTBC-drug resistant. This study aimed to determine the optimal concentration of clove flower powder added to TURLM medium to prevent the degradation of MTB bacteria shape.

METHOD

This was an experimental study conducted in Microbiology Laboratory of Medical Laboratory Technician Department in Politeknik Kesehatan Kementerian Kesehatan, Mataram, Indonesia. This study was using an alternative media for MTB growth, the Anchovy Sweet Seaweed Medium a.k.a. TURLM. This media contains basic ingredients of anchovy flour, purple sweet potato and seaweed. The formula were 50.1 grams of sea anchovy flour, 45 grams purple sweet potato flour, 4.5 grams of seaweed flour, 15 grams of agar – agar, 12 ml of glycerol, 100 ml of egg homogenization, and 1000 ml of aquadest (Rohmi and Diarti, 2017). The TURLM media were prepared for the study with the add of clove flowers powder in 6 variations (0,5g, 1 g, 2 g, 3 g, 4 g, and 5 g).

Figure 1. TURLM Media with variation of clove flower powder

A total of 30 sputum samples with positive MTB results were accommodated. Vortex was used to mix the samples. 4% NaOH was added to the sputum, stirred for 15 minutes in an upright position. Vortex was homogenized for 15 minutes. The supernatant was discarded after 15 minutes of rotation in a 3000-rpm centrifuge. The solution was neutralized with buffer phosphate (pH six point eight) before being centrifuged at 3000 rpm for 15 minutes and the supernatant discarded. This procedure was repeated three times, with the last round of supernatant being discarded and the pH of the sediment being checked using pH indicator paper (pH is expected to be neutral 7)

Positive smear sputum was inoculated using 0.1 ml inoculum wire into the media tube. Formula for variations in the content of clove powder was added to TURLM and Loweinstein – Jensen as a control. From the first week of incubation until the end of the eighth week, all culture tubes were incubated at 37°C and observed daily. Observations were made by looking at the growth of the colony. Microscopic examination

with ZN staining was used to confirm MTB bacteria

RESULT AND DISCUSSION

The number of complete forms of MTB vegetative cells was determined using the slide calculation method, which involved making preparations on a 1 cm^2 glass object and staining with Ziehl-Neelsen (ZN) staining. The calculation is done by counting the number of broken or incomplete vegetative cells (fragmented) and complete vegetative cells in a 1 cm^2 area.

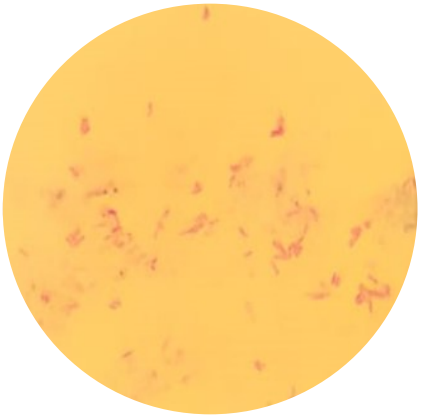

Figure 2. Form of MTBC vegetative cells in

TURLM media with the addition of clove flower powder

There was a difference in the average number of vegetative cells from MTB on TURLM media of clove flower powder with a concentration variation of 0.5 g (Mean=39.27; SD=2.32), 1 g (Mean=16.93; SD=2.30), 2 g (Mean=14.07; SD=2.20), 3 g (Mean=11.03; SD=1.45), 4 g (Mean=0.00; SD=0.00), and 5 g (Mean=0.00; SD=0.00). These results showed that complete form of vegetative cells from MTB was mostly found in TURLM media with the addition of clove flower powder of 0.5 g, while in the group with a concentration of 4 and 5 g there was no complete form of vegetative cells from MTB. The mean difference in the number of vegetative cell forms from MTB on TURLM media with variations in the addition of clove flower powder (0.5 g, 1 g, 2 g, 3 g, 4 g, and 5 g) was reported to be different and statistically significant. ($p<0.001$).

Table 1. Kruskal-Wallis analysis of the number of complete vegetative cells of MTB colonies in TURLM media with the addition of clove flower powder.

The concentration of clove flower powder	Number of complete vegetative cells of MTB colonies		
	Mean	SD	p-value
0.5 g	39.27	2.32	<0.001
1 g	16.93	2.30	
2 g	14.07	2.20	
3 g	11.03	1.45	
4 g	0.00	0.00	
5 g	0.00	0.00	

According to the findings, the higher the dose of clove flower added to TURLM media, the more MTB vegetative cells degraded into fragmented MTB, which is shaped rods, thin, straight or slightly bent, granular, and not sheathed and has a size of 0.5-4 x 0.3-0.6. These bacteria have a unique nature in that they can withstand acid and alcohol color washing, earning them the moniker "acid-fast bacteria" (MBT). Furthermore, these bacteria are resistant to dry and cold environments. These bacteria can survive for months in the house or in a humid and dark environment, but they are not resistant to light, sun, or airflow and will die if exposed to them. (Widoyono, 2011).

Mycobacterium leprae (M. leprae) that is solid or intact is considered a living bacterium, while fragmented or nonsolid is considered a dead bacterium, according to a literature report. *M. leprae* can live for 2 to 9 days outside the body (Robertuji,

2004). The higher the dose of clove flower used, the more the vegetative form is degraded into the fragmented form. This cannot be used directly to justify or generalize the findings so that further research is needed regarding the degradation or changes experienced in the form of Fragmented MTB bacteria at higher clove flower concentrations.

CONCLUSION

The higher the dose of clove seeds added to TURLM media, the greater the number of MTB vegetative cells that were degraded to form fragmented MTB.

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