1. INTRODUCTION

1.1. Background

The crisis of antibacterial resistance is an inevitable consequence of the commercial usage of antibacterial agents in human health-related settings and agriculture. This crisis is exacerbated by the misuse and inappropriate disposal of antibacterial which allows Darwinian selection for bacteria with resistance strategies against antibacterial (Amábile-Cuevas, 2013). One of the oldest strategies to combat this crisis is to screen for novel antibacterial compounds with novel drug classes or structures from natural isolates. This method has led to discoveries of antibacterial compounds, many of which are isolated from terrestrial/soil *Actinomyces*/*Streptomyces* bacteria (Bérdy, 2005; Barka et al., 2016). However, the frequent terrestrial *Actinomyces* screening have led to a decrease of number in identified novel structures as seen in its absence from screening attempts in recent years (Ibnouf et al., 2022, Kumari et al., 2021). *Actinomyces* ability to produce many antibiotic compounds with variation drug classes and mechanisms of action are mediated by their biosynthetic gene clusters which will be expressed into an enzyme complex that is capable of performing multiple chemical modifications (Belknap et al., 2020). Commercial antibiotics that have been isolated from *actinomycetes* include vancomycin, streptomycin, carbapenem, erythromycin, kanamycin, and neomycin (Quinn et al., 2020).

Overuse of terrestrial samples as antimicrobial screening targets and its increasing difficulty to isolate a novel antimicrobial drug class has led to a shift in screening trends of antibiotic screening from terrestrial to marine samples (Kashfi et al., 2020). *Nudibranch*, an ordo of sea slug, has been less studied for its associated microbe ability to produce novel antibacterial compared to popular marine screening samples such as sediments and sea sponges (Kashfi et al., 2020; Anteneh et al., 2021). *Nudibranch* bacterial community contains vertically transferred bacteria that cannot be found in sea waters nor sediment, its mantle collects planktonic *actinomyces* which has also been studied minimally, and its diet which consists of sponges may have picked up some of the sponge-associated bacteria. Nudibranch is also known to be able to deposit antimicrobial compounds obtained from its diet and possibly associate microbes to its mantle (Böhringer et al., 2017). It has also been reported that in one case actinobacteria genus constitutes 10% of the total *nudibranch* bacterial community (Stuij et al., 2023; Jagannathan et al., 2021; Bérdy, 2005).

In this thesis, *nudibranch* glycerol preserve (*Phylidia varicosa*) was screened for its associate bacteria using gram-positive selective mediums. All culturable bacteria/ isolates are tested for its metabolite's antibacterial property towards test pathogens which are *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Propionibacterium acne* (ATCC 6919), *Streptococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Mycobacterium smegmatis*, and *Pseudomonas aeruginosa* (ATCC 27853). All pathogens used are either a surrogate model or an actual clinically significant human pathogen. After the isolate exhibiting antibacterial activity to test pathogens is identified in the agar plug assay, downstream experiments will be conducted to extract and evaluate the activity of the antibacterial compound (Balouiri et al., 2016).

Extraction of the antimicrobial metabolite was done by ethyl acetate extraction on the bacterial supernatant (Ambarwati et al., 2020). Ethyl acetate is a slightly nonpolar semi-polar solvent that had been used for extraction of antimicrobial metabolites, it has the capability of dissolving most number of compound compared to other solvent system such as n-hexane and chloroform, which is

suitable with the experimental setting as it is in our best interest to isolate as many compounds as possible during the extraction process since there is no preliminary information on the antimicrobial compound polarity that will be isolated nor we targeted antibiotic with specific polarity (Bachtiar et al., 2020).

1.2. Research question

The Research questions in this report are as follows:

1. Are there culturable *Actinomyces* bacteria from *nudibranch* isolate
2. Does the cultured *Actinomyces* possess antimicrobial metabolites
3. Is it possible for the antimicrobial metabolites to be isolated with ethyl acetate extraction

1.3. Research aims

This report aims to screen for antimicrobial-producing microbes with selective conditions for *Actinomycetes* from nudibranch isolate followed by isolating and measuring the activity of the antimicrobial compound it produces.

1.4. Hypothesis

H0*:* *Nudibranch* does not host antimicrobial-producing bacteria belonging to the actinomyces group.

H1: *Nudibranch* hosts antimicrobial-producing microorganisms belonging to the *actinomycetes/ Streptomyces* group.

H0: Subsequent extraction of the microbe metabolites would fail to isolate the antibacterial compound

H1: Subsequent extraction of the microbe metabolites would allow isolation of the antibacterial compound.