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Abstract. The aim of this study was to examine the effect of different media and solidifying agents as growth media of a rare thermophilic actinobacterium, strain *Microbispora rosea* subsp. *rosea* SL3-2-R-1, on its antibacterial activity. To investigate the antibacterial potential, the strain was grown on four media, e.g., International *Streptomyces* Project (ISP) 1, ISP 2, ISP 3, and Bennett's medium, solidified with agar and gellan gum, at 45 °C for 21-days. The antibacterial activity screening was performed using the agar plug diffusion method against four bacterial test strains (*S. aureus*, *B. subtilis*, *K. rhizophila*, and *E. coli*) grown on Mueller-Hinton agar, incubated at 30 °C for 17 h. The antibacterial activity was observed on strain grown on ISP 1 gellan gum, ISP 2 gellan gum, ISP 3 agar, ISP 3 gellan gum, and Bennett's agar against *B. subtilis*. The strain grown on ISP 1 gellan gum, ISP 3 agar, and ISP 3 gellan gum inhibited the growth of *K. rhizophila*, while on ISP 1, gellan gum and ISP 3 agar were positive against *S. aureus*. However, on all media, the strain showed no inhibition against *E. coli*.

1. Introduction

Natural products persist in being reliable sources of novel bioactive compounds potent for antimicrobial agents to counter the rising number of antibiotic-resistant pathogenic microorganisms. Currently, a rigorous attempt to find new secondary metabolites with novel chemical structures was done by exploring natural extreme temperature environments to isolate and investigate rare and novel taxa of thermophilic *Actinobacteria* [1]. Thermophilic *Actinobacteria*, especially filamentous actinomycetes group, were known for their pharmaceutically and industrially significant parts as producers of important bioactive compounds ranging from antibiotics, anti-inflammatory compounds, immunostimulants, immunosuppressors, enzyme inhibitors, to clinically and highly thermostable enzymes [2-3]. Rare thermophilic actinomycetes are members of class *Actinobacteria* excluding streptomycete strains, with high-difficulties and low-isolation rate using standard methods and growth at high temperatures ranging

from 40 to 80 °C [1, 2]. Known genera of rare actinomycetes including *Actinomadura*, *Amycolatopsis*, *Saccharomonospora*, *Microbiospora*, *Kutzneria*, *Nocardia*, *Pseudonocardia*, *Saccharopolyspora*, *Saccharothrix*, and many more [1].

Natural environments with extreme temperatures are still rarely explored, thus greater prospect for isolation of novel rare thermophilic actinomycetes taxa, leading to the finding of secondary metabolites with novel structures and potent applications [1, 4]. Diversity and potential applications of thermophilic microorganisms from the Cisolok geothermal area in Sukabumi, West Java, are still rarely reported, hence offering a strong research area. Twenty-five thermophilic *Actinobacteria* isolates were previously obtained from soil samples in Cisolok geothermal area, West Java, Indonesia. Among 25 isolates, 15 belonged to the non-*Streptomycetaceae* family, including strain SL3-2-R-1, which was identified as *Microbispora rosea* subsp. *rosea* [5]. Genus *Microbispora*, the member of the family *Streptosporangiaceae*, was recognized for its roles in providing humus and nutrients for plants through the biodegradation process. It also contributed as a biocontrol agent by producing bioactive compounds against plant pathogens. Other potent secondary metabolites known produced by the member of the genus *Microbispora* are antibiotics, enzyme inhibitors, and antidiabetic compounds [6].

Study regarding the potential of *Microbispora rosea* subsp. *rosea*, isolated from soils of geothermal areas in Indonesia, as bioactive compounds producer has not yet been described. Previously, strain *Microbispora rosea* subsp. *rosea* SL3-2-R-1 was observed for the colony development, substrate mycelium formation, and sporulation on four tested media, e.g., ISP 1, ISP 2, ISP 3, and Bennett's, solidified with agar and gellan gum after incubation for five days [7]. The formation of aerial mycelium and spore in actinomycetes were affected by the availability of sufficient nutrients in growth media and often related to the production of secondary metabolites [8]. This preliminary study examined the potency of *Microbispora rosea* subsp. *rosea* strain SL3-2-R-1 in producing antimicrobial compounds on various tested media and solidifying agents.

2. Methods

2.1. Microorganisms

A rare thermophilic actinobacterium, strain SL3-2-R-1, isolated from forest soil under the Bamboo tree (6°56'00.0"S 106°27'12.8"E) in the geothermal area of Cisolok, West Java, was used in this study. Based on the partial sequence of the 16S rRNA gene and phylogenetic analyses, the strain shared 99.62% rDNA similarity with *Microbispora rosea* subsp. *rosea* ATCC 12950^T [5]. The strain was cultivated and maintained on International *Streptomyces* Project (ISP) 1 medium [9] at 45 °C and room temperature. Long-term preservation was performed using agar blocks in 20% (v/v) glycerol at -80 °C, and as lyophilized cells [10]. Strain SL3-2-R-1 was deposited at the Universitas Indonesia Culture Collection (UICC), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Kampus UI Depok, Indonesia. Test strains including *Kochuria rhizophila* NBRC 12078, *Staphylococcus aureus* NBRC 100910, *Bacillus subtilis* NBRC 13719, and *Escherichia coli* NBRC 3301 from NITE Biological Resource Center (NBRC) Culture Collection, Japan, were used for the antibacterial assay.

2.2. Assessment for antibacterial activity

Strain SL3-2-R-1 was cultivated in four different media including ISP 1, ISP 2, ISP 3 [9], and Bennett's medium [11] solidified both with 2% agar and gellan gum, at 45 °C for 21-days. Pre-culture of tested bacterial strains was prepared in nutrient broth (NB) medium, grown at 30 °C shaking incubator overnight. The agar plug diffusion method [12] was performed for antibacterial assay of strain SL3-2-R-1 using 21-days old cultures. The agar plugs were arranged on Mueller-Hinton agar's surface containing bacterial test strains, then incubated at 30 °C. The inhibition zone was observed and measured (mm) after 17 h of incubation.

3. Results and discussion

Formation of aerial mycelium in strain SL3-2-R-1 was observed in all tested media solidified with gellan gum and ISP 3 agar after five days of incubation [7]. In this study, prolonged incubation of strain SL3-2-R-1 for up to 21-days was conducted on four tested media solidified with agar and gellan gum to ensure aerial mycelium and spore formation. Agar plugs from 21-days incubation were then subjected to screening antibacterial activity towards Gram-positive, *K. rhizophila*, *S. aureus*, *B. subtilis*, and Gram-negative *E. coli* strains. Morphology of strain SL3-2-R-1 on representative media, ISP 1 and ISP 3 media, solidified with agar and gellan gum, is shown in Figure 1. Strain SL3-2-R-1 displayed good growth, and sporulation on media solidified with gellan gum compare to agar.

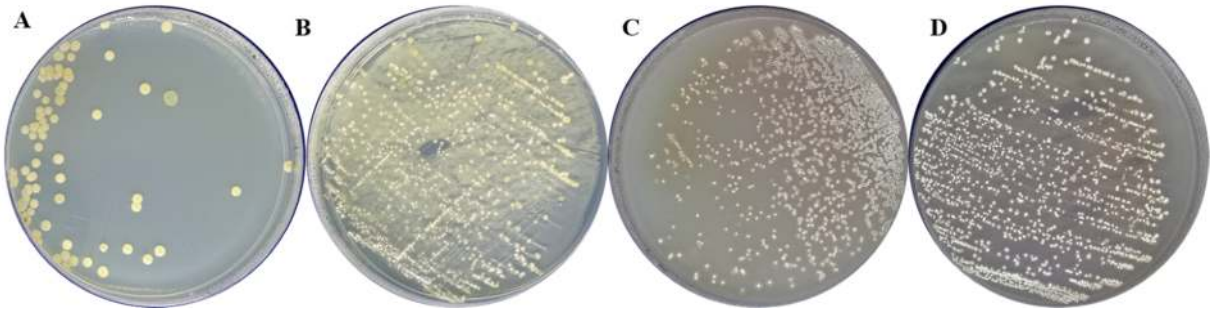

Figure 1. Morphology of strain SL3-2-R-1 cultivated on (A) ISP 1 agar, (B) ISP 1 gellan gum, (C) ISP 3 agar, (D) ISP 3 gellan gum media at 45 °C.

The ability of strain SL3-2-R-1 in exhibiting antibacterial activity is presented in Table 1 and Figure 2. Antibacterial activity was strongly observed on ISP 3 agar with positive results against all tested Gram-positive bacterial strains. Activity against *B. subtilis* was displayed on ISP 1, ISP 2, ISP 3 gellan gum, and Bennett's agar. Inhibition zones were also detected on ISP 1 gellan gum against *S. aureus*. However, an inhibition zone was not observed on all tested media against the Gram-negative *E. coli*. The results showed that inhibition zones' rate against tested bacterial strains varied depending on the medium and solidifying agents used.

Table 1. Antibacterial activity of SL3-2-R-1 cultivated on various media, solidified with agar and gellan gum, against four test strains after 17 h of incubation at 30 °C.

No	Medium	Inhibition zones against test strains (mm)			
		S. aureus NBRC 100910	*B. subtilis* NBRC 13719	*K. rhizophila* NBRC 12078	*E. coli* NBRC 3301
1	ISP 1 agar	-	-	-	-
2	ISP 1 gellan	8.55	20.59	nd	-
3	ISP 2 agar	-	-	-	-
4	ISP 2 gellan	-	9.63	-	-
5	ISP 3 agar	8.78	18.06	10.95	-
6	ISP 3 gellan	-	12.80	nd	-
7	Bennett's agar	-	13.53	-	-
8	Bennett's gellan	-	-	-	-

Notes: (-): no inhibition zone; (nd): not determined

The current study was conducted based on an approach termed 'One Strain-Many Compound (OSMAC) as an attempt to discover potentially new secondary metabolites from the isolated, rare thermophilic actinobacterial strains. This simple method was applied by altering parameters used in strain cultivation, such as media components [13]. Modification of media compositions that affected secondary metabolism production was reported in rare actinomycetes strains, including new sesquiterpenoid producer *Lentzea violacea* AS08 [14] and several *Streptomyces* sp. strains [15]. In this study, we employed standard International *Streptomyces* Project media [9] to designate morphology characteristics, e.g., formation of the substrate and aerial mycelium, spore, colony, and diffusible pigment color [16] for the cultivation of strain SL3-2-R-1. Meanwhile, Bennett's medium was known previously for inducing the aerial mycelia production in actinomycetes [11]. Solidifying agents were also renowned for affecting the formation of aerial mycelia in several rare genera of actinomycetes. Isolation media solidified by gellan gum was previously effective in gaining strains of actinomycetes potent as secondary metabolites producers [17].

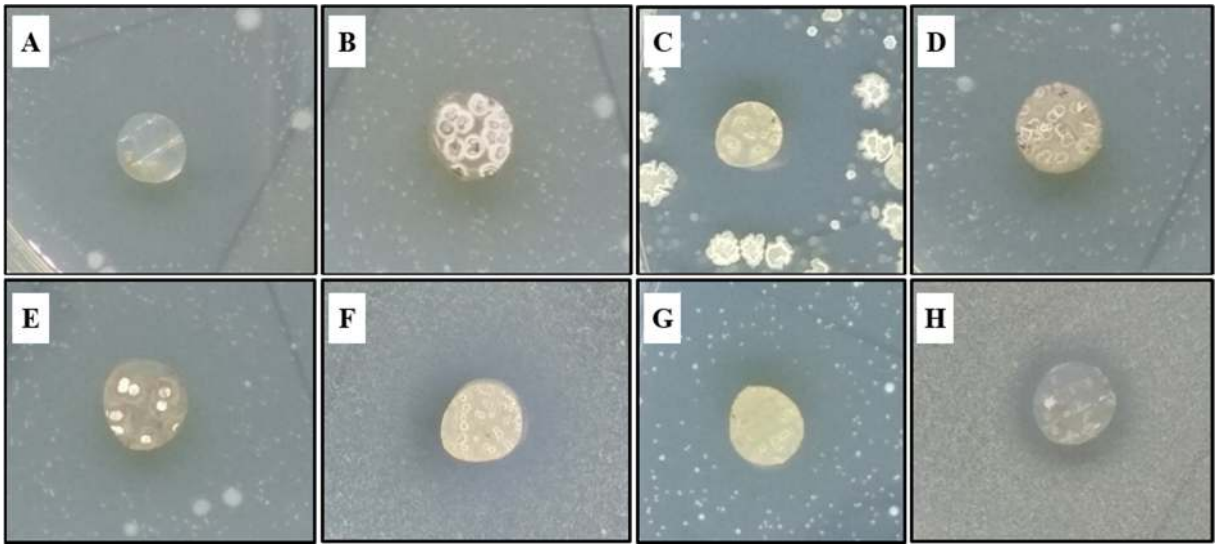

Figure 2. Antibacterial assay of SL3-2-R-1 on different media against *B. subtilis* on (A) ISP 1 gellan gum, (B) ISP 2 gellan gum, (C) ISP 3 agar, (D) ISP 3 gellan gum, (E) Bennett's agar; against *K. rhizophila*; on (F) ISP 3 agar; against *S. aureus* on (G) ISP 3 agar, and (H) ISP 1 gellan gum.

Several members of the genus *Microbispora* are recognized for their secondary metabolites production, including hibarimicin, a signal transduction inhibitor, producer *Microbispora rosea* [18], novel diketopiperazine producer *Microbispora aerata* [19], novel linfuranone A producer *Microbispora* sp. GMKU 363 [20], and novel diterpenoid compound producer *Microbispora hainanensis* CSR-4 [6]. These previous studies demonstrated promising results in exploring bioactive compounds from the genus *Microbispora* to discover novel secondary metabolites.

4. Conclusion

This preliminary study indicated that the strain *Microbispora rosea* subsp. *rosea* SL3-2-R-1 exhibited antibacterial activity supported by the formation of inhibition zones on tested bacterial strains. The current strategy successfully supported the effectiveness of the OSMAC approach in altering secondary metabolites production of rare actinobacterial strain SL3-2-R-1. Examination of additional cultivation parameters, e.g., temperature, pH, and incubation time, is required to induce the production of the bioactive compound in rare actinomycetes.

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