Production of antimicrobial compound from two potential actinomycetes SRM 2 and SD 17 using soluble and local starch

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Abstract. Fermentation of antimicrobial compound from two potential actinomycetes, SRM 2 and SD 17, has been carried out using a local carbon source and commercial soluble starch. Three local carbon sources, rice, corn, and tapioca starch were screened to determine the best local carbon source for growth medium of actinomycetes. Determination was based on Amylolytic Index Activity (AIA) using Iodine test. Result from screening local starch test of both actinomycetes SRM 2 dan SD 17 showed that rice starch was the best carbon source with AIA value (0.87 and 2.30) higher than corn starch (0.50 and 1.43) and tapioca starch (0.38 and 1.71). Antimicrobial compound of actinomycetes SRM 2 and SD 17 was produced in CSM medium containing soluble starch (CSMs) and rice starch (CSMb). Filtrate of the growth medium was tested for antimicrobial activity by using cylinder method against certain Gram positive and negative bacteria or yeasts. The results showed that filtrate of medium CSMs and CSMb from actinomycetes SRM 2 were most effective to inhibit Kocuria rhizophila with Antimicrobial Index (AI) 2.79 and 2.66, respectively. The filtrate was less effective against to Escherichia coli with AI 0.92 and 0.89. Filtrates of medium CSMs and CSMb from actinomycetes SD 17 were also most effective to inhibit K. rhizophila with AI 1.75 and 3.16 respectively. Meanwhile, the filtrates were less effective to inhibit Candida albicans with IA 0.67 for CSMs and 0.95 for CSMb, but not effective to inhibit Saccharomyces cerevisiae.

Keywords: Actinomycetes, antimicrobial fermentation rice flour soluble starch

1. Introduction
Antibiotics are chemical compounds that in low concentration are able to inhibit the growth and kill microorganisms. Antibiotics have an important role in modern medicine, but currently the effectiveness of some types of antibiotic have decreased. WHO [1] informs that antibiotic resistance has become a global problem and new types of antibiotics have not been found in the past three decades. Therefore it is necessary to explore new antibiotic compounds to overcome the problem of resistance. The marine environment has high diversity of microorganisms that is potential as source of new antibiotic compounds or bioactive compounds. Mangrove habitat is a marine ecosystem which has potential to be explored for microorganisms.
The Mangrove ecosystem has unique salinity due to tidal influences, contains high levels of organic carbon, organic matters, and nutritional properties. According to Azman et al. [2], in mangrove ecosystems there are rare actinomycetes which can isolated for bioactive compounds. Actinomycetes are known as a source of antimicrobial compounds and contribute almost 45% of bioactive compounds found today. About 10,000 antibiotics of 23,000 antibiotics have been found were produced from orders Actinomycetales [3].

Fermentation medium using carbon source from natural material is one of the most important factors in the fermentation process. In pharmaceutical industry, such as production of penicillin, about 58% of total cost was provided for raw material procurement [4]. Previous study by Fadhilah et al. [5] showed that the actinomycetes SRM 2 has antibacterial activity to inhibit Gram positive and negative bacteria. The actinomycetes SD 17 has antimicrobial activity against Gram positive bacteria and yeast [6].

The purpose of research is to evaluate local starch which can substitute soluble starch as carbon source for production of antimicrobial compound.

2. Materials and method

2.1. Microorganisms

The actinomycetes isolates SRM 2 and SD 17 were provided by Microbiology laboratory Department of Biology, Universitas Indonesia. Both isolates were used to produce antimicrobial compound against to Gram positive bacteria, Staphylococcus aureus NBRC 100910 and Kocuria rhizophila NBRC 12708. The growth filtrate of SRM 2 would be tested also against Gram negative bacteria Escherichia coli NBRC 3301. The growth filtrate of SD 17 would also be tested against yeasts Candida albicans UICC Y-29 and Saccharomyces cerevisiae UICC Y-17.

2.2. Screening of local starch

Screening of rice, corn, and tapioca starch were done using amylolytic assay method in Starch Agar (SA) medium. The medium contained 20 g local starch, 1 g KNO₃, 0.5 g K₂HPO₄, 0.5 MgSO₄.7H₂O, 0.5 g NaCl, 0.01 g FeSO₄.7H₂O, and 15 g agar in 1000 mL aquades [7]. Actinomycetes SRM 2 and SD 17 were inoculated at the center of medium and then incubated for 7 days. Amylolytic activity of isolate on SA medium was seen as a yellowish clear zone formation after flooding by 1% iodine solution. The diameter of the yellowish zone was measured using a caliper and calculated to obtain Amylolytic Index Activity (AIA) using the formula [8]:

$$AIA = \frac{\text{Clear zone width} - \text{colony width}}{\text{colony width}}$$

The best local starch will be determined by the highest AIA value and would be used for fermentation medium in producing antimicrobial compound of actinomycetes SRM 2 and SD 17. For comparison, the antimicrobial compound will also be produced in medium with soluble starch as carbon source.

2.3. Fermentation

Actinomycetes SRM 2 and SD 17 were fermented in Cross Streak Media (CSM) medium to produce antimicrobial compounds. The CSM medium containing 8 g soluble starch (CSMs) or 8 g the best local starch (CSMb), 3 g yeast extract, 3 g peptone, 8 g soluble starch, 3 g casein, 0.5 g K₂HPO₄, 0.5 g MgSO₄.7H₂O, 2 g NaCl in 1000 mL aquades [9]. About 10% (v/v) spores suspension of each isolate was inoculated in CSMs and CSMb medium, then incubated at 30 °C until 5 day for actinomycetes SRM 2 [5] and actinomycetes 7 days for SD 17 [6].

2.4. Antimicrobial activity test

Antimicrobial activity test was done using the cylinder method [10]. The filtrates of CSMs and CSMb growth medium of actinomycetes SRM 2 and SD 17 were centrifuged at 13,000 rpm for 10 minutes.
to obtain the supernatant. Bacterial cells used for antimicrobial test were inoculated in Muller Hinton Agar (MHA) and the yeast cells were inoculated in Sabouraud Dextrose Agar (SDA) in a petri dish. About 100 μL of supernatant were dropped into the cylinder. Antimicrobial activity can be observed as a clear zone. The Antimicrobial Index (AI) was calculated according to Ragasa et al. [11]:

\[ AI = \frac{\text{clear zone diameter} - \text{cylinder diameter}}{\text{cylinder diameter}} \]

3. Results and discussion

3.1. Screening of local starch

The results of amylolytic assay showed that both actinomycetes SRM 2 and SD 17 were able to degrade all the local starch (figure 1). The highest Amylolytic Index Activity (AIA) was obtained from medium with rice starch. This means that rice starch was the best local carbon source which could be used as carbon source for fermentation medium of antimicrobial compound. Nevertheless the AIA value from rice starch was still lower than soluble starch (table 1).

![Figure 1. Amylolytic assay of actinomycetes SRM 2: (a) soluble starch, (b) rice starch, (c) corn starch, (d) tapioca starch; and actinomycetes SD 17: (e) soluble starch, (f) rice starch, (g) corn starch, (h) tapioca starch.](image)

| Isolates | Amylolytic Index Activity (AIA) |
|----------|---------------------------------|
|          | Soluble starch | Rice starch | Corn starch | Tapioca starch |
| SRM 2    | 1.00            | 0.87        | 0.50        | 0.38          |
| SD 17    | 2.50            | 2.30        | 1.43        | 1.71          |
3.2. **Antimicrobial activity test**

The results of antimicrobial activity test of actinomycetes SRM 2 can be seen on figure 2. Antimicrobial activity from actinomycetes SRM 2 inhibited growth of *K. rhizophila* and *S. aureus* as Gram positive bacteria and *E. coli* as Gram negative bacteria. Table 2 showed that Antimicrobial Index (AI) activity from actinomycetes SRM 2 from filtrate CSMb was higher than CSMs medium. The clear zone formation in figure 3 showed that actinomycetes SD 17 can inhibit Gram positive bacteria, *K. rhizophila* and *S. aureus*, and yeast *C. albicans*. Antimicrobial activity test results from actinomycetes SD 17 in table 3 also showed that AI value from filtrate CSMb has higher result than CSMs medium.

![Figure 2. Antimicrobial activity of actinomycetes SRM 2 from CSMs filtrate against:](image)

(a) *K. rhizophila*, (b) *S. aureus*, (c) *E. coli*; and CSMb filtrate against: (d) *K. rhizophila*, (e) *S. aureus*, (f) *E. coli*.

| Medium | Test microorganism | Control | Replication 1 | Replication 2 | Replication 3 | Replication 4 | Mean (μ) | SD |
|--------|--------------------|---------|---------------|---------------|---------------|---------------|----------|----|
| CSMs   | *K. rhizophila* NBRC 12708 | -        | 2.76          | 2.33          | 2.92          | 3.14          | 2.79     | 0.34 |
|        | *S. aureus* NBRC 100910  | -        | 1.01          | 0.82          | 1.00          | 1.11          | 0.98     | 0.12 |
|        | *E. coli* NBRC 3301     | -        | 1.21          | 0.95          | 0.75          | 0.78          | 0.92     | 0.21 |
| CSMb   | *K. rhizophila* NBRC 12708 | -        | 2.37          | 2.47          | 2.94          | 2.85          | 2.66     | 0.28 |
|        | *S. aureus* NBRC 100910  | -        | 1.13          | 0.88          | 0.90          | 0.96          | 0.97     | 0.12 |
|        | *E. coli* NBRC 3301     | -        | 0.80          | 0.82          | 1.09          | 0.83          | 0.89     | 0.14 |
Figure 3. Antimicrobial activity of actinomycetes SD 17 from CSMs filtrate against:
(a) K. rhizophila, (b) S. aureus, (c) C. albicans; and CSMb filtrate against:
(d) K. rhizophila, (e) S. aureus, (f) C. albicans.

Table 3. Antimicrobial activity of actinomycetes SD 17.

| Medium  | Test microorganism         | Control | Replication | \(\bar{x}\) | SD |
|---------|---------------------------|---------|-------------|-------------|-----|
| CSMs    | K. rhizophila NBRC 12708  | -       | 1.60        | 1.47        | 2.07 | 1.87 | 1.75 | 0.27 |
|         | S. aureus NBRC 100910     | -       | 1.66        | 1.34        | 1.19 | 1.46 | 1.41 | 0.20 |
|         | C. albicans UICC Y-29     | -       | 0.75        | 0.59        | 0.76 | 0.56 | 0.67 | 0.10 |
|         | S. cerevisiae UICC Y-17   | -       | -           | -           | -    | -    | -    | -    |
| CSMb    | K. rhizophila NBRC 12708  | -       | 3.08        | 3.28        | 3.12 | 3.15 | 3.16 | 0.01 |
|         | S. aureus NBRC 100910     | -       | 2.79        | 2.90        | 3.36 | 3.15 | 3.05 | 0.26 |
|         | C. albicans UICC Y-29     | -       | 1.34        | 0.83        | 0.80 | 0.83 | 0.95 | 0.26 |
|         | S. cerevisiae UICC Y-17   | -       | -           | -           | -    | -    | -    | -    |

Actinomycetes SRM 2 was able to degrade rice, corn, and tapioca starch. The ability to use different kinds of starch is caused by amylase enzymes which was produced by actinomycetes SRM 2 [5]. The highest AIA value was observed in SA containing rice starch as a carbon source. This indicated that the rice starch was easier to degrade compared to corn or tapioca starch. Based on size of molecules, rice starch has smallest molecules [12] compared to molecules of corn and tapioca starch [13]. The smallest molecules will have higher ratio of surface area and the volume, and would be more effective for enzymes-substrate reaction. Moreover the rice starch have more amylose molecules compared to corn.
and tapioca starch which have more amylopectin [13]. Amylose with straight chains is easier to degrade than amylopectin which has branched chains. Figure 1 shows the presence of yellowish clear zones around the isolates on media which contained starch. The yellowish zone represents starch degradation by actinomycetes SRM 2 and SD 17. Starch will be degraded into simpler compounds, such as glucose, maltose, or oligosaccharides. Iodine solution will react with starch to give a blue to violet color but will give a yellowish to orange color if the starch has been degraded. In addition, a larger zone and brighter yellowish color indicate more active enzyme was produced and this correlates to higher Amylolytic Index Activity (AIA). The more active enzyme will cause the cells to grow better. The growth of microorganisms is directly proportional to antimicrobial productivity.

Results of antimicrobial activity showed that both filtrate of medium CSMs and CSMs from SRM 2 and SD17 is effective to inhibit Gram positive *K. rhizophila* and *S. aureus*. *Kocuria rhizophila* is the most sensitive bacteria to the filtrate of actinomycetes SRM 2 and SD17. According to Young et al. [14], *K. rhizophila* has high sensitivity to some types of antibiotics, especially the β-lactam group, so it is good for antibiotic test. The use of rice starch as a carbon source in the fermentation media gave different effect for antimicrobial production. Based on antimicrobial activity test results of actinomycetes SRM 2, there seemed no significant different between AI value from CSMs with AI value from CSMb for each test microorganism. These results are different with the results of fermentation filtrate from actinomycetes SD 17. The use of rice starch in fermentation of actinomycetes SD17 apparently produced more antimicrobial substances as indicated by AI value. The AI value from CSMb media contained rice starch increased significantly compared to AI value from CSMs media contained soluble starch. Antimicrobial Index value of *K. rhizophila* (1.75), *S. aureus* (1.41), and *C. albicans* (0.67) which resulted from CSMs filtrate are much lower than AI value of *K. rhizophila* (3.16), *S. aureus* (3.05), and *C. albicans* (0.95) from CSMb filtrate media. The increased of AI value from CSMb filtrate media for *K. rhizophila*, *S. aureus*, and *C. albicans* are 80.57 %, 116.31 % and 41.79 % respectively. Based on the antimicrobial activity test, the rice starch is potential to substitute soluble starch as a carbon source for antimicrobial production from actinomycetes SD17.

4. Conclusion

Based on the AIA, actinomycetes isolates SRM 2 and SRM 17 were able to use rice starch as carbon source better than corn or tapioca starch. The use of rice starch in fermentation medium of actinomycetes SRM 2 did not affect the production of antimicrobial compound. The rice starch has potential to substitute soluble starch as a carbon source in antimicrobial compound produced by actinomycetes SD 17.

Acknowledgments

This work was financially supported by Universitas Indonesia under research grant PITTA 2017 to Dr. rer. nat. Yasman, M.Sc. with grant contract number No. 0705/SK/R/UI/2017 and Laboratory of Centre of Excellence Indigenous Biological Resources-Genome Studies FMIPA UI.

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