

The isolation and identification of endophytic bacteria from mangrove (*Sonneratia alba*) that produces gelatinase

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Abstract. Gelatinase is an enzyme that hydrolyze gelatin into gelatin hydrolyzate. The purpose of this study was to isolate and to identify endophytic bacteria from *Sonneratia alba* mangrove which able to produce gelatinase enzyme. *Sonneratia alba* mangroves was obtained from Bajul Mati Beach, Malang Regency. The samples in this study were, stems, and leaves. Pure cultured bacteria were investigated for its capability for producing gelatinase enzyme by using gelatin media. Best producer would further be analyzed its species using microbact system. Screening process resulted in 3 positive isolates, namely code isolate of R, B, and L. R which was isolate from root of *S. alba* was the best producer for gelatinase. Identification process with morphology and microbact system revealed that A. SBM is a Gram-negative bacterium that has a basil cell shape, with a diameter colony of 2.19 mm. Based on the microbact system test carried out, the bacteria is *Pseudomonas aeruginosa*.

1. Introduction

The metalloproteinase gelatinase is an important enzyme not only for the chemical industry, but also for the food industry as a solvent, binder, stabilizer, water binder as well as an anticancer, antibiotic, and other medicinal products [1]. Hence, the exploration of new sources of this enzyme is important and is always attracting the interests of not only microbiologists, but food chemists and pharmacists as well. The gelatinase enzyme can be produced by animals, plants and microorganisms. Microorganisms such as *Micrococcus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus subtilis*, *Corynebacterium*, *Salmonella*, *Vibriococcus*, *Chromobacterium violaceum*, *Staphylococcus aureus* and *Proteus* are widely known as gelatinase producers [2]. Microorganisms, especially bacteria is a potential enzyme producer due to the fact that it can rapidly grow. Hence, the efficiency for mass production is very promising.

Mangroves are reported to have unique bioactives that have the potential to produce several enzymes for industry. Mangroves grow and develop in coastal areas and have a unique adaptation to environmental stress. Mangroves live in two different habitats, namely the terrestrial and ocean habitats at the same time. This harsh environment may trigger the production of the microorganism to produce unique bioactives such as enzymes [3].

This study investigates the probability of the bacterial endophytic mangrove in producing gelatinase enzyme. The isolate would further be identified its species by using morphology and the microbact system analysis.



2. Methodology

Materials for this study were the leaves, bark and root of the mangrove *Sonneratia alba* from Bajul Mati Beach, Malang Regency which is located in East Java, Indonesia. Other media and reagents were Luria Bertani agar (LB agar), and (0.15 grams yeast extract, 0.3 grams pepton, 0.3 grams NaCl, 0.45 gram agar) and medium for gelatinase screening (0.15 gram pepton, 0.09 gram beef extract, 3.6 gram gelatin) per 30 mL aquades that were pure grade.

2.1. Isolation of endophytic bacteria

The isolation of endophytic bacteria was carried out according to the method of Rajendra et al [4] with modification. One gram of sample (leaves, bark and root) were grinded and diluted until 10^{-4} . The sample was then cultured in LB agar and incubated for 48 hours at the temperature of 37 °C. Each colony was pure cultured by using the streak quadrant method. The pure isolates were kept at 4°C until further used.

2.2. Screening of gelatinase-producing bacteria

The pure culture of bacteria was further analyzed to discover its capability for producing gelatinase by using a medium for gelatinase screening following the method of Ballan *et al.* and Della *et al.* [2, 5] with slight modification. The best producer of gelatinase would be identified by using morphology and the microbact analysis system.

2.3. Species identification

Species identification was performed with morphology and biochemical characterization. The Gram stain was performed according to the standard method. The microbact sistem is a method for identifying microorganisms based on their biochemical characteristics and depends on the outcome of the oxidase and non-oxidase bacteria. If it is a positive oxidase, the microbact 24E system is the most appropriate; while microbact 12E should be used when the bacteria shows a negative oxidase. All protocols followed the manufacturer's instructions [6].

3. Result and discussion

Based on the results of the streak plate quadrant, the isolation steps obtained 3 bacteria isolates. The code of R, B, and L were the isolates from the root, bark and leaves of *S. alba*, respectively. The three isolates which were successfully isolated from the mangrove *S. alba* were further analyzed for its capability to produce gelatinase. The gelatinase test was performed on the three isolates and results indicated that all of the isolates produced gelatinase. The production of gelatinase was marked with the remaining liquid media on the gelatinase screening media after the incubation at 4 °C. The results showed that only two isolates were positive for producing gelatinase (figure 1), namely from the root and bark. It also showed that the isolate from the root (isolate R) was the best producer of gelatinase.

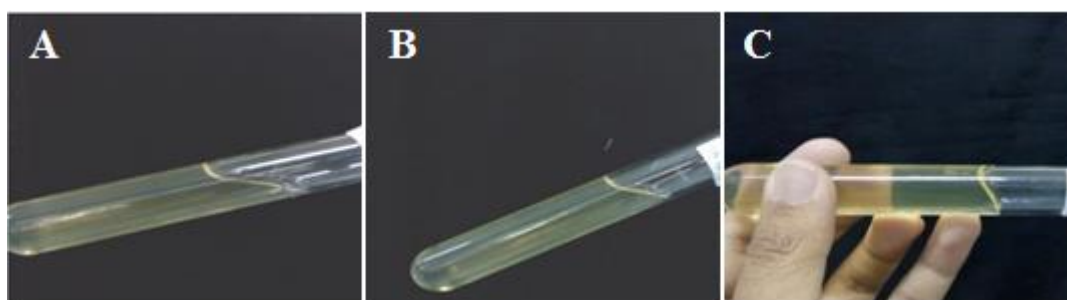


Figure 1. the result of screening of gelatinase-producing bacteria A isolate R, B isolate B, C isolate L.

Further analysis of the species was performed only on the best producer. Hence, isolate R was analyzed by its morphology and biochemical characteristics in order to determine the species. Gram positive and morphology analysis revealed that the isolate R is a gram negative bacteria (figure 2.) with a diameter of about 2.19 mm.

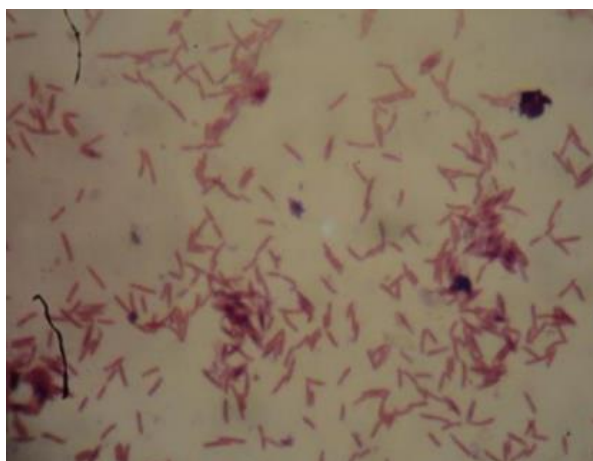


Figure 2. Gram identification of the isolate R.

The endophytic bacteria from the root of *S. alba* (isolate R) was an oxidase negative bacteria, therefore the 24E microbact system was used. An analysis of the oxidase, spores, nitrate, lysine, ornithin, H₂S, glucose, mannitol, xylose, ONPG, indole, V-P, citric, TDA, gelatin, moalonat, inositol, rhammnosa, sucrose, lactose, arabinose, adonitol, raffinosa, salicin, arginine, catalase, coagulase, hemolysis, starch hydrolysis, and casein hydrolysis was conducted. The results of the microbact analysis are described in table 1.

Table 1. The results of the biochemical characteristic of isolate R based on Microbact analysis system.

No.	Biochemical test	Result	Bergey's
1	Spore	+	+
2	Oxidase	+	+
3	Motility	-	+
4	Nitrate	+	+
5	Lysine	-	Tidak diuji
6	Ornithine	-	Tidak diuji
7	H ₂ S	-	Tidak diuji
8	Glukose	+	+
9	Manitol	+	+
10	Xylose	+	+
11	ONPG	+	Tidak diuji
12	Indole	-	Tidak diuji
13	Urease	-	Tidak diuji
14	V-P	+	Tidak diuji
15	Citric	-	+
16	TDA	-	Tidak diuji
17	Gelatine	-	+
18	Malonate	-	Tidak diuji

19	Inositol	-	Tidak diuji
20	Rhamnose	-	Tidak diuji
21	Sucrose	-	+
22	Lactose	-	-
23	Arabinose	+	+
24	Adonitol	-	Tidak diuji
25	Raffinose	-	Tidak diuji
26	Salicine	-	Tidak diuji
27	Catalase	+	+
28	Arginin	-	Tidak diuji
29	Coagulase	-	Tidak diuji
30	Beta Hemolysis	+	Tidak diuji
31	Novobiosin sensitivity	-	Tidak diuji
32	Starch hydrolysis	+	+
33	Casein hydrolysis	+	+

Based on the analysis of the microbact system, the isolate R is *Pseudomonas aeruginosa*. It has the characteristic of positive in glucose, xylose, d malonic and rhamnosa fermentative. Contrastingly, it is negative in mannitol, arabinose, inositol, sucrose, lactosa, adonitol, raffinose fermentation, salicin and arginine. Simple sugar was used by bacteria as a source of carbon source. In this bacteria, it seemed that if the source of simple sugar is absent then it will utilize the complex sugar.

Pseudomonas aeruginosa is a gram negative bacteria, rod-shaped in form, and aerobic. It has flagella for their motility. This bacteria is capable of making their own pili type IV that serves as a process of the adhesin to bind on the host cell. *P. aeruginosa* is able to attach and colonize on the various types of cells [7]. This bacteria is a pathogen bacteria and causes acute ventilator-associated pneumonia and chronic lung infections in patients with cystic fibrosis (CF). It is also difficult to treat naturally because it has developed protective shield from neutrophils [8, 9].

4. Conclusion

Gelatinase-producing bacteria was successfully isolated from the mangrove of *S. alba*. The highest bacteria which produced gelatinase was *P. aeruginosa*. Although this bacteria is a potential gelatinase producer, it is unrecommended for further development due to safety issues.

5. References

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