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Biodiversity of Indigenous Amylolytic and Cellulolytic Bacteria in Sago Waste Product at Susupu, North Moluccas

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Abstract: People at Susupu, North Moluccas prepare the sago (*Metroxylon sago*) in traditionally way for consumption. The residue of processed sago usually thrown away on the ground, so it was caused pollution. Some amylolytic bacteria species and cellulolytic bacteria species could be founded in sago waste product. The purpose of this research are: 1) to identify the indigenous amylolytic bacteria species in sago waste product; 2) to identify the indigenous cellulolytic bacteria species in sago waste product; 3) to test the amylum hydrolysis ability of each amylolytic bacteria species; 4) to test the cellulose hydrolysis ability of each cellulolytic bacteria species. This research was conduct at the Microbiology laboratory, Biology Department-FMIPA-State University of Malang and the Microbiology laboratory-Faculty of Medicine-Brawijaya University. The research samples are sago waste product from Susupu, North Moluccas. The samples were grinded and diluted in nutrient broth, and then the suspension was diluted gradually until 10^{-10}. The suspension was inoculated 0.1 mL each on nutrient agar medium in 37 ºC during 1 × 24 h. Each bacteria colony that grows on the medium were isolated and determined to know which one were the amyloliytic bacteria and the cellulolytic bacteria. The amylum hydrolysis index of each amylolytic bacteria species were counted as well as the cellulose hydrolysis index of each cellulolytic bacteria species. Each amylolytic bacteria and cellulolytic bacteria species were identified. This research result shows that: 1) there are 5 indigenous amylolytic bacteria species, i.e., *Bacillus mycoides*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus alvei* and *Serratia liquefaciens*; 2) there are 4 indigenous cellulolytic bacteria species, i.e., *Serratia liquefaciens*, *Acinetobacter iwofii*, *Bacillus licheniformis* and *Bacillus cereus*; 3) *Serratia liquefaciens* has the highest amylum hydrolysis index, i.e., 3.08; 4) *Acinetobacter iwoffii* has the highest cellulose hydrolysis index, i.e., 2.01.

Key words: Amylolytic bacteria, cellulolytic bacteria, sago waste product.

1. Introduction

People at Susupu, North Moluccas prepare the sago (*Metroxylon sago*) to make the sago powder and making some sort of processed food in traditionally manner. Commonly the sago waste products were thrown away on the ground or stream so it contributed to the environment pollution, Amitum a cellulose in the sago waste product could be degrade, so it can be diluted easily by using the amylolytic and cellulolytic indigenous bacteria.

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The sago waste product contains carbohydrate, so it is good for substrate of amylolytic and cellulolytic indigenous bacteria from sago waste product. Some bacteria species were founded in the sago waste product, but not all the bacteria species have amylolytic and cellulolytic characters. Based on this fact, it is needed to isolate and identify the amylolytic and cellulolytyc indigenous bacteria from sago waste product at Susupu, North Moluccas. According to Apun et al., there is *Bacillus amyloliquifaciens*, an amylolitic and cellulolytic bacteria that have been isolated from sago pith waste from Pusa, Sarawak, Malaysia [1]. There is a possibility that another

amylolitic or cellulolytic bacteria species were founded in the sago waste product at Susupu, North Moluccas. Furthermore, it is important to measure the amylumhydrolisis ability of the amylolytic bacteria species based on the amylum hydrolysis Index and the cellulitic hydrolysis ability of the cellulytic bacteria species based on the Cellulose Hydrolyysis Index. This research is done to: 1) identify the indigenous amylolytic bacteria species in sago waste product; 2) identify the indigenous cellulytic bacteria species in sago waste product; 3) test the amylum hydrolisys ability of each cellulolytic bacteria species.

2. Material and Method

2.1 Material

Sago waste product from Susupu, North Maoluccas. Nutrient broth medium, nutrient agar plate medium, carboxyl metyl cellulose (CMC) plate medium, 0.1% congo red solution, 1 M NaCl solution, 70% alcohol, amylum Agar medium, iodium solution.

2.2 Method

Sago waste product was collected in three serile bottles 25 g each. Each sample were homogenized with shaker in 100 rpm and acclimated during 1 × 24 h. The suspension was diluted at 10^{-1} to 10^{-10}. Each diluted sample suspension were inoculated in 0.1 mL each on Nutrient Agar plate (NA) medium and inoculated in 37 ^{o}C during 1 × 24 h.

Each bacteria colonies that grow on NA plate medium were isolated and determined their amilum hydrolytic ability in the manner of inoculated the isolate on the amylum Agar medium by streak method and incubated in 37 ^{o}C for 1 × 24 h, then added with iodine solution, if there is a clear zone surround the bacteria colony, it indicated the bacteria have an ability to hydrolyze the amylum. Each bacteria colonies were also determined their cellulose hydrolytic ability in the manner of inoculated the isolate on the CMC medium by streak method and incubated in 37 ^{o}C during 1 × 24 h. Furthermore, each colony was flooded with 1% congo red solution [1, 2]. If there is a clear zone surrounded the bacteria, it indicated the bacteria have an ability to hydrolyze the cellulose.

Each amylolitic bacteria as isolate were measured their hydrolyzed Index by inoculated on the amylum Agar with quadrant streak method and incubated at 37 ^{o}C during 1 × 24 h, then added with iodium solution. Furthermore each cellulolytic bacterias isolated were measured their cellulose Hydrolysis Index by inoculated on the CMC medium with quadran streak method and incubated at 37 ^{o}C during 1 × 24 h. Each bacteria colony was flooded with 1% congo red solution for 15 min and washes with 1 M NaCl solution. The cellulose Hydrolisis Index or amilolytic Hidrolisis Index were measured with the formula:

$$\frac{(\square \text{ clear zone} + \square \text{ bacteria colony}) - \square \text{bacteria colony}}{\square \text{ bacteria colony}}$$

$\square$ = diameter

Each amylolitic and cellulolytic bacteria isolates were indetify with Microbact GNB 12/B/E Identification Kits.

3. Results and Discussion

3.1 Isolation of Indigenous Bacteria from Sago Waste Product

Based on the isolation result of indigenous bacteria species, it was found 6 isolates bacteria species and the codes are: B, D, G, H, M and O. Furthermore, each bacteria isolate were determined to know the amylolytic species, and the cellulolytic species.

3.2 Determination of Amilolytic and Cellulolytic Bacteria Species

There are 5 amylolytic bacteria isolates, each amylolytic bacteria show the clear zone surround their colonies on amylum agar medium after that added with iodine solution. Then the amylum hydrolysis Index of each amylolytic bacteria isolates were measured.

The cellulolytic bacteria isolates also determined there are 4 cellulolytic bacteria isolates, each cellulolytic bacteria show the red colony colour that surround by clear zone on CMC medium after flooded with 1% congo red solution and washes with 1 M NaCl solution [1]. It also found that there are 3 isolates have amylolytic besides cellulolytic characters. Then the cellulose hydrolysis Index of each cellulolytic bacteria isolates were measured. Furthermore, all amylolytic and cellulolytic bacteria were identified. Table 1 show the amylolytic and cellulolytic bacteria species from sago waste product at Susupu, North Molucccas.

3.3 Measurment of the Amylum Hydrolysis Index and the Cellulose Hydrolysis Index of the Indigenous Bacteria Species

The amylum hydrolysis index of each amylolitic bacteria species were measured to know the bacteria species that has the highest amylum hydrolysis index. Table 2 show the amylum hydrolysis index of each amylolytic bacteria. *Serratia liquefaciens* has the highest amylum hydrolysis Index, i.e., 3.81.

The cellulose hydrolysis Index of each cellulolytic bacteria species were also measured. Table 3 show the cellulose hydrolysis index of each cellulolytic bacteria, *Acinotobacter iwofii* has the highest cellulose hydrolysis index, i.e., 2.009.

The sago waste product that have thrown away to the ground or the stream would be contribute the environment pollution. However the sago waste product could degrade by soil insect and followed by the indigenous bacteria. This research's result: there are amylolytic and cellulolytic indigenous bacteria species in sago waste product. There are also found

Tabel 1 The amylolytic and cellulolytic bacteria species from sago waste product.

Number	Code	Species	Character	
			Amylolityc	Cellulolytic
1	B	*Bacillus mycoides*	+	-
2	D	*Serratia liquefaciens*	+	+
3	G	*Bacillus alvei*	+	-
4	H	*Acinetobacter iwofii*	-	+
5	M	*Bacillus licheniformis*	+	+
6	O	*Bacillus cereus*	+	+

+: have an amylolytic or cellulolytic character
-: doesn't have an amylolytic or cellulolytic character

Tabel 2 The amylum hydrolysis index of each amylolytic bacteria species from sago waste product.

Number	Code	Species	Amylum Hydrolysis Index				
			UI	UlI	UlII	∑	ẍ
1	B	*Bacillus mycoides*	0.066	0.056	0.066	0.188	0.063
2	D	*Serratia liquefaciens*	3.509	2.796	2.740	9.245	3.081
3	G	*Bacillus alvei*	1.279	2.378	3.00	6.657	2.219
4	M	*Bacillus licheniformis*	0.739	0.572	0.043	1.354	0.451
5	O	*Bacillus cereus*	0.964	0.688	1.257	2.909	0.970

Table 3 The cellulose hydrolysis index of each cellulolytic bacteria species from sago waste product.

No	Code	Nama spesies	Cellulose Hydrolysis Index				
			UI	UlI	UlII	∑	x
1	D	*Serratia liquefaciens*	0.550	0.509	2.00	3.059	1.019
2	H	*Acinetobacter iwofii*	2.181	1.275	2.571	6.029	2.009
3	M	*Bacillus licheniformis*	1.320	0.954	0.818	3.092	1.031
4	O	*Bacillus cereus*	1.200	1.357	1.029	3.586	1.195

some bacteria species that have amylolitic as well as cellulolytic character. These bacteria species play a role in bioremediation process.

This research's result shows that there are 5 indigenous amylolytic bacteria species, i.e., *Baciilus mycoides*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus alvei*, and *Serratia liquefaciens*. There are also found 4 indigenous cellulolytic bacteria species, i.e., *Serratia liquefaciens, Acinetobacter iwofii*, *Bacillus licheniformis,* and *Bacillus cereus*. Bacteria species that belong to the genus Bacillus commonly have an ability to degrade the carbohydrate, protein and lipid compounds [3] *Bacillus licheniformis* and *Bacillus cereus* were the amylolytic, proteolytic, and lipolytic bacteria species; both of them can produce amylase, protease, and lipase enzymes [4]. The result also proved that these bacteria have an amylolytic and cellulolytic characters.

The amylum hydrolysis ability was differ between each amylolytic bacteria species. The amylum hydrolysis index of 5 bacteria species showed in the range of 0.063 to 3.081, and *Serratia liquefaciens* has the highest amylum hydrolysis index, i.e., 3.081. The cellulose hydrolysis ability was also differ between each, cellulolytic, bacteria species. Cellulose hydrolysis Index of 4 cellulolytic bacteria species is in the range of 1.019 to 2.009. *Acinetobacter iwofiiis* also found in soil [5].

The amylolytic bacteria should produce amylase enzyme, which has a function as a biocatalyzator in the biodegradation process of amylum to become glucose. The degradation process: amylum → maltose → glucose [6]. In sago waste product, there found amylum and fibers. The indigenous amylolytic bacteria species could be used in the amylum biodegradation process to become glucose, so it will help to eliminate the waste product that contains amylum.

The materials contains of cellulose especially contains cellulose, hemicelluloses, and lignin [6]. The cellulolytic bacteria species could produce cellulose enzyme, that has a function as a biocatalyzator in the bidegradation process begins from the degradation of cellulose to become cellobiose, then cellobiose was degrade to become glucose-1-phosphat [6]. Glucose-1-phosphat was more soluble in the water, so this biodegradation process will also help to eliminate the waste product that contains glucose.

Bacteria species that have been isolated from sago waste pith waste product at Pusa, Serawak, Malaysia is *Bacillus amyloliquefachiens* [1]. This bacteria produce enzymes that play a role in the amylum and cellulose biodegradation, so it can be used for amylum and cellulose degradation in sago pith waste product. Some Bacillus species that produce cellulase enzyme. According to Yin, et al., cellulose enzyme has been isolated from *Bacillus subtilis* [7]. *Bacillus polymyxa*, can produce β-amylase enzyme, so it is an amylolytic bacteria [8]. The 4 indigenous cellulolytic bacteria cellulolytic, i.e., *Serratia liquefaciens*, *Acinetobacter iwofii*, *Bacillus licheniformis*, and *B. cereus* can also be used in cellulose biodegradation, so it will also help to eliminate the sago waste product that contains cellulose.

This research results had been proved that there are indigenous amylolytic bacteria species and indigenous cellulolytic bacteria species in sago waste product at Susupu, North Moluccas, some bacteria species are amylolytic as well as cellulolytic, i.e., *Serratia liquefaciens*, *Acinetobacter iwofii*, *Bacillus licheniformis*, and *Bacillus cereus.* Based on these facts, actually the sago waste product problem could be solved by using the indigenous amylolytic and cellulolytic bacteria species, because they have an ability to degrade the amylum and cellulose. It was important to watch over the environment surrounding the sago waste product, in order not to pollute by toxic compound that will kill the indigenous bacteria species.

4. Conclusions

Based on this research result, the conclusions are:

(1) There are 5 indigenous amylolytic bacteria species, i.e., *Bacillus mycoides*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus alvei* and *Serratia liquefaciens*.

(2) There are 4 indigenous cellulolytic bacteria species, i.e., *Serratia liquefaciens*, *Acinetobacter iwofii*, *Bacillus licheniformis* and *Bacillus cereus*.

(3) *Serratia liquefaciens* has the highest amylum hydrolysis index, i.e., 3.08.

(4) *Acinetobacter iwoffii* has the highest cellulose hydrolysis index, i.e., 2.01.

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