Short Communication

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Biochemical and Fe-resistant characteristics of indigene bacteria from a high iron concentration landfill in Indonesia

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Abstract

Bacteria isolated from leachate with high heavy metal concentration mostly have potential for bioremediation. The aim of this study was to isolate bacteria from leachate of a landfill containing high concentration of Fe. The leachate sample was collected from Gampong Jawa Landfill, Aceh, Indonesia and used to isolate the bacteria by spread method. The purification of the isolates was carried out through four quadrant plate method. Pure isolates were characterized based on the microscopic or macroscopic appearance and biochemical tests. Fe-resistance was tested by disc diffusion method and constructing the growth curve based on the optical density of broth culture media. A total of 27 pure isolates were obtained and identified, representing eight different genera: *Pseudomonas, Bacillus, Neisseria, Micrococcus, Staphylococcus, Proteus, Acinetobacter*, and *Escherichia*. Of eight isolates (each genus was represented by a single isolate), *Pseudomonas* sp. and *Bacillus* sp. were considered resistant to Fe exposure (15 ppm) with inhibition zones of 1.00 and 0.81 mm, respectively. At Fe concentration of 20 ppm the inhibition zones exceeded 1 mm, but *Bacillus* sp. had smaller inhibition zone than that of *Pseudomonas* sp. (1.15 mm versus 2.86 mm). Growth inhibition of *Bacillus* sp. was concentration-dependent on Fe exposure but could survive at Fe exposure up to 20 ppm. In conclusion, *Bacillus* sp. had a resistance against Fe exposure and its bioremediation potential is subjected for future studies.

Keywords: Leachate, indigene bacteria, ferrous metal, *Bacillus* sp, bioremediation

Introduction

Increase in population has led to the overwhelming number of municipal solid waste which is a threat to environment and human health [1]. Although efforts have been made to decrease the solid waste, such as by implementing the "reduce", "reuse", and "recycle" (3R) principles, the issue persists and becomes more concerning [2]. In managing solid waste, several options including incineration and plasma gasification have been used; however, these options are not sustainable since they emit harmful air pollutants and require high energy [3]. Bioremediation by using bacteria with zero energy requirement and emission have been a promising alternative [3]. However, the application is challenged by its limited applicability against various types of solid waste [4]. Therefore, exploring bacterial candidates that are capable for the bioremediation of solid waste is up most important [5].

Leachate generally is characterized by chemical oxygen demand, biological oxygen demand, electrical conductivity, total suspended solid, heavy metals, acidity (pH), and microorganisms [6]. The level of each aforementioned parameter depends on the waste

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processing which could be performed via chemical, biological, or physical methods [3]. In one of the landfills in Indonesia, Gampong Jawa landfill which is located in Banda Aceh, the leachate could contain an extremely high iron concentration (12.26 ppm) [7]. Ideally, the concentration of iron in water is not higher than 0.3 ppm, otherwise, it may impose toxicity to the environment and humans [7]. The presence of iron with such high concentration could induce the adaptation mechanism of the bacteria, hence possessing Fe-resistant characteristics [8]. Resistance to heavy metal is an important characteristic in regards for its used in solid waste bioremediation [8]. Bacteria isolated from the waste is called indegene bacteria which are mostly used in bioremediation. Therefore, the aim of this study was to isolate and characterize the bacteria from the leachate of Gampong Jawa landfill.

Methods

Materials

The materials used in this study were leachate waste samples, $FeSo_4$, spirits, nutrient agar (NA) medium, nutrient broth medium, Mueller-Hinton agar medium, sulfide-indole-motility medium, triple sugar iron agar medium, Simmons' citrate agar medium, Methyl Red Voges-Proskauer (MR-VP) broth medium, H_2O_2 (30%), ethanol (70%), distilled water, Gram staining, Kovac's reagent, α-naphthol, NaCL, safranin, iodine, KOH, and crystal violet. Otherwise mentioned specifically, all materials were used in analytical grade and procured from Sigma-Aldrich (Selangor, Malaysia). The leachate (26°C and pH 7) was collected from Gampong Jawa Landfill, Banda Aceh, Indonesia with purposive sampling.

Study design

This study combined qualitative and quantitative approaches in examining bacterial isolates of leachate from Gampong Jawa landfill, Indonesia. Morphological characteristics were observed macroscopically and microscopically. Biochemistry tests on the bacterial characteristics were carried out qualitatively. The inhibition zone was measured as quantitative data in the resistance test against Fe.

Bacterial isolation and biochemistry characterization

Isolation was carried out using the spread method by adding leachate from dilutions of 10^{-2}, 10^{-3}, and 10^{-4}. Each diluted leachate was taken for 1 mL, and spread to a petri disk containing NA medium that had been enriched with $FeSo_4$ 5 ppm (incubation, 37°C for 24 hours). One bacterial parent was taken and streaked to four quadrants plate of 5 ppm Fe-enrich NA medium, and subsequently incubated (30°C for 24 hours). The results of the purification were duplicated on slanted NA in test tubes and incubated for 24 hours at 30°C. Then Gram staining was carried out, and biochemical testing with sulfide-indole-motility testing, Methyl Red Voges-Proskauer (MR-VP) test, urease test, Simmons' citrate agar test, catalase test, triple sugar iron agar test by following the protocol reported previously [9].

Fe resistance test

The bacterial isolates resistance against Fe was tested through disc diffusion method. The paper disc was soaked in $FeSO_4$ solutions with concentrations of 10, 15, and 20 ppm, respectively (distilled water was used as control). Pure isolates obtained from a previous isolation was inoculated into Mueller-Hinton agar medium. Thereafter, the soaked paper was placed on the inoculum-containing medium and incubated (37°C; 24 hours). Vertical and horizontal diameters of the formed inhibition zone was determined on a digital caliper as suggested previously to get the average inhibition zone [10]. Isolate with inhibition zone of ≤1 mm was considered Fe-resistant [10].

The bacterial resistance against Fe was also observed through the growth curve of the bacteria inoculated in nutrient broth medium. The culture was prepared in two stages. Starter culture I, comprising 10^8 isolates CFU/mL, was initiated with the inoculation of the most iron (Fe)-resistant strains. After incubating in 10 mL of nutrient broth at room temperature on a rotary shaker (100 rpm) for 24 hours, the bacterial culture was prepared for stage II. In this phase, 10 mL of the culture was aseptically transferred into an Erlenmeyer flask containing 90 mL of nutrient broth medium with varying concentrations of iron (Fe) (10, 15, and 20 ppm), while the control had no added iron. Viability tests were conducted by measuring optical density (OD) every 6 hours for 48 hours at 600 nm on a spectrophotometer. The resulting OD data were utilized to construct a growth curve, depicting time (t) on the *x*-axis and OD values on the *y*-axis.

Results

Characterization and resistance test of isolated bacteria to iron metal (Fe)

There were 27 pure isolates obtained from the leachate from the landfill, where the apparent characteristics of the colonies are presented in **Table 1**. Most of the colonies had round shape, but those with irregular or spotted shapes were observed as well. The edge was mostly flat and the elevation – convex. The colony diameter of each isolate ranged from 0.7 to 1.7 mm. The identification results suggest that the 27 indigene isolates were from eight genera: namely *Pseudomonas, Bacillus, Neisseria, Micrococcus, Staphylococcus, Proteus, Acinetobacter*, and *Escherichia* (**Table 2**).

Test resistance bacteria indigenous to metal iron

The screening results of bacterial resistance against Fe are presented in **Table 1**. At 10 ppm, isolates LB1 (*Pseudomonas* sp.), LD1 (*Bacillus* sp.), LF2 (*Staphylococcus* sp.), LD4 (*Proteus* sp.), and LC3 (*Escherichia* sp.) were resistant, but not with LB2 (*Neisseria* sp.), LE4 (*Micrococcus* sp.), and LC4 (*Acinetobacter* sp.). The only isolates resistant at 15 ppm were LB1 (*Pseudomonas* sp.) and LD1 (*Bacillus* sp.). None of the isolates were resistant at 20 ppm, however, LD1 (*Bacillus* sp.) had higher Fe-resistant than LB1 (*Pseudomonas* sp.) as suggested by the inhibition zone (1.15 mm versus 2.86 mm).

Table 1. Characteristics of indigenous bacteria in leachate waste at in Gampong Java landfill, Banda Aceh, Indonesia

Label	Shape	Color	Edge	Elevation	Diameter (mm)
LA1	Round	Yellow	Flat	Convex	1.20
LA2	Round	Cream	Flat	Convex	0.90
LA3	Irregular	Cream	Choppy	Convex	1.12
LB1	Spotted	Cream	Flat	Convex	1.70
LB2	Irregular	Cream	Choppy	Convex	0.95
LB3	Rhizoids	Cream	Threaded	Convex	1.20
LB4	Irregular	Cream	Choppy	Convex	1.16
LB5	Round	Light brown	Choppy	Convex	0.90
LB6	Round	Dark yellow	flat	Convex	0.96
LC1	Round	Cream	flat	Convex	1.30
LC2	Round	Cream	flat	Convex	1.12
LC3	Round	Light brown	Rippling	Convex	1.87
LC4	Irregular	Cream	Threaded	Flat	1.11
LC5	Round	Cream	Choppy	Convex	0.90
LD1	Round	Cream	Choppy	Flat	1.80
LD2	Round	Cream	Flat	Convex	0.70
LD3	Irregular	Cream	Choppy	Convex	0.70
LD4	Irregular	Yellow	Choppy	Convex	0.70
LE1	Round	Cream	Flat	Convex	0.95
LE2	Irregular	Cream	Choppy	Flat	1.25
LE3	Round	Light brown	Choppy	Convex	0.70
LE4	Round	Bright yellow	Flat	Convex	0.80
LF1	Irregular	Cream	Choppy	Convex	0.98
LF2	Irregular	Yellow	Choppy	Convex	1.40
LF3	Spotted	Cream	Flat	Convex	0.90
LF4	Round	Yellow	Flat	Convex	0.98
LF5	Round	Cream	Flat	Flat	1.20

Table 2. Results of identification of genus of indigenous bacteria in leachate waste at Gampong Jawa landfill from several sources

Characteristics	Isolate							
	LA1, LA2, LB6, LF2, LF5	LA3, LB2, LD3, LF3	LB6, LB4, LC4, LD1, LF1, LF4	LB3, LC3, LE3	LC1, LC5, LE2	LB1, LC2, LD1,LE1	LD4	LE4
Gram staining	+	-	+	-	-	-	-	+
Cellular shape	Spherical	Rod	Rod	Spherical	Rod	Rod	Rod	Spherical
Glucose	-	-	+/-	+	-	+	+	+
Lactose	+	-	+/-	-	+	-	-	-
Sucrose	+	+	+/-	-	+	-	-	+
Gas	+/-	-	-	-	-	-	-	-
H_2S	-	-	-	+	-	-	-	-
Indole	+/-	-	-	-	+	-	-	-
Motility	-	-	+/-	-	+	+	+	+
Methyl red	+	+	+/-	-	+	-	+	+
Voges-Proskauer	+	-	+/-	-	-	-	-	-
Urease	+	+	-	+	-	-	+	-
Citrate	-	-	-	+	-	+	-	+
Catalase	+	+	+	+	+	+	+	+
Endospores	ND	-	+	ND	-	-	-	ND
Genus	*Staphylococcus*	*Neisseria*	*Bacillus*	*Acinetobacter*	*Escherichia*	*Pseudomonas*	*Proteus*	*Micrococcus*

ND, not detected

Table 3. Resistance test for indigenous bacteria in leachate waste at Gampong Jawa landfill

Isolate	Fe concentration	Average inhibition zone (mm)	Remark
LB1	Control	0	Resistant
Pseudomonas sp.	10 ppm	0.68	Resistant
	15 ppm	1.00	Resistant
	20 ppm	2.86	Sensitive
	Control	0	Resistant
LD1	10 ppm	0	Resistant
Bacillus sp.	15 ppm	0.81	Resistant
	20 ppm	1.15	Sensitive
LB2	Control	0	Resistant
Neisseria sp.	10 ppm	2.18	Sensitive
	15 ppm	4.58	Sensitive
	20 ppm	5.70	Sensitive
LE4	Control	0	Resistant
Micrococcus sp.	10 ppm	1.40	Sensitive
	15 ppm	2.13	Sensitive
	20 ppm	3.21	Sensitive
LF2	Control	0	Resistant
Staphylococcus sp.	10 ppm	1.00	Resistant
	15 ppm	2.07	Sensitive
	20 ppm	3.21	Sensitive
LD4	Control	0	Resistant
Proteus sp.	10 ppm	0.49	Resistant
	15 ppm	1.79	Sensitive
	20 ppm	3.51	Sensitive
LC4	Control	0	Resistant
Acinetobacter sp.	10 ppm	1.75	Sensitive
	15 ppm	2.05	Sensitive
	20 ppm	3.21	Sensitive
LC3	Control	0	Resistant
Escherichia sp.	10 ppm	0.52	Resistant
	15 ppm	1.77	Sensitive
	20 ppm	3.24	Sensitive

Growth profile of the isolated *Bacillus* sp.

The growth profiles against $FeSO_4$ exposure (10, 15 and 15 ppm, respectively) was observed for isolate LD1 (*Bacillus sp.*), where the curve is presented in **Figure 1**. The log phase was observed after 12 hours to 30 hours of incubation. Following 30 hours to 42 hours of incubation, the bacterial growth experienced a stationary phase. Decline in the absorbance, indicating a death phase, was observed between 42 hours and 54 hours of incubation. Following the log phase, the OD stayed at the lowest when the $FeSO_4$ concentration was 20 ppm. The OD was concentration-dependent toward $FeSO_4$. Increasing the concentration from 10 to 20 ppm decreased 28.7% of the OD at 42-hour observation.

Discussion

In this present study we found 27 indigene bacteria which were divided into eight genera, *Pseudomonas, Bacillus, Neisseria, Micrococcus, Staphylococcus, Proteus, Acinetobacter*, and *Escherichia*. These findings are in line with previous study that isolated the bacteria from different source of waste [11, 12]. In a previous study, three genera, *Aeromonas, Pseudomonas and Bacillus*, were isolated from the landfill leachate [13]. Previous studies have even explored the ability of the isolated bacteria in bioremediation, including biodegrading microplastics [11-13].

Herein, *Bacillus sp.* was found to be the most resistant to Fe exposure. Although the growth was inhibited, the bacteria could survive when exposed with 20 ppm $FeSO_4$. In a previous study, bacteria from this genus could even survive Fe exposure with higher concentrations (50, 100 and 150 ppm) [14]. A previous study suggested that *Bacillus* sp. was able to reduce the concentration of Cd^{2+} [15]. Genus *Bacillus* sp. can be differentiated from other bacterial genera, namely the production of oval or cylindrical endospores [16]. The cell surface in the genus *Bacillus* sp. consists of a protein (glycoprotein) called the S layer, and not all *Bacillus* sp. have S layer. The S layer has an important role in the interaction of *Bacillus* sp. with metal [16].

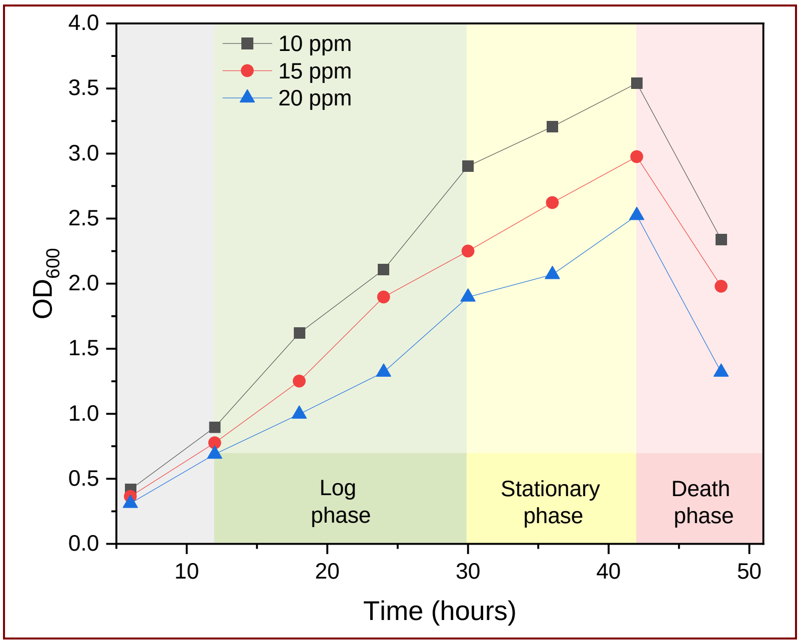

Figure 1. Growth curve of isolate LD1 (*Bacillus* sp.) exposed with $FeSO_4$ with different concentrations.

This study has several limitations. Resistance of heavy metal was only performed using Fe, though our data can be indicative to other metals, the generalization cannot be made. The sampling was carried out once, different season and weather conditions might change the bacterial compositions in the leachate. Moreover, the bacteria observed in this study are only those which are culturable in Fe-enriched media, while those with resistance to different heavy metals might have been missed. Future studies must be carried out to determine the resistance mechanism and molecular characteristics of the isolates. Application of the isolates for bioremediating the solid waste should be investigated as well.

Conclusions

Leachate in Gampong Jawa landfill is rich with Fe-resistant bacteria which probably developed their resistance mechanism following the intense and chronic exposure to Fe. The twenty-seven bacterial isolates collected were found to belong to eight genera, namely of *Pseudomonas*, *Bacillus*, *Neisseria*, *Micrococcus*, *Staphylococcus*, *Proteus*, *Acinetobacter* and *Escherichia* Among the investigated isolates, *Bacillus sp*. was found to yield highest resistance against Fe. The bacteria could survive even when the concentration was increased to 20 ppm, though growth inhibition was observed. It is therefore interesting to further investigate the potential of *Bacillus sp*. in bioremediating solid waste.

Ethics approval

Not required.

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Competing interests

The authors declare that they have no known conflicts of interest related to the publication of this work.

Underlying data

All underlying data have been presented.

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