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Bacillus as Siderophore and Iron-bioremoval Bacteria

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Abstract. Some *Bacillus* strains can produce siderophore. Siderophore is a chelating agent for ferric iron as a response to low iron environment. *Bacillus* has ability as iron bioremoval. The aim of this research was to get siderophore *Bacillus* strain which could resist to iron and to know the ability of its bioremoval.

This research used *Bacillus* isolated from Kalimas Surabaya ie: A6, DA11, and SS19. The strains were screened for siderophore bacteria in Fe-CAS agar medium. Ferric bioreduction was analysed on medium contained $FeCl_3.6H_2O$ 50; 100; and 150 mg/L. Ferric bioremoval was measured by Atomic Absorption Spectroscopy method.

Bacillus A6, DA11, and SS19 could produce siderophore and also stand to media containing 150 mg/L $FeCl_3.6H_2O$. *Bacillus* DA11 had the highest ability of ferric bioremoval, which was 26.841 mg/L from 33.365 mg/L concentration, with efficiency 80.5%.

Keywords: *Bacillus*, Bioremoval, $FeCl_3.6H_2O$, Siderophore

Introduction

Bacillus bacteria are particularly interesting because their genetic background confers variable tolerance to metal [1]. Some genera can produce antibiotic, insecticide, siderophore and so on [2]. Some strain of *Bacillus* had been reported that they can resist to heavy metals such as Hg, Cd, Pb, and Cu [3]. Siderophore is a chelating agent for ferric iron as a response to low iron environment, so siderophore also can hide Fe in rhizosphere and inhibit pathogenic bacteria growth [4]. Iron is one of micronutrient as cytochrome pigment and enzyme cofactor [5].

Bacillus A6, DA11, and SS19 were isolated from Kalimas Surabaya and resisted to Hg, Pb, Cd, and Cu but these bacteria have not been known their potential as siderophore bacteria and Fe bioremoval agent.

Material and Method

Siderophore Screening

Bacillus A6, DA11, and SS19 were inoculated aseptically into Fe-CAS media by streak plate method, and incubated in room temperature (24 hours). Siderophore was shown by yellow to orange colony which was contrast with the blue colour of Fe-CAS media [6].

Fe Bioremoval

Forty five (45) ml *Bacillus* culture (starter) was inoculated aseptically into 180 ml nutrient broth media and incubated until μ hours based on growth curve. Before *Bacillus* culture was given $FeCl_3.6H_2O$, the cell density was counted with *Haemocytometer.* Fifty (50) ml culture cell was given $FeCl_3.6H_2O$ with concentration 50 and 100 mg/L then incubated (24 hours) on rotary shaker (100 rpm). After 24 hours, the culture was added with 5 drops of HNO_3 and heated with temperature ≤ 85°C (15 minutes), then centrifuged 4000 rpm, 20 minutes. Fe^{3+} concentration in supernatant was measured with Atomic Absorption Spectrophotometry, wavelength (λ) 248.3 nm. Fe^{3+} concentration in supernatant was residue of Fe^{3+} (K_s) which was not removed by *Bacillus*. Concentration which could be removed by *Bacillus* and the efficiency of bioremoval was counted with formula:

$$R = K_0 - K_s \qquad E = \left(\frac{R}{K_0}\right) \times 100\%$$

R = Fe^{3+} concentration which could be removed by *Bacillus*
E = Efficiency of Fe^{3+} bioremoval

K_0 = Fe^{3+} concentration in media without *Bacillus*
K_s = Fe^{3+} concentration in supernatant after centrifugation

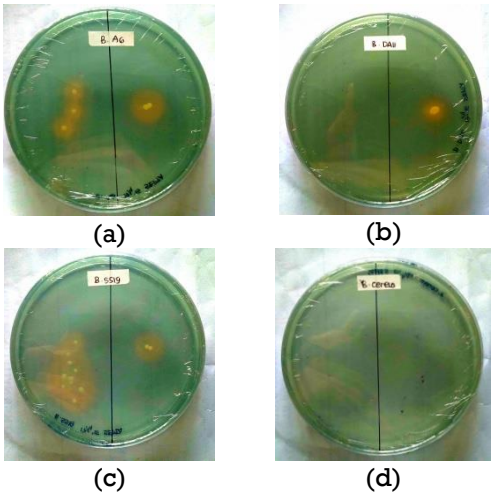
Viability

The $FeCl_3.6H_2O$ treated *Bacillus* was taken 100 µL and inoculated with pour plate method into nutrient agar without $FeCl_3.6H_2O$. The plate count could be used to determine the number of viable bacteria to $FeCl_3.6H_2O$ and it was counted with Colony Forming Units (CFU) method.

Result and Discussion

Bacillus as siderophore

Bacillus A6, DA11 and SS19 produced siderophore and formed yellow to orange colony on Fe-CAS agar (**Fig. 1**). The siderophore zone on Fe-CAS agar media was various, its diameter in 48 hours was bigger than in 24 hours incubation (**Table 1**)



(a) (b) (c) (d)

Fig. 1. *Bacillus* as siderophore bacteria after 48 hours incubation (a. *Bacillus* A6, b. *Bacillus* DA11, c. *Bacillus* SS19, d. *Bacillus cereus* ATCC 1178 as control and not producing siderophore)

The yellow to orange colour in Fe-CAS-agar is caused by producing siderophore by *Bacillus*. Chrome Azurol S (CAS) and HDTMA form complexes with ferric so Fe-CAS agar has blue colour, if there is strong Fe chelating agent like siderophore, then siderophore will take Fe from the blue dye complex which causes Fe-CAS agar colour changes into yellow to orange [7]

Table 1. Siderophore zone

No	Bacillus	Siderophore zone diameter	
		24 h incubation (cm)	48 h incubation (cm)
1	A6	0.6	1.23
2	DA11	0.4	0.55
3	SS19	0.4	0.98
4	*B. cereus* ATCC1178	-	-

Siderophore is Fe^{3+} chelating agent produced by bacteria in low iron condition [8]. Fe-CAS media composition as siderophore test media, only has 0.0027 gr/L of Fe, so that test bacteria can produce siderophore. It is supported by [9] that *Bacillus* does not produce siderophore in high concentration Fe condition.

Fe Bioremoval

This method used nutrient broth and 10^6 *Bacillus* cell density when $FeCl_3.6H_2O$ was added. AAS analysis in nutrient broth without *Bacillus* showed reduction (**Table 2**), so that Fe^{3+} concentration was used as treatment concentration. The decrease concentrations of Fe^{3+}, because the components of nutrient broth will ionized and they can make ligands with metal [10]

Table 2. Fe^{3+} concentration without *Bacillus*

$FeCl_3.6H_2O$ concentration (mg/L)	Measure concentration from AAS* (mg/L)
50	9,496
100	33,365

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Bacillus A6, DA11 and SS19 performed Fe^{3+} bioremoval. The more $FeCl_3.6H_2O$ was added, the higher Fe^{3+} concentration was removed and also its efficiency (**Table 3**).

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Table 3. Bioremoval Fe^{3+}

No.	Bacillus	Treatment Concentration (mg/L)	Supernatant Concentration (mg/L)	Bioremoval Concentration (mg/L)*	Bioremoval Efficiency (%)*
1.	A6	9.496	4.995	4.501[a]	47.4[a]
		33.365	6.642	26.723[b]	80.1[b]
2.	DA11	9.496	4.540	4.956[a]	52.2[a]
		33.365	6.524	26.841[b]	80.5[b]
3.	SS19	9.496	4.440	5.056[a]	53.2[a]
		33.365	7.481	25.884[b]	77.6[b]

*Number with different alphabet in same significantly column ($p < 0.05$).

Based on anova analysis, *Bacillus* A6, DA11 and SS19 are not significantly different in Fe removal ($p > 0.05$) and also their efficiency. Concentration $FeCl_3.6H_2O$ showed that the bacteria had significant difference in Fe^{3+} removal and its efficiency. It showed that concentration had influence on bioremoval ability and bioremoval efficiency in *Bacillus* A6, DA11 and SS19. All of *Bacillus* had more 70% bioremoval efficiency of $FeCl_3.6H_2O$ (33.365 mg/L).

Microbial removal of metal depends on biomass, metal tolerant, and interaction with metal [11]. Generally the process of bioremoval can be described as biological ion exchange with binding groups present on the surface of cell wall, carboxyl, sulfonate, phosphoryl, amido, amino, imidazole. Those group have negative charge which will interact with positive charge ion and form ligand bond [12]

Bacillus Viability

All of *Bacillus* A6, DA11 and SS19 grew in nutrient agar without Fe with CFU more than 300. It showed that Fe in media did not inhibit the growth of *Bacillus*. Fe is one of micronutrient which is needed for bacteria growth [13].

Conclusion

Based on this research, hat Bacillus A6, DA11, and SS19 could produce siderophore which indicated that they attached Fe. Bacillus DA11 had the best Fe^{3+} bioremoval ability with bioremoval efficiency 80.5%.

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