Bacillus as Siderophore and Iron-bioremoving 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 potency as iron bioremoval. The aim of this research is to get siderophore *Bacillus* strain which can 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 applied on medium contained $FeCl_3.6H_2O$ 50; 100; and 150 mg/L. Ferric bioremoval was measured by Atomic Absorption Spectroscophy method.
Bacillus A6, DA11, and SS19 could produce siderophore and also resisted 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 are isolated from Kalimas Surabaya and resist 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 had contrast with the blue color of Fe-CAS media [6].

Fe Bioremoval

Forty five (45) ml *Bacillus* culture (starter) was inoculated aceptically 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 centrifugated 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:

$$R = K_0 - K_s \qquad E = (R/K_0) \times 100\%$$

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 centrifugating

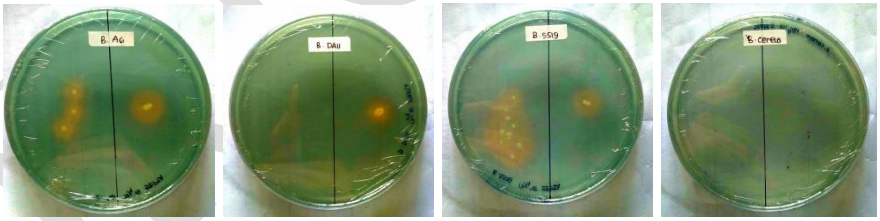
Viability

The *Bacillus* which had been given $FeCl_3.6H_2O$ was taken 100 µL and inoculated with pour plate method into nutrient agar without $FeCl_3.6H_2O$. The plate count can 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 can produce siderophore and form yellow to orange colony on Fe-CAS agar (**Fig. 1**). The siderophore zone on Fe-CAS agar media is various, its diameter in 48 hours is 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 is as control and not producing siderophore)

The yellow to orange color 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 color, if there is strong Fe chelating agent like siderophore, then siderophore will take Fe from the blue dye complex which causes Fe-CAS agar color 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 uses nutrient broth and uses 10^6 *Bacillus* cell density when $FeCl_3.6H_2O$ is added. AAS analysis result in nutrient broth without *Bacillus* shows decrease (**Table 2**), so that Fe^{3+} concentration is 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 can do Fe^{3+} bioremoval. The more $FeCl_3.6H_2O$ is added, the higher Fe^{3+} concentration is removed and also its efficiency (**Table 3**).

Table 3. Bioremoval Fe^{3+}

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

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

Based on manova analysis, *Bacillus* A6, DA11 and SS19 are not different significantly in Fe removal ($p > 0,05$) and also their efficiency. Concentration $FeCl_3.6H_2O$ shows that the bacteria have significant difference in Fe^{3+} removal and its efficiency. It shows that concentration has influence on bioremoval ability and bioremoval efficiency in *Bacillus* A6, DA11 and SS19. All of *Bacillus* have 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 grow in nutrient agar without Fe with CFU more than 300. It shows that Fe in media does not inhibit the growth of *Bacillus*. Fe is one of micronutrient which is needed for bacteria growth [13].

Conclusion

Based on this research, it can be conclude that *Bacillus* A6, DA11, and SS19 can produce siderophore which indicates that they can attach Fe. *Bacillus* DA11 has the best Fe^{3+} bioremoval ability with bioremoval efficiency 80,5%.

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