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Heavy Metal Degradable Halophilic Bacteria Isolation from Soil in Samut Sakhon, Thailand

Jaruwan Chutrtong

Abstract*: This research was conducted to select halophilic microorganisms that can reduce heavy metals in water to be used in wastewater treatment. Halophilic bacteria from salt field soil were isolated in nutrient broth containing 5 heavy metals compound (Zn, Co, Cr, Hg, and Cu). 20 isolates which growth in heavy metal containing media were screened. After that, experiment was conducted to determine the maximum concentration of heavy metals that the isolated could growth. The concentration of heavy metals was obtained from 50-500 mg/L. In media which add Zn, Co, Cr and Cu compound, the isolated were found to be able to grow at concentrations of 450-500 mg/L but they did not grow in media which add Hg (50-500 mg/L). To check the heavy metal degrading ability, the isolates were cultured in nutrient broth which add the most concentrated of heavy metal that they could grow and incubated at 37 ° C for 15 days. Measured the remaining heavy metals and counted the number of microorganisms on days 3, 5,7,10 and 15 were done. It was found that microorganisms were capable to reduce two heavy metals, Cr and Co. Cr reduced by the isolated HL7 and 10-20. Co reduced by the isolated HL1, 3, 4, 5, 7, 8, 10, 11 and 15. Zn and Cu decreased at the decimal level, which were not significant. The results of this research showed that the isolated of halophilic bacteria which isolated from soil in Samut Sakhon had the ability to reduce some heavy metals, which can be applied in wastewater treatment.

***Index Terms*: Halophilic bacteria, Heavy metal, Soil, Thailand**

I. INTRODUCTION

In the present situation, chemicals are used increasingly to facilitate life. They are used in many industries such as smelting industry, tannery industry, dying industry, agricultural products' industry (insecticides, pesticides, chemical fertilizers) and household products' industry [1]. These products may contain more or less heavy metal contaminants. Using heavy metal contaminants products can cause contamination in the environment which harmful to life, for example, arsenic from smelting outflow in water resources in Ron Piboon District in Thailand that caused black fever [2]. Because heavy metals are stable substances, they do not decompose by natural processes in a short time. It must be treated before released to environments to reduce toxicity [3]. There are many ways to treat heavy metal contaminated wastewater, but the friendly way is biological treatment using microorganisms, although it takes longer to heal [4]. It can save money and does not cause any chemical contamination. From the former studies, salinity tolerant microorganisms were able to grow in heavy metals such as copper, lead, cadmium, chromium and zinc [5]. It also found that saline tolerant microorganisms could transform toxic substances into non-toxic substances and absorbed into their cells. Halomonas spp., salinity-sensitive bacteria, which isolated from the San Joaquin community in California, was able to grow in water containing 2M of Selenium (Se). The concentration of Selenium at this level could kill fish and poultry. This type of microorganism could convert Selenium to dimethyl selenite form, non-toxic substance. The rate of change was 1.65 mg/hr. [6]. These show that saline-tolerant microbes have the ability to reduce heavy metals. Therefore, the researcher was interested to find microorganisms that can degrade other heavy metals in water. This is the source of this study by separating the salt tolerant bacteria that is capable to grow in the presence of heavy metals and studying its ability to reduce heavy metals in the water of the microorganisms. If the selected microorganism is highly capable, it will be applied in the treatment of heavy metal contaminated wastewater.

II. PROCEDURE

In this research, salt-tolerant microorganisms from soil which collected from salt pan were isolated. Subsequently, cultured the isolates in broth to study the efficiency of heavy metals reducing in liquid environments. The steps are as follows

A. *Isolation of Halophilic Bacteria in Metallic Conditions*

1. Weigh 10 g of soil, which collected from salt pan in Samut Sakhon province in Thailand.
2. Put the weighing soils in flask containing 100 mL of 0.5% NaCl Nutrient broth.
3. Shake flask to mix soil with media.
4. Incubate at 37 ° C for 5 days.
5. Take 500 µl of culture from 4. Inoculate in nutrient broth flask which supplemented with 0.05 mg /ml. of Co, Cu, Hg, Cr and Zn.
6. Incubate at 37 ° C for 72 hours.
7. Take 100 µl of culture from 6. Inoculate on nutrient agar with 0.5% NaCl. Spread over the plate and then incubated at 37 ° C for 24 hours.
8. Select different types of colonies and re-streak on nutrient agar with 0.5% NaCl to get pure colonies.

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* Correspondence Author
Jaruwan Chutrtong*, Industrail Microbiology, Suan Sunandha Rajabhat University, Bangkok, Thailand, jaruwan.ch@ssru.ac.th.

9. Incubate at 37 ° C for 24 h. Study physical character by Gram's stain and microscope.

B. *Study Resistance of Halophilic Bacteria on Heavy Metal by Plate Diffusion Method*

1. Culture the purely isolated bacteria in 0.5% NaCl nutrient broth. Incubated at 37 ° C for 18 h for nutrient broth.
2. Divide nutrient agar plate with 0.5% NaCl to 6 equal arts.
3. Spread 100 µl of 18-hour-old broth from 1. on nutrient agar in 2.
4. Drill a hole on 6 divided part of Nutrient Agar plates in 3.
5. Drop 40 µl of CoSO4 • 7H2O, ZnSO4 • 7H2O, Hg (NO3) 2, Cr (NO3) 3 • 9H2O and CuSO4 • 5H2O per hole at different concentration (50,100,150, 200, 250, 300, 350,400, 450,500 mg /ml).
6. Incubate at 37 °C for 24 h. Check clear zone around the hole.

C. *Testing the ability to degrade heavy metals in water*

1. Add the highest concentration of heavy metal solution from the plate diffusion method to 0.5% NaCl Nutrient broth.
2. Inoculate 1 ml of 108 CFU/ml of the isolates and incubate at 37 ° C for 15 days.
3. Examine the remaining heavy metals in the sample on days 3, 5,7,10 and 15 with Atomic Absorption Spectrophotometer (AA).

III. RESULT

A. *Isolation of Halophilic Bacteria in Metallic Conditions*

After a few days of cultivation, bacteria were isolated and purified. Different morphology colonies were selected and re-cultured for purification. Total 20 different pure bacterial isolates were collected. Primary screening was performed by Gram's staining and microscopic examination. Characterization of isolate colonies were carried out and the results are shown in table 1 and 2.

Table 1. Isolate colonies form, elevate and margin.

sample	form	elevate	margin
HL 1	circular	raised	undulate
HL 2	circular	raised	entire
HL 3	circular	raised	undulate
HL 4	circular	raised	undulate
HL 5	circular	raised	entire
HL 6	circular	raised	entire
HL 7	circular	raised	entire
HL 8	circular	raised	entire
HL 9	circular	convex	undulate
HL 10	circular	raised	undulate
HL 11	circular	raised	undulate
HL 12	circular	raised	entire
HL 13	circular	convex	undulate
HL 14	circular	raised	entire
HL 15	circular	raised	undulate
HL 16	irregular	flat	undulate
HL 17	irregular	flat	entire
HL 18	irregular	convex	entire
HL 19	irregular	convex	entire
HL 20	circular	raised	entire

Table 2. Isolate colonies color, Gram and shape.

sample	color	Gram	shape
HL 1	creamy	positive	rod
HL 2	creamy	positive	rod
HL 3	creamy	positive	rod
HL 4	greyish	positive	rod
HL 5	greyish	positive	rod
HL 6	creamy	positive	rod
HL 7	white	negative	rod
HL 8	white	positive	rod
HL 9	creamy	positive	rod
HL 10	greyish	positive	rod
HL 11	white	positive	rod
HL 12	white	positive	rod
HL 13	greyish	positive	rod
HL 14	creamy	positive	rod
HL 15	white	positive	rod
HL 16	greyish	positive	rod
HL 17	white	positive	rod
HL 18	white	positive	rod
HL 19	greyish	positive	rod
HL 20	greyish	positive	rod

B. *Study Resistance of Halophilic Bacteria on Heavy Metal by Plate Diffusion Method*

Result of growth performance of 20 isolates in the presence of cobalt, chromium, mercury and zinc are shown in the table 3. and 4.

Table 3. Capability to grow in heavy metal at concentrations 50-250 mg /ml.

sample	Co	Cr	Hg	Cu	Zn
HL 1	-	-	+	-	-
HL 2	-	-	+	-	-
HL 3	-	-	+	-	-
HL 4	-	-	+	-	-
HL 5	-	-	+	-	-
HL 6	-	-	+	-	-
HL 7	-	-	+	-	-
HL 8	-	-	+	-	-
HL 9	-	-	+	-	-
HL 10	-	-	+	-	-
HL 11	-	-	+	-	-
HL 12	-	-	+	-	-
HL 13	-	-	+	-	-
HL 14	-	-	+	-	-
HL 15	-	-	+	-	-
HL 16	-	-	+	-	-

HL 17	-	-	+	-	-
HL 18	-	-	+	-	-
HL 19	-	-	+	-	-
HL 20	-	-	+	-	-

- = do not found clear zone + = found clear zone

Table 4. Capability to grow in heavy metal at concentrations 250-500 mg /ml.

sample	Co	Cr	Hg	Cu	Zn
HL 1	-	-	+	-	-
HL 2	-	-	+	-	-
HL 3	-	-	+	-	-
HL 4	-	-	+	-	-
HL 5	-	-	+	-	-
HL 6	-	-	+	+ *	-
HL 7	-	-	+	-	-
HL 8	-	-	+	-	-
HL 9	-	-	+	-	-
HL 10	-	-	+	-	-
HL 11	-	-	+	-	-
HL 12	-	-	+	-	-
HL 13	-	-	+	-	-
HL 14	-	-	+	-	-
HL 15	-	-	+	-	-
HL 16	-	-	+	-	-
HL 17	-	-	+	-	-
HL 18	-	-	+	-	-
HL 19	+	-	+	-	-
HL 20	-	-	+	+	-

- = do not found clear zone + = found clear zone
* = found clear zone at 500 mg/ml

C. ***The ability to degrade heavy metals in water***

Result of ability to degrade heavy metals in water are shown in the table 5.

Table 5. Heavy metal residue in Nutrient broth

Sample	Day	Remaining quantity in broth (ppm)			
		Zn	Co	Cr	Cu
HL1	3	19.73	77.69	69.51	23.92
	5	19.72	77.67	69.36	22.78
	7	19.75	77.63	69.54	22.08
	10	19.73	73.76	69.42	22.93
	15	19.73	73.71	69.21	21.88
HL2	3	19.59	73.95	64.95	24.74
	5	19.57	73.06	64.41	24.46
	7	19.56	72.95	64.12	24.42
	10	19.55	72.90	64.08	24.21
	15	19.53	72.51	63.92	24.14
HL3	3	19.45	77.29	75.84	25.45
	5	19.44	76.91	75.61	25.15
	7	19.43	75.86	75.42	23.72
	10	19.43	75.63	75.11	23.36
	3	19.41	75.21	75.10	23.23
HL4	3	19.58	76.64	68.62	25.91
	5	19.56	76.05	68.51	25.61
	7	19.54	75.99	68.45	23.78
	10	19.53	74.93	68.44	23.17
	15	19.52	74.16	68.19	23.07
HL5	3	19.68	78.49	65.91	24.44
	5	19.67	78.28	65.81	24.04
	7	19.67	75.51	65.47	24.27
	10	19.66	74.63	65.41	23.55
	15	19.66	74.19	65.27	23.54
HL6	3	19.75	73.68	58.88	24.81
	5	19.74	73.66	58.71	24.72
	7	19.73	73.53	58.55	23.40
	10	19.73	73.25	58.52	23.81
	15	19.73	73.16	58.28	22.37

Table 5. (continue)

Sample	Day	Remaining quantity in broth (ppm)			
		Zn	Co	Cr	Cu
HL7	3	19.94	80.85	77.38	22.88
	5	19.94	80.82	72.49	22.60
	7	19.78	79.14	61.42	22.36
	10	19.71	78.21	51.19	21.38
	15	19.71	76.09	49.78	20.05
HL8	3	19.86	78.37	77.64	24.64
	5	19.85	78.28	77.36	24.46
	7	19.83	75.66	77.37	24.15
	10	19.79	75.18	77.04	23.74
	15	19.53	75.10	77.02	22.31
HL9	3	19.76	76.73	70.59	23.76
	5	19.75	76.23	70.49	23.40
	7	19.74	75.98	70.74	23.13
	10	19.66	75.61	70.18	23.10
	15	19.65	75.15	70.07	23.08
HL10	3	20.10	77.73	88.93	24.52
	5	19.85	77.13	88.51	24.21
	7	19.76	77.05	61.31	24.41
	10	19.55	75.31	53.05	23.15
	15	19.55	75.71	49.66	22.06
HL11	3	19.92	78.98	82.71	23.63
	5	19.91	78.24	77.93	23.19
	7	19.91	77.53	63.64	23.16
	10	19.85	77.10	63.92	22.52
	15	19.72	76.72	38.89	22.33
HL12	3	19.70	77.72	88.13	23.91
	5	19.67	77.12	78.20	23.08
	7	19.63	77.47	77.02	22.98
	10	19.60	77.05	50.57	22.41
	15	19.58	76.84	50.45	22.01
HL13	3	19.92	77.18	79.37	23.16
	5	19.81	77.09	75.97	22.95
	7	19.77	77.08	73.46	22.91
	10	19.66	77.04	57.34	22.56
	15	19.54	77.01	55.40	22.51
HL14	3	19.93	77.60	76.62	23.90
	5	19.93	77.58	75.28	23.53
	7	19.83	77.28	70.87	23.16
	10	19.75	77.08	70.57	22.92
	15	19.68	76.92	70.03	22.65
HL15	3	19.77	78.21	79.30	22.86
	5	19.76	76.24	73.98	22.80
	7	19.76	76.09	71.09	22.63
	10	19.71	76.09	59.49	22.27
	15	19.70	76.09	56.42	22.11
HL16	3	19.86	77.08	82.91	23.51
	5	19.85	77.07	82.38	23.17
	7	19.83	77.06	76.36	23.03
	10	19.76	77.02	62.99	22.22
	15	19.62	77.01	62.24	22.08
HL17	3	19.75	76.64	75.72	24.45
	5	19.72	76.56	74.60	23.19
	7	19.66	76.47	72.15	22.87
	10	19.66	76.41	66.24	22.67
	15	19.60	76.04	63.68	22.34
HL18	3	19.96	76.60	76.60	22.84
	5	19.91	76.51	76.43	22.83

	7	19.71	76.46	76.47	22.53
	10	19.67	76.30	59.56	22.42
	15	19.60	76.12	58.21	22.02
HL19	3	19.72	76.16	84.19	24.64
	5	19.71	75.85	84.15	24.02
	7	19.66	75.53	75.50	23.77
	10	19.59	75.43	63.26	22.70
	15	19.59	74.91	62.24	22.53

Table 5. (continue)

Sample	Day	Remaining quantity in broth (ppm)			
		Zn	Co	Cr	Cu
HL20	3	19.74	77.37	87.45	21.80
	5	19.68	77.34	74.26	21.34
	7	19.63	76.51	74.03	21.16
	10	19.63	76.47	70.65	21.01
	15	19.62	76.08	70.23	20.68

IV. CONCLUSION

The major focus of this study is to isolate and identify bacterial that help recovery of surroundings from heavy metal contamination. 20 isolates of halo-tolerance microorganisms which could grow in medium contain 5 heavy metals (Cu, Cr, Co, Zn and Hg) were isolated from salt soil in Samut Sakorn province. Characters of colonies were varied. All of them had rod shape. Only HL 7 was Gram's negative. All of them were tested for the highest concentration of heavy metals that they could tolerance by Plate diffusion method in nutrient agar. Incubated at 37 ° C for 15 days. All of the isolates, HL1-20, could not grow on media which had Hg. The best Co reducing isolate was HL1, which reduced Co quantity from 77.68 to 73.76 (5.05 %). Reduction of Co conducted from the tenth day to the fifteenth day. The best Cr reducing isolate was HL7, which reduced Cr from 77.38 to 49.78 (35.66 %). The decline of Cr was continuous from the beginning to the last day of experiment (15 days). The best Co reducing isolate was HL1, which reduced Co quantity from 77.68 to 73.76 (5.05 %). Reduction of Co conducted from the tenth day to the fifteenth day. For Zn and Cu, all the isolates (HL1-20) reduced heavy metals at the decimal level, which was not significant. These four heavy metals (Cu, Cr, Co, and Zn) are used in a variety of industries. Exposure to these substances causes harm to health. Getting Zn into the body causes liver and kidney failure and may also cause anemia. Cr causes inflammation of the skin and irritate epithelium of the internal organs. Getting Cu into the body causes red blood cells breaking. If the body has copper accumulated in the amount of 25-30 mg/kg of body weight, the symptoms of cirrhosis and mania may occur. Getting Co into the body can cause allergies, asthma, difficulty breathing and also cause skin allergy. For this reason, wastewater contaminated with these four heavy metals needs to be treated before released to natural water source. Therefore, the microorganisms isolated from this experiment can be utilized to decrease the heavy metal pollutant in future. However, more study on this matter should be executed in order to reconfirm the bioremediation activity by these bacteria, the presence of the bacteria, as well as bacteria identification genotype and the concentration of heavy metal pollution in the environment.

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AUTHORS PROFILE

Jaruwan Chutrtong. Birth place: Bangkok Thailand. Bachelor degree: Bachelor of Science (B.Sc.) in biology (microbiology) fromSrinakharinwirot University, Bangkok Thailand in 1985. Master degree: Master of Science (M.Sc.) in microbiology from Kasetsart university, Bangkok Thailand in 1994. She worked as MEDICAL SCIENTIST in Regional Medical Sciences Center in Nakhon Ratchasima between 1985-1988. In 1988-1994, she worked as MEDICAL SCIENTIST in Department of Medical Sciences, Ministry of Public Health in Bangkok. After that, from 1994 – now, she is LECTURER in industrial microbiology department, faculty of science and technology Suan Sunandha Rajabhat University, Bangkok Thailand. Previous publications:

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Asst. Prof. Jaruwan Chutrtong: memberships – committee of Educational Professional Foundation.