Evaluation of Yeast Isolates as Indicators for Measurement of Bioavailable Cadmium

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***Abstract*—** Cadmium (Cd) in the environment significantly increased from the anthropogenic activities and its detection in the environment is essential. This study aims to characterize the phenotypic changes of 34 yeast isolates when grown with and without Cd. Seven isolates were able to grow on Endo agar (EA) with 30 µg/mL kanamycin (KAN) and Congo red agar (CRA) with 100 µg/mL vancomycin (VAN) when treated with 0-10 mg/L Cd. On EA with KAN, K12 showed a pink colony on agar without Cd but colorless on medium with Cd. Two isolates (K9 and K11) changed the color from orange to red colonies on CRA with VAN and 0-1 mg/L Cd after incubation for 48 h. The observed visible colors of yeast growth using different media indicated their certain sensitivity and specificity to bioavailable Cd. Thus, additional growth conditions will be further investigated and evaluated as the indicators of Cd in contaminated samples.

***Index Terms*—** Cadmium, Yeast, Phenotypic change, Sensitivity, Specificity, Biodetection.

I. Introduction

CADMIUM (Cd) is one of heavy metals that is highly toxic and widespread in the environment in recent decades and trends to accumulate in soils due to its low mobility and non-degradability. The environmental contamination with Cd has been increased from the anthropogenic activities which mainly caused by batteries industry, pigments, metal coatings plastics and mining [1]. In the environment, Cd commonly forms a complex with lead, zinc, sulfide and carbonate and normally is not present as a pure form [2]. Cd is efficiently bound by high molecular weight proteins including albumin and by non-protein sulfhydryl groups in the human body. Cd can accumulate in the kidney and liver and Cd increased in the kidney can also result in a higher calcium excretion leading to a higher risk of kidney stones [3][4].

The measurement of heavy metals effecting on the health and environment has been the subject of many studies. Cd is widely spread in the environment and can interact with the soil and living organisms through several pathways [5]. Thus, the effective detection of Cd in the contaminated samples could help reducing risk of living organism to its toxicity. A biosensor can be used to detect the bioavailability of heavy metals concentration using microbial and biomolecules, one of the most significant parameters regarding the environmental aspects [6]. Most importantly, their advantages include bioavailable measurement, inexpensive, sensitive and suitable for a field work [7].

In this study, phenotypic characterization of yeast grown on different media treated or untreated with Cd will be investigated. We proposed that the phenotypic change of yeast according to Cd response could be observed and indicate the amount of bioavailable Cd in the yeast cell. So, the altered phenotypes could be applied and developed as a tool for Cd detection.

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II. Materials And Methods

A. Yeast and selective media

The total of 34 yeast isolates were cultivated in nutrient broth (NB) with shaking 150 rpm at 30°C until it reached a mid-log phase. Then, 5 µL of yeast cell suspensions were dropped onto two types of media, Endo agar (1% peptic digest of animal tissue, 1% lactose, 0.25% dipotassium phosphate, 0.25% sodium sulfite and 0.05% basic fuchsin) with or without 30 µg/mL kanamycin (KAN) and Congo red agar. The Congo red agar test developed by Freeman et al. (1989) is based on the subculture of the yeast isolates on brain heart infusion agar (BHIA), supplemented with sucrose and Congo red dye and with/without 100 µg/mL vancomycin (VAN). Both media were added with 0-50 mg/L $CdCl_2$ and the yeast cultures were incubated at 30°C for 48-72 h.

B. Effect of lactose on yeast growth

Endo agar (30 µg/mL kanamycin) and Congo red agar (100 µg/mL vancomycin) containing various concentrations of lactose 1-5% (w/v) and supplemented with $CdCl_2$ ranged from 0-50 mg/L was used to detect $CdCl_2$ by yeast. After incubated at 30°C for 48-72 h, the change of colony color was observed.

C. Yeast cell preservation

The single colony of yeast was grown in NB at 30°C. The cell pellet was harvested by centrifugation at 7,500 rpm at 4°C and the supernatant was discarded. The 10^9 CFU/mL cell suspensions were prepared in 5, 10 and 20% (w/v) skim milk, then 30 µL was dropped on the paper disc or 100 µL were added into 1.5 mL polypropylene microtube. Then, the cell suspension was dried either using speed vacuum or freezing at -70°C and followed by freeze dryer for 24 h. After that, they were stored at 4°C until used for Cd detection on 48 well-microtiter plates.

D. Cd detection by preserved yeast cell

For the preserved yeast cell in the polypropylene microtubes, 100 µL of LB broth was added and mixed by vortex and incubation 30°C for 18 h and used as a starter. An amount of 10 µL re-suspended starter yeast cells were dropped on the EA and CRA agar and followed by the Cd solution. In contrast, the paper disc immobilized with yeast cells were directly placed on EA and CRA agar and dropped with Cd solution. After incubation at 30°C for 24, 48 and 72 h, the change of color colony or paper disc was observed.

III. Results

A. Growth and phenotype of yeast

On Endo agar, the total of 34 yeast isolates were able to grow when untreated with Cd but 20, 2, and 8 isolates did not grow when 12.5, 25, 50 mg/L Cd was present, respectively (Table. I). Four isolates (K10, K15, K30 and K31) were able to grow on Endo agar containing Cd at concentration higher than 50 mg/L. Fifteen yeast isolates were able to grow when treated or untreated with Cd on Endo agar with KAN. While five yeast isolates (K9, K11, K12, K26 and K34) grown when untreated with Cd and showed pink colony but they could not grow at concentration range of 12.5-50 mg/L (Table. I). Seven isolates (K7, K9, K11, K12, K14, K26 and K34) were able to grow and changed color of colony on Endo agar with various Cd concentration (0-12.5 mg/L). Three isolates (K9, K11 and K12) exhibited the red and pink colonies on Endo agar containing Cd concentration 0-6.25 mg/L but did not grow when treated with 12.5 mg/L Cd (Table. II).

Six isolates were able to grow on Congo red containing Cd below 50 mg/L, while 28 isolates were able to grow at Cd concentration above 50 mg/L. K4 is the only isolate that did not grow on Congo red after incubation for 24 h and a similar result was also observed when tested on Congo red with KAN. The 3 isolates (K12, K14 and K34) exhibited dark red colony on Congo red agar treated with 0-1 mg/L Cd but they were pallid red colony when treated with Cd at the concentration above 5 mg/L. Three isolates (K7, K9 and K11) showed a dark red colony when treated with Cd concentration below 5 mg/L after incubation for 48 h (Fig. 1).

B. Effect of lactose on growth of yeast

Out of 34, 3 yeast isolates (K7, K11 and K34) developed a red colony on the Endo agar supplemented with 2-4% (w/v) lactose and untreated Cd but they exhibited white colonies on medium treated with Cd concentration above 0.39 mg/L after incubation for 24 h. The white colony was observed on Endo agar containing 1 and 5% lactose either with or without Cd. While, 4 isolates (K9, K12, K14 and K26) showed the white colonies when Cd was above 0.39 mg/L on Endo agar containing 2-4% lactose after incubation at 30°C for 48 h (Table III).

TABLE I
CHARACTERISTICS OF 34 YEAST ISOLATES ON ENDO AGAR WITH OR WITHOUT 30 µG/ML KANAMYCIN AND TREATED WITH CADMIUM

Isolate	Growth							
	Endo				Endo with 30 µg/mL kanamycin			
Cd (mg/L)	0	12.5	25	50	0	12.5	25	50
K1	+	-	-	-	-	-	-	-
K2	+	+	+	-	+	+	+	+
K3	+	+	+	-	+	+	+	+
K4	+	-	-	-	-	-	-	-
K5	+	-	-	-	-	-	-	-
K6	+	-	-	-	-	-	-	-
K7	+	-	-	-	+	+	+	+
K8	+	+	+	-	+	+	+	+
K9	+	-	-	-	+	-	-	-
K10	+	+	+	+	+	+	+	+
K11	+	-	-	-	+	-	-	-
K12	+	-	-	-	+	-	-	-
K13	+	-	-	-	-	-	-	-
K14	+	-	-	-	+	+	+	+
K15	+	+	+	+	+	+	+	+
K16	+	-	-	-	-	-	-	-
K17	+	-	-	-	-	-	-	-
K18	+	-	-	-	-	-	-	-
K19	+	+	-	-	+	+	+	+
K20	+	+	+	-	+	+	+	+
K21	+	+	+	-	+	+	+	+
K22	+	+	+	-	+	+	+	+
K23	+	+	+	-	+	+	+	+
K24	+	+	-	-	-	-	-	-
K25	+	-	-	-	-	-	-	-
K26	+	-	-	-	+	-	-	-
K27	+	-	-	-	-	-	-	-
K28	+	-	-	-	-	-	-	-
K29	+	+	+	-	+	+	+	+
K30	+	+	+	+	+	+	+	+
K31	+	+	+	+	+	+	+	+
K32	+	-	-	-	-	-	-	-
K33	+	-	-	-	-	-	-	-
K34	+	-	-	-	+	-	-	-

Colony color: + growth, − no growth.

TABLE II
PHENOTYPE OF 7 YEAST ISOLATES WHEN GROWN ON ENDO AGAR (1% LACTOSE) AFTER INCUBATION AT 30°C FOR 24 H.

Isolates	Cd concentration (mg/L)				
	0	1.56	3.12	6.25	12.5
K7	colorless	colorless	colorless	colorless	no growth
K9	red	pink	pink	pink	no growth
K11	red	pink	pink	pink	no growth
K12	red	pink	pink	pink	no growth
K14	colorless	colorless	colorless	colorless	no growth
K26	colorless	colorless	colorless	colorless	no growth
K34	colorless	colorless	colorless	colorless	no growth

C. Cell stability

The survival of K9, K11 and K12 on the paper disc and the polypropylene microtubes showed a similar result and the number of viable cells was not different. The cells preserved with 5, 10 and 20% (w/v) skim milk and immobilized on polypropylene microtubes were able to survive better when dried using freeze dryer compared with a speed vacuum. While the cell survival was not different when preserved with 5, 10 and 20% skim milk.

The preserved cell on microtube of K9 and K11 were able to change from orange to red color on Congo red agar containing

100 µg/mL vancomycin and supplemented with 0-1 mg/L Cd after incubation at 30°C for 48 h. The K12 isolate was able to change from pink to red colony on Endo agar treated with Cd concentration at 0.1 mg/L or higher and showed a similar result between Endo agar with or without 30 µg/mL kanamycin (Fig. 2). In contrast, the yeast cell immobilized on paper disc did not show any changes in color. between untreated and treated Cd. The highest sensitivity was observed when a starter was prepared from the cells preserved on polypropylene microtube and the fresh culture was then tested for Cd detection.

TABLE III
DETECTION LIMIT OF CADMIUM (MG/L) BY 6 YEAST ISOLATES WHEN GROWN ON ENDO AGAR WITH 1-5% LACTOSE AFTER INCUBATION AT 30°C FOR 24 H.

Isolates	Lactose (w/v)				
	1%	2%	3%	4%	5%
K7	> 12.5	< 0.39	< 0.39	< 0.39	< 12.5
K9	> 12.5	< 12.5	< 12.5	< 12.5	< 12.5
K11	> 12.5	< 0.39	< 12.5	< 12.5	< 12.5
K12	> 12.5	< 12.5	< 12.5	< 12.5	< 12.5
K14	> 12.5	< 12.5	< 12.5	< 12.5	< 12.5
K26	> 12.5	< 12.5	< 12.5	< 12.5	< 12.5
K34	> 12.5	< 0.39	< 0.39	< 0.39	< 12.5

IV. DISCUSSION

K12 produced red colony due to the ability to ferment lactose on Endo agar containing 30 µg/mL kanamycin and Cd below 0.1 mg/L. Normally, yeast produces pink colony when grown under lactose fermentation. K12 exhibited colorless on Endo agar supplemented with Cd at higher concentration than 0.1 mg/L suggesting that Cd might be slightly toxic to yeast. It has been reported that many heavy metals are toxic to yeast cell during fermentation processes including copper, cobalt, cadmium, zinc, arsenic and lead [8]. However, the degree of toxicity depends on type, concentration and bioavailability of heavy metals.

K9 and K11 showed dark red colony on Congo red agar with 100 µg/mL vancomycin when treated Cd concentration below 5 mg/L. It has been reported that when yeasts produced a biofilm on Congo red, they showed many colony colors from brown to black. K9 and K11 showed colony colors ranging from red to dark red, indicating that they were the non-biofilm producers [9]. The cations can promote the biofilm formation by facilitating exopolysaccharide polymerization in *Staphylococcus epidermidis* [10]. The colony color modification of yeast was observed on Congo red when added with Cd suggests that at higher concentration, Cd may have the effects on growth and induction of biofilm formation. Hence, K9 and K11 are the potential isolates that should be further investigated under other growth conditions.

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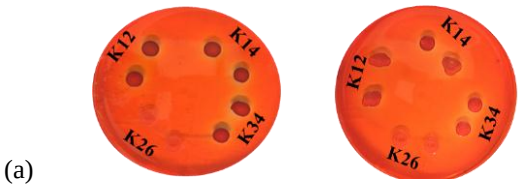

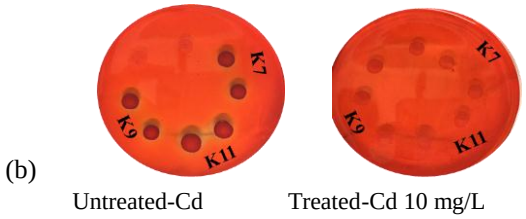

Fig. 1 Phenotypic characterization of 7 yeast isolates (a) K12, K14 and K34 immobilized on microtube exhibited dark red colony on Congo red agar with untreated Cd but pale color on agar treated with 5 mg/L Cd, (b) K7, K9 and K11 showed dark red colony on agar with untreated Cd but pale colony when treated with 10 mg/L Cd after 48 h.

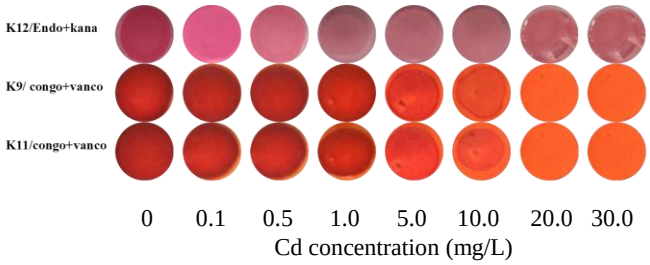

Fig. 2 Color change of K9, K11 and K12 when cells preserved on microtube were used as starters and tested on Endo agar and Congo red when tested with 0-30 mg/L Cd after incubation at 30°C for 48 h.

V. CONCLUSION

K9, K11 and K12 are the interesting yeast isolates because they behave and response differently to Cd. These 3 isolates changed colony color on Endo agar and Congo red when Cd was present at a certain concentration. The detection limit of Cd by K12 on Endo agar was below 0.1 mg/L after incubation at 30°C for 48 h. The phenotypic properties of some yeast isolates when grown under certain level of Cd observed in this study can be easily observed and defined, however their specificity on Cd has not yet been investigated. Thus, the genotypic features of yeast in response to Cd could give more supportive information and eventually, the detection tool could be developed and applied for the measurement of Cd in the contaminated samples.

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