



Research Article

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Isolation, antimicrobial activity and bioremediation of heavy metal Cadmium (Cd) by using lactic acid bacteria from Dadih Origin Lareh Sago Halaban, Payakumbuh, West Sumatera, Indonesia

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ABSTRACT

Isolation of Lactic Acid Bacteria (LAB) were taken from dadih lareh Sago Halaban, have been identified in MRS solid agar medium. The total colony found was 80×10^8 cfu/mL. Six colonies were picked as isolates (S1, S2, S3, S4, S5, S6), concerning their good growth and were identified as lactic acid bacteria based on the morphology, gram stained, and catalase test. Antimicrobial activity screening of LAB isolates were used disc-diffusion method against pathogen bacterias such as *Staphylococcus aureus*, *Salmonella thypii* and *Escherichia coli* in low pH. The six isolate have antimicrobial activity againts pathogen bacteria with varieties of clear zone, and one of them showed the potential as good probiotic and has ability to remove heavy metal of cadmium ions in the solution. The potential one isolate named S5.

Keywords: Dadih, Probiotic, Antimicrobial activity, Bioremediation, Cadmium

INTRODUCTION

Dadih is one buffalo dairy products traditionally processed in the area of West Sumatra. Dadih has a protein content of 39.8% which complete essential amino acids, calcium, and vitamins B and K, that produce from fermentation process. Lactic acid bacteria also consist of *Lactobacillus casei subsp casei*, *Leuconoctoc paramasentereroides*, *Enterococcus faecalis subspecies liquefaciens*, *Lactococcus lactis lactis sub sp* [1]. BAL on Dadih shows anti-mutagenic activity was also able lower blood cholesterol levels both in vitro about (34%) and in vivo [2].

Dadih consist of LAB are probiotics that used as a probiotic food. Probiotic food is beneficial to health used probiotic bacteria are in living conditions and available in high concentrations and minimal total colony about 10^8 CFU /g products[3]. Probiotic bacteria grow in intestine (adhesion) on epithelial cells and make colonies in human gut antagonism against pathogenic bacteria[4], and prevent colonization of pathogenic bacteria in wall of intestinal microbial[5]. Probiotics help to protect the host from various intestinal diseases and disorders while increasing the number of beneficial bacteria and make the balance steady again[6].

Cadmium and lead are both highly toxic metals. Cadmium is present in almost all foods and also cooking equipment, but the concentrations vary depending on the type of the area and the level of environmental contamination [7]. Food from plants generally contains higher concentrations of cadmium than meat, eggs, milk, dairy products, and fish[8,9]. Smoking is another major source of cadmium exposure. One cigarette may contain 1 to 2 µg cadmium, but this varies based on the brand. It is estimated that a person smoking 20 cigarettes per day will absorb about 1µg of cadmium daily. Oral exposure to Cd may cause renal damage[10], osteoporosis [11], and possibly prostate [12] and renal[13] cancer. Chronic exposure to even low levels of cadmium could also lead to adverse renal [14] and negative bone effects[10][14]. Many lactic acid bacteria (LAB) strains have been classified as probiotics due to their potential to inhibit bacterial pathogen and their ability to grow in the intestine. The aim of this work was to isolate lactic acid bacteria as probiotic and to determine antimicrobial activity and bioremediation of cadmium isolated from *dadih* fermented buffalo milk.

EXPERIMENTAL SECTION

Isolation and characterization of lactic acid bacteria from *dadih*.

Dadih samples were obtained from Lareh Sago Halaban, Limapuluh Kota, West Sumatera, Indonesia. For isolation of LAB, serial dilution technique was used. 1 g of sample was dissolved into 9 ml of MRS broth. After dissolving, they were shaken homogeneously and were incubated at 37°C for 24 hours in an aerobic condition. Serial dilution of 10^{-2} until 10^{-8} were made by pipetting 0.1 ml of previous dilution into 0.9 ml of peptone water. 0.1 ml of final dilution was inoculated to MRS agar plates and incubated at 37°C for 48 hours for bacterial growth. The plates were observed for appearance of colonies and number of colonies produced on plate. Bacteria were purified by streak plate method on MRS agar and incubated at 37°C for 48 hours and then maintained in refrigerator at 40°C until further analysis. Six colonies are randomly chosen. All isolated were initially identified with the classical microbiological methods of Gram stain, catalase reactions, and growth phase[15].

pH tolerance

LAB isolates are grown on MRS broth then incubated for 24 hours at 37°C. 1 mL of the culture was inoculated into 9 ml of MRS broth which has been set up pH of 3 and 4. Observe bacterial growth by using a spectrophotometer at 600 nm at 0, 2, 4, 12 and 24 hours[15].

Antimicrobial Activity of isolated lactic acid bacteria against pathogen bacteria

Antimicrobial activity of LAB against *E.coli*, *S. aureus*, and *S. typhi* was determined using disc diffusion assay. Six single isolated colonies were selected from MRS agar plates and transferred to grow in sterile MRS broth. The broth culture was incubated anaerobically at 37°C for 24h. The indicator microorganism (*E.coli*, *S.aureus*, *S.thypi*) were grown in Nutrient Broth at 37°C for 24h. Using sterile cotton swab, the indicator microorganism swabbed into the surface of MRS agar. The sterile paper disc (6mm) dipped into LAB culture and into sterile MRS broth as negative control, and put onto the surface of swabbed MRS agar. After 24 h incubation, each plate then evaluated and diameters of inhibition zone including diameter of the discs then measured.[16]

Bioremediation of Cadmium (Cd)

Effect of pH

Selected LAB isolates were cultured in the media 10 mL MRS broth and incubated for 48 hours. Supernatant was discarded, while the pellet was washed with buffer solution (*disodium hydrogen phosphate/potassium hydrogen phosphate*) twice. The pellet was then added 10 mL of sterile water which contain 10 mg/L of Cd with varying pH (2; 3; 4; 5; and 6). Vortex the suspension, and incubated at 37 ° C for 2-3 hours. The solution was then centrifuged, the supernatant was measured using Atomic Absorption Spectrophotometer (AAS)[15].

Effect of Cd Concentration

Selected LAB isolates were cultured in the media 10 mL MRS broth and incubated for 48 hours. centrifuged at a speed of 8000 rpm. Supernatant was discarded, while the pellet was washed with buffer solution (*disodium hydrogen phosphate/potassium hydrogen phosphate*) twice. The pellet was then added 10 mL of sterile water to set the pH 3 with varying concentrations of Cd (10; 30; 50 mg/L). Vortex the suspension, and incubated at 37 ° C for 2 hours. The solution was then centrifuged, the supernatant was measured using Atomic Absorption Spectrophotometer (AAS) [15].

RESULTS AND DISCUSSION

Isolation and characterization of lactic acid bacteria from dadih

The strains retained give small colonies of approximately white or milky color, smooth surface and a regular circular circumference were observed on solid medium. The microscopic examination reveals that the tested strains were gram positive, with a cellular *coccus* form associated in pairs or in chains (Table 1). Total colony was 80×10^8 cfu/mL. Six colonies were picked as isolates were assigned as lactic acid bacteria based on the morphology, gram stained, and catalase test. They were observed Gram positive *coccus* and catalase negative (figure 1). All isolates showed the exponential growth phase between 4-18th hours and stationary phase start from 18th hour (Fig. 1). The results demonstrated the same growth curve of isolation of *L.acidophilus* ATCC4796 [16].

Table 1. Morphological characteristics of the lactic acid bacteria isolated from Dadih in Lareh Sago Halaban

Strain's code	Cell's formed	Catalase test
S1	cocci	negative
S2	cocci	negative
S3	cocci	negative
S4	cocci	negative
S5	cocci	negative
S6	cocci	negative

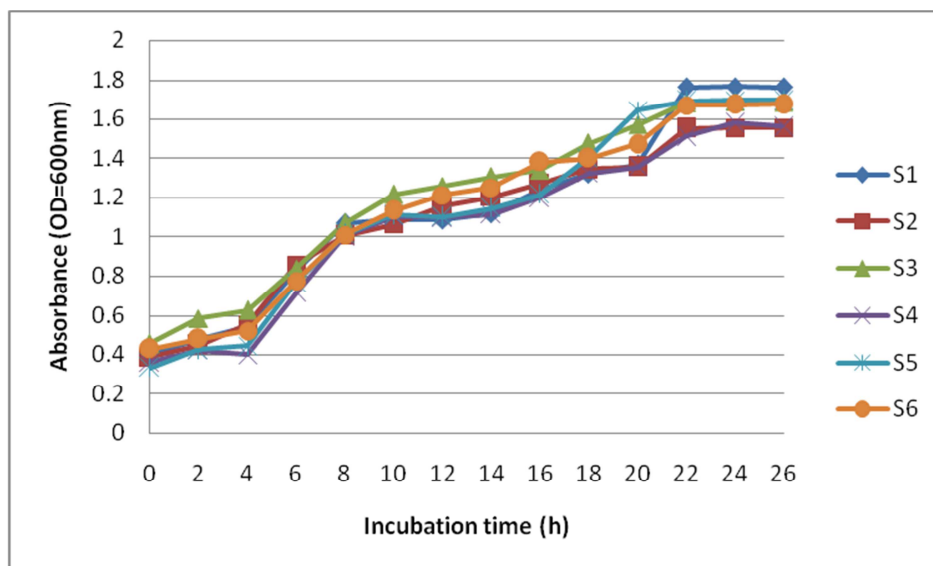


Fig 1. Growth curve of LAB isolates

pH tolerance

In this study, it was observed that LAB has the capability of resistance to acids. At pH 4, the cell density indicated by the absorbance was greater when compared with pH 3. If both are compared to the control, the growth of LAB is strongly influenced by pH (Fig. 2). In order to exert positive health effects, LAB should resist the stressful conditions of the stomach and upper intestine that contain bile [17]. Acidity is believed to be the most detrimental factor affecting the growth and survival of LAB, because their growth falls significantly below pH 4.5 [18]. However, the bacteria are still able to survive and these strains are expected to survive the acidic conditions in fermented food products or the stomach.

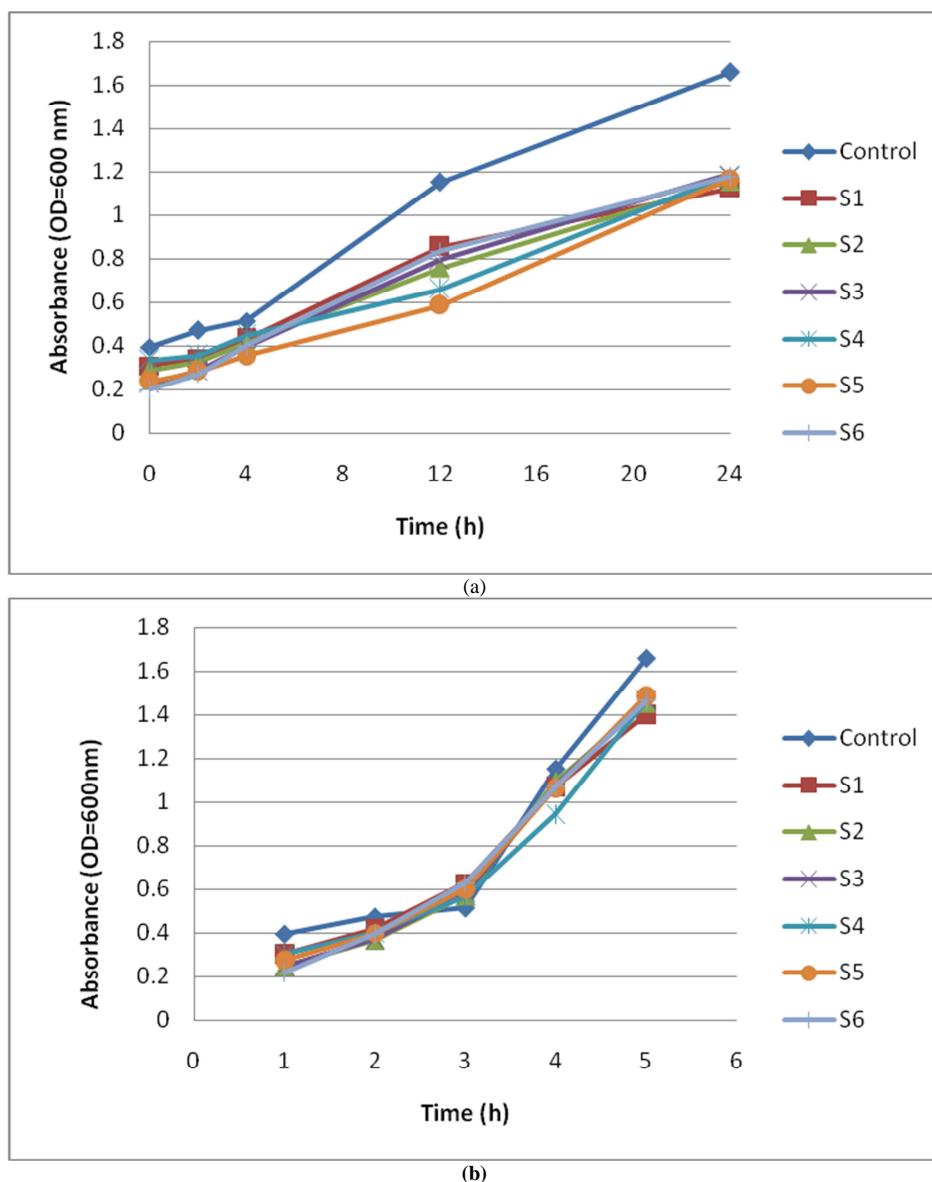


Fig 2. Effect of pH on the growth of LAB: (a) pH 3; (b) pH 4

Antimicrobial Activity of isolated lactic acid bacteria against pathogen bacteria

The antimicrobial activity was directly evaluated against food spoilage bacteria and food-born pathogen including three pathogen bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella thypii*) is shown in (Fig 3). expressed an inhibition growth of *Staphylococcus aureus*, *Salmonella thypii* and *E. coli* on six isolate (S1,S2, S3, S4, S5 and S6) The strongest inhibition zone against *S.aureus* was on S5 (22 mm) and the weakest was on S6 (12 mm). The strongest inhibition zone against *E.coli* was on S3 (21 mm) and the weakest was on S6 (8 mm). The strongest inhibition zone against *S.thypii* was on S1 (18 mm) and the weakest was on S6 (11mm). Generally the median values recorded by measuring the diameter of the inhibition zones, show that the Gram positive bacteria (*Staphylococcus aureus*) are sensitive to the compared to the negative gram bacteria (*E. coli* and *S.thypii*). Diameter inhibition of bacteria of gram-positive greater than gram-negative because gram-negative bacteria more resistant with antimicrobials compare gram positif because several mechanisms of resistance, such as natural permeability barrier properties on exterior layer can inhibits the entry of anti-microbial compounds, as well as the mechanisms inactivate specific resistance of the compound to prevent penetrate the cytoplasmic membrane or prevent binding to intracellular[19]. The results are almost identical by Syukur et al [16]. Antimicrobial activity of LAB against *E.coli*, *S. aureus*, and *S. typhii* was determined using disc diffusion assay six colonies (E1,E2, E3, E4, E5, and

E6) the result are inhibition zone against *S.typhii* were between 10.5mm-14mm, the strongest inhibition zone was on E1 and the weakest was on E4. The strongest inhibition zone against *S.aureus* was on E4 (14mm) and the weakest was on E1 (12.5mm). The strongest inhibition zone against *E.coli* was on E1 (12mm) and the weakest was on E3 and E5 (10.5mm). Generally *S.aureus* was more sensitive against LAB compared with *E.coli* or *S. typhii* [16]. Inhibition zone started to appear at 12 hours of incubation time and became more prominent at 24 hours incubation. The inhibition zone however depletes after 36 hours incubation maybe due to death phase of the bacteria thus producing unstable metabolite. The reason of LAB isolates which capable of inhibiting the growth of pathogenic bacteria is that it can produce lactic acid and acetic acid which is acidic to pathogens and thus suppress their growth. This metabolite was exerts by the bacteria during the early phase of their life cycle [20]. Overall, only three isolates (S1, S3, and S5) showed good antimicrobial activity; ± 17 mm against *S.thypi*, ± 21 mm against *E.coli*, and ± 22 mm against *S. Aureus*. Inhibition zone against *S. aureus*(Gram positive bacteria) are greater than on *E. coli* and *S. typhii* (Gram negative bacteria). The antimicrobial activity of lactic acid bacteria may be due to a number of factors. Among these are decreased pH levels, competition for substrates and the production of substances with a bactericidal or bacteriostatic action, including bacteriocins. The drop in pH arising from the production of lactic acid can be enough to inhibit certain strains. This is because the non-dissociated form of lactic acid triggers a lowering of the internal pH of the cell that causes a collapse in the electrochemical proton gradient in sensitive bacteria, hence having a bacteriostatic or bactericidal effect [21].

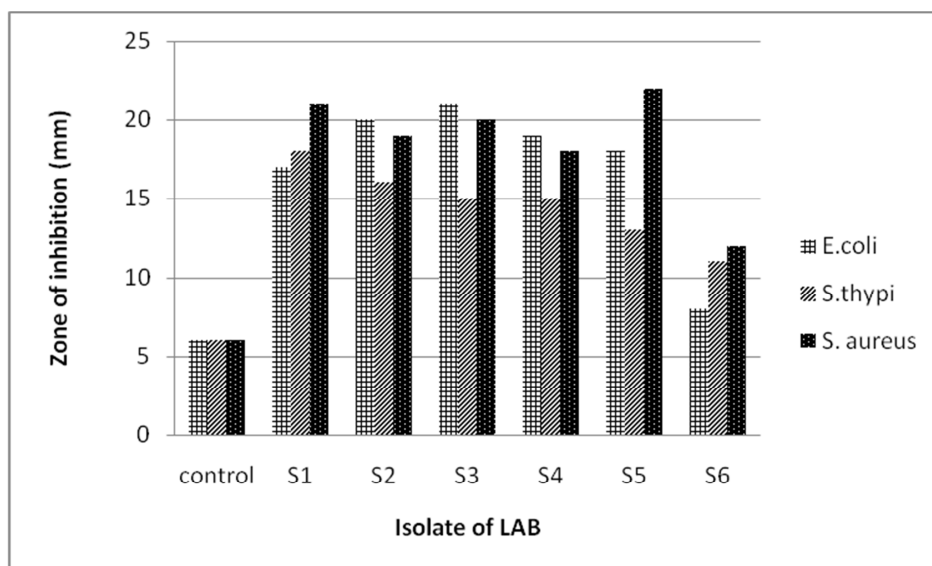


Fig 3. Inhibition zone of LAB isolates against pathogenic bacteria: S1-S6 = isolates

Bioremediation

pH effect

Isolate S5 shows greater absorption of the metal with increasing pH, which shows high absorption efficiency 87.70% on pH 6. This is according to *L.rhamnosus*, *L.fermentum*, *B.lactis* and *B.logum* tested on various pH 2, 3, 4, 5 and 6 get highest absorption at pH6 [22]. This is due to the competition on the bond between the negatively charged heavy metal cations and protons [23]. Some strain *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* produce exopolysaccharide containing different charged groups, including carboxyl, hydroxyl, oxyl, and phosphate, which make a greater percentage of negatively charged groups increase the number of ligands capable of binding cationic metals [24].

Concentration effect

Increasing cadmium concentration enhanced the binding of cadmium (Fig. 5). The increase in metal removal with increasing biomass may be explained by a higher number of binding sites. Incubation had no effect on metal removal, indicating that the binding process is energy-independent). Similar observations were made with a *Citrobacter* strain in lead, cadmium and zinc binding [20]

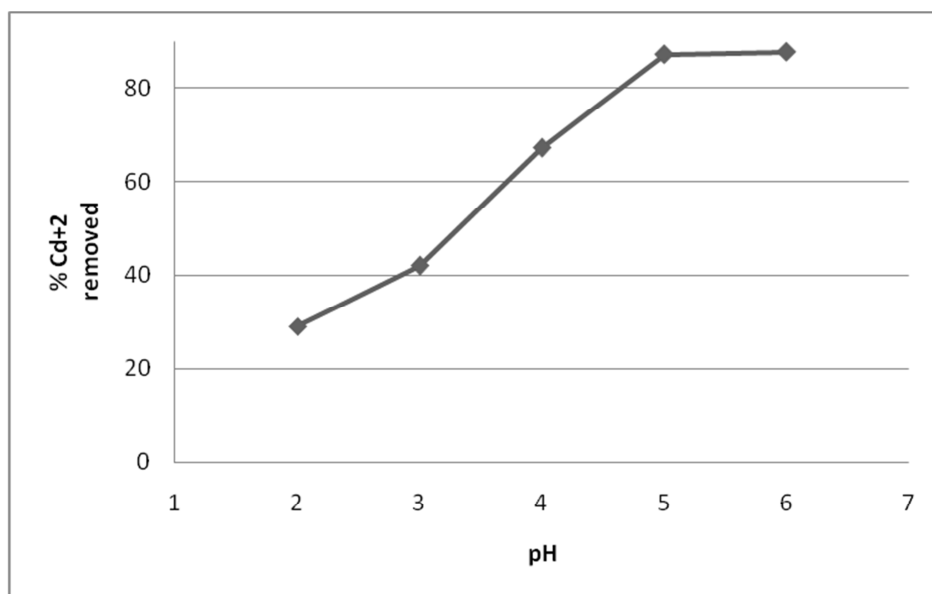


Fig 4. Effect of pH on cadmium binding by isolate S5, Cd ⁺² = 10 mg/L

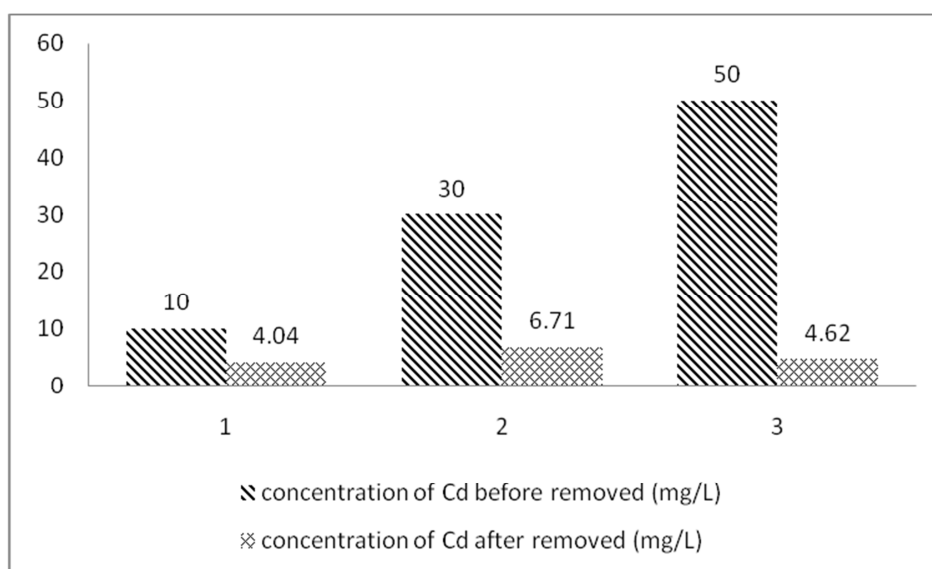


Fig 5 . Effect of concentration Cd ⁺² binding by isolate S5

CONCLUSION

All isolates that showed antibacterial activity against pathogenic bacteria and showed tolerance to acidic pH. It is claimed that they are potentially probiotic isolates. Growth of LAB slightly inhibited at low concentrations of Cd, whereas about 86,33 % of LAB growth is inhibited if given Cd concentration of 100 mg/L. Largest Cd uptake occurs at pH 6 with efficiency decreases with decreasing pH. The concentration of a given metal is also influential, the greater the concentration, the efficiency of absorption is reduced.

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