Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 3, 15-29, 2024

The Role of Resistance and Para-Probiotics in Nickel Biosorption by Probiotic Lactobacilli

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Received: 7 May 2024 Accepted: 24 August 2024

ABSTRACT: Nickel is a major pollutant of water and food, playing a destructive role in the health disorders of humans. Recently, probiotic bacteria have been recognized as a highly secure and eco-friendly approach to nickel biodetoxification. Four *Lactobacillus* strains, namely *L. brevis 205*, *L. mucosae* 226, *L. plantarum* 78, and *L. casei 303* were investigated to assess their nickel resistance through disk diffusion and MIC methods. Strains with the highest and lowest resistance were selected for Bioremediation assays including Biosorption, Desorption, and Bioaccumulation. *L. brevis* 205 and *L. casei* 303 exhibited the highest and lowest sensitivity to nickel, respectively. Both of them exhibited a plentiful performance in Biosorption assays, with 82.22% for *L. brevis* 205, and 72% for *L. casei* 303. The bioremoval assay with the para-probiotic (dead) biomass of the two strains exhibited a Biosorption yield of about 69% for *L. brevis* 205 and 75% for *L. casei* 303. Both probiotic and para-probiotic biomass demonstrated excellent nickel Biosorption capability and *L. casei* 303 para-probiotic biomass outperformed *L. brevis* 205. Thus, probiotic *Lactobacillus* strains of this study could be brilliant candidates for nickel bioremoval in water, food, and pharmaceutical industries, regardless of bacterial resistance or viability.

Keywords: Biosorption, Desorption, *Lactobacillus*, Nickel, Probiotic, Para Probiotic.

Introduction

Heavy metal contamination is one of the foremost important challenges within modern times, which has been pulled into consideration by edibles and environmental researchers (Jacob *et al.*, 2018). Nickel is one of the most common environmental pollutants, which is widely found in the human environment due to its wide use in various industries such as battery making, petrochemicals, steel containers, and even fake jewelry (Ray, 2009). Nickel moves around human habitation by industrial and manmade activities (Duda-Chodak & Blaszczyk, 2008) and enters into drinking and irrigation water. In turn, it intrudes in agricultural crops and edibles, causing different disorders or even life-threatening diseases (Henderson et al., 2012). So far, various solutions have been invented and applied to clean nickel from edibles and

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environment, which can be classified into three physical, chemical, and biological methods. Some physical methods include reverse osmosis, sedimentation, and membrane filtration. Chemical methods are also performed utilizing solvents and chemical compounds (Yadav *et al*., 2021). Although there have been many innovations in this field, due to the possible hazards caused by chemicals and the leaving of toxic compounds in the environment, these methods do not have enough reliability for food and medicinal uses (Aryal & Liakopoulou-Kyriakides, 2015). Currently, biological methods that are performed especially by using microorganisms have attracted the attention of researchers in this field (Vaid *et al*., 2022). Microorganisms, with exceptionally different metabolic pathways and interesting removal techniques, have an extraordinary capability to evacuate heavy metals like nickel (Massoud & Zoghi, 2022; Yaashikaa *et al*., 2022). For years, chemolithotrophic environmental bacteria have been successfully used to clean different environments. These bacteria consume various polluting chemical compounds as a source of carbon and energy and convert them into unharmed or reduced toxicity compounds (Verma & Kuila, 2019). Unfortunately, they could not be used in drinking water and food detoxification due to their special growth conditions and their probable pathogenicity (Monachese *et al*., 2012a).

Lactic acid bacteria (LAB) are a diverse group of probiotic bacteria that are especially popular in the food, dairy, and fermentation industries (Cui *et al*., 2015; Gänzle, 2015; Grujović *et al*., 2022). Some of the LAB's beneficial impacts on human health include stimulating and inducing the immune system, improving intestinal inflammation, and preventing various allergies (Kerry *et al*., 2018). Moreover, LAB have exceptionally great capacities within the field of removing heavy metals (Afraz et al., 2021; Gasong *et al*., 2017). Cellular polymers like the cell wall and EPS have superior performance as natural biosorbents. The functional groups on the cell walls of LAB include carboxyl, phosphonate, amine, and hydroxyl by which the adsorption of the metal ions to the cell surface takes place (Qu *et al*., 2022; Vijayaraghavan & Yun, 2008). EPS in LAB is composed of galactose, glucose, fructose, and rhamnose, which can be very fruitful in the Biosorption of heavy metals (Suresh Kumar *et al*., 2007). Heavy metals are deposited on the surface of LAB which are then transported into their cells using siderophores and other metal transporters and bioaccumulated. Also, LAB could convert hazardous metallic compounds into safer or less toxic molecules through various chemical and molecular pathways (Raklami *et al*., 2022).

Bacterial Bioremediation takes place through Biosorption and Bioaccumulation. Biosorption happens based on ionic interactions between the extracellular surface of the cells and the metallic ions. As a result, the quantity of impurities adheres to the cell's outer layer rather than undergoing metabolism reactions (Saba *et al*., 2019). Bioaccumulation is a more complicated strategy for heavy metal immobilization which is thoroughly dependent on the energy and metabolism pathways of the bacterial cell. Of course, both strategies appear to have profitable impacts on metal bio-removal (Chojnacka, 2010).

Polak-Berecka *et al*. in 2017, investigated 11 strains of *Lactobacillus with* different species for their resistance and Biosorption of cadmium ions. Strains had been isolated from the human gastrointestinal tract, previously. They

used $CdCl_2$ for the metal-resistance assay which culminated in 7 strains with the top resistance to $CdCl_2$. Then the results of the Biosorption assay revealed *L. gallinarum* and *L. crispatus* did the most cadmium bioremoval with an 85% rate (Polak-Berecka *et al*., 2017).

With many such examples (Bhakta *et al*., 2012a; Elsanhoty *et al*., 2016; Tian *et al*., 2015), it can be conceived that LAB have a successful detoxification potential of various chemicals and metals, including nickel and its compounds (Tian *et al*., 2012).

Meanwhile, the use of the dead biomass of these microorganisms has recently received special attention. The dead biomass could be an even better choice for Bioremediation assays by reducing the possibility of any type of harmful infections and cost-effectiveness (Tavana et al., 2020; Wang & Chen, 2009). There are reports of successful Bioremediation trials with dead biomass of bacteria in heavy metal bio-removal scope. Seltina et al. used *Streptomyces rimosus* for cadmium, lead, and nickel Biosorption. They treated the dead biomass of this bacterium with NaOH (0.1 M) and then used it through Cd-diluted solutions. As a result, they detected that 63.3 mg of Cd, 135 mg of lead, and 32.6 mg of Ni were removed by each gram of treated dead biomass, individually (Selatnia *et al*., 2004).

One critical factor frequently studied in metal Biosorption by bacteria is the metal resistance of the strain. Bacteria possess different mechanisms to resist metal ions, and the efflux pumps are the most common approach employed by bacteria to remove excessive toxic metal ions (Hobman & Crossman, 2015). Metal resistance tests have been a primary experiment in designating a strain for Biosorption assays, almost in all of the studies (Ahmed et al., 2017; Ameen *et al*., 2020).

With this background, we aimed to investigate two objectives: the correlation between nickel resistance in LAB and the rate of nickel Biosorption and the Biosorption capabilities of dead LAB biomass. Based on this, we investigated the resistance to nickel in probiotic *Lactobacillus* strains. Then using living and dead biomass of sensitive and resistant strains, we compared their nickel Bioremediation.

Materials and Methods

– *Lactic Acid Bacteria*

Four strains of *Lactobacillus* species including *L. brevis 205*, *L. mucosae* 226, *L. plantarum* 78, and *L. casei 303* (belonging to the Bacteriology department of Pasteur Institute of Iran) were selected for this study. These strains were extracted from the feces of healthy individuals and have been approved as probiotic strains after undergoing specific examination (Rohani *et al*., 2015). The strains were first recovered from the lyophilized stock and cultured on the specific medium of De Man, Rogosa, and Sharpe (MRS) broth. To refresh the strains, cultivation was carried out in three consecutive days. A gross culture was then prepared from a single colony of each strain, and the next day, the stocks of the strains were prepared in MRS-glycerol vials and stored in a freezer at -20° C to use in upcoming tests.

– *Nickel solutions*

Nickel chloride powder (NiCl2, 6 H2O) (Merck, Germany) was purchased. 50 grams were then weighed and dissolved in 100 ml of double distilled water. The concentration of the prepared solution was determined using an atomic absorption spectrophotometer (AAS). The concentrations of 160, 120, 80, and 40 g/l

were then made from the original stock and named A, B, C, and D, respectively.

– ***Preparation of nickel resistance discs***

Using different concentrations of nickel chloride, nickel discs were made in four groups A to D, according to our previous study. Briefly, 40 microliters of each nickel solution were gently injected into each group of autoclaved discs using a mechanical pipette, and then the discs were left to be dried (Beglari *et al*., 2022).

– ***Nickel resistance test***

24-hour culture of studied *Lactobacillus* strains (*L. brevis 205*, *L. mucosae* 226, *L. plantarum* 78, and *L. casei 303*) were prepared for nickel resistance test. Briefly, the pellets of the strains were prepared from the bacterial culture using a centrifuge (5000 rpm, 10 minutes). The supernatant was then discarded and the pellets of bacteria were washed twice using double distilled water by a centrifuge. In the next stage, 0.5 McFarland suspension (1.5 x 10^8 cfu/ml) was prepared from each strain, and with the help of a sterile swab, was cultivated on MRS-Muller mixed medium agar. Each plate was divided into four areas and the prepared discs at different concentrations of nickel chloride were placed in the designated quadrants. At the end, the plates were incubated at 37°C for 24 hours. The day after, strains with the narrowest and widest growth inhibition zone were selected as the most and the least resistant strains of *Lactobacillus,* respectively. They were then applied to the MIC tests.

– ***MIC test***

Four LAB strains were prepared for the minimum inhibitory concentration (MIC) test using a higher concentration of nickel chloride. In brief, serial dilutions of nickel chloride from a 1000 g/L (4.2 M) solution were transferred into the 96-well plates. The first well took 300 mL of nickel chloride. The next wells received 150 mL of 0.5 McFarland standard (1.5 x 10^8 cells/mL) of LAB strains up to the tenth well. 150 mL of nickel chloride of the first well was then transferred to the next well after appropriate ups and downs and this procedure was repeated to the tenth well. Wells 11 and 12 were filled with MRS broth and were considered negative controls. The plate was incubated at 37°C for 18 hours. The lowest concentration of the nickel in which no growth was detected was designated as MIC of that strain (Cockerill, 2012). Strains with the highest and the lowest MIC values were selected for Biosorption assays.

– ***Bioremediation assays***

The Biosorption assay was performed according to Kinoshita's description, with minor modifications (Kinoshita, 2019). The whole stages of the Bioremediation assay are illustrated in Figure 1.

– ***Biosorption with active biomass***

- Two strains selected from the previous test were cultured in MRS broth and incubated at 37 ° C for 24 hours under aerobic conditions. The 24-hour cultivation process was carried out for 3 consecutive days.
- On the fourth day, the bacterial suspensions were centrifuged (6000 rpm, 4 °C, 5 min.). The supernatants were discarded and the pellets were washed two more times using distilled water.
- The pellets were resuspended in 5 ml of distilled water, and then their concentrations were adjusted to (OD_{600} = 1).

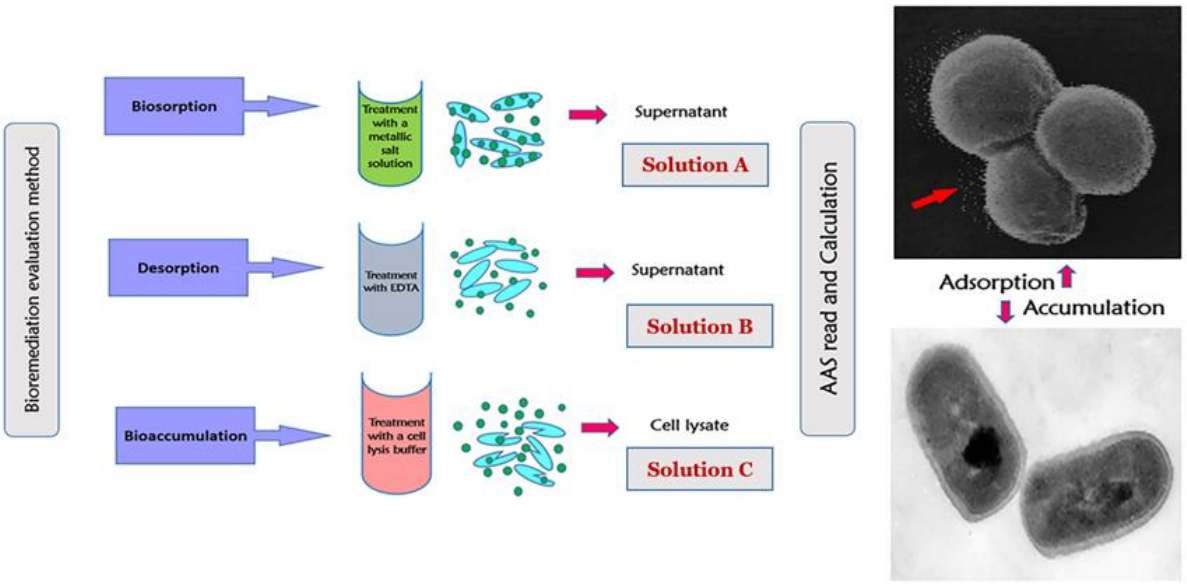

Fig. 1. Schematic picture of the Biosorption, desorption, and Bioaccumulation protocol of nickel. Absorption: bacterial cell pellets were treated with nickel chloride and incubated for 1 h during which, nickel ions were taken to the cell surface and/or accumulated inside the cell. The decreased nickel of the supernatant was estimated by AAS read. Desorption: cell pellets of the previous section were treated with EDTA to chelate the adsorbed nickel ions and free them from the solution. Then the nickel amount in the supernatant was estimated by AAS. Cell lysis: using a specific lysis buffer, bacterial cells were lysed completely, the accumulated nickel was released and AAS estimated the concentration of the nickel.

- 1 ml of the adjusted suspension of each strain was transferred to the sterile conical tubes.
- The suspensions in the conical tubes were centrifuged (6000 rpm, 4 °C, 5 min.) and the supernatant was discarded.
- Three ml of 5 ppm nickel chloride was added to the pellet of each strain and vortexed.
- The reaction suspensions in the conical tubes were incubated for one hour in a 37°C incubator.
- After 1 hour, they were taken out of the incubator, and after centrifugation with the previous pattern, their supernatants (solution A) were transferred to sterile conical tubes. The pellets were kept for the Bioaccumulation test.
- Supernatants were diluted with 500 microliters of 2% nitric acid.
- The samples were examined to determine the amount of remaining nickel in the suspension using AAS. The nickel chloride solution was used as the negative control.

The Biosorption rate of nickel by bacteria was calculated using the following equation:
Biosorption rate (%) = (NC value–sample value)/NC value X 100

(NC = Negative Control)

At the end, the amount of Biosorption of resistant and sensitive strains to nickel was determined. For each strain, each test was performed in triplicates, and the average read was analyzed as the final result.

– ***Time-dependent desorption assay***

A Biosorption assay was performed for each of the two strains in five conical tubes, following the same procedure as in the previous section. After collecting solution A from each conical tube, 10 mM EDTA was added to the remaining pellets and placed on a shaker (FINEPCR, speed 9) at room temperature for five continuous hours so that the metal ions attached to the surface of the cells were released and entered into the liquid around the bacteria by time passage. After each hour, one of the conical tubes of each strain was removed from the shaker, and centrifugation was performed (6000 rpm, 4 °C, 5 min.). The supernatant (solution B) was taken away for analysis of nickel ions desorbed from the cell surface. Finally, five samples were achieved for each strain which made ten samples in total. Each solution was analyzed using AAS to determine desorption timing and the percentage of ions attached to the cell surface (Bhakta *et al*., 2012b).

– ***Bioaccumulation assay***

The pellets of the previous stage were treated for cell lysis. The cells were first washed with 500 µl of a mixture of 2 mM EDTA and 30 mM NaCl (pH 8) and then resuspended in a special lysis buffer for *Lactobacillus* and rested for 2 hours in an incubator at 37 °C. The composition of the lysis buffer was: TE buffer (Tris-HCL 20 mM, EDTA 2 mM, pH 8.0), lysozyme (20 mg/ml), and Triton X100 (1% V/V) (Alimolaei & Golchin, 2016). After two hours of incubation at 37 °C, proteinase K solution (20 mg/ml) was added to the mixture and allowed to rest overnight at room temperature. At the end, the bacterial suspensions were centrifuged and the supernatants (solution C) were collected to be analyzed by AAS. This process determines the approximate amount of ions that have accumulated inside the cell.

– ***Biosorption test with inactive Biomass***

The performance of the dead biomass of the two *Lactobacillus* strains and their Biosorption ability was investigated for the selected strains. Briefly, bacterial suspensions with the concentration of OD_{600}=1 were prepared from the overnight culture of the two strains and then 500 µl of each strain suspension was transferred to a sterile conical tube. The intended conical tubes were then well-covered in aluminum foil and autoclaved. Thereafter, heat-killed and inactive biomass of the strains were obtained.

A Biosorption test was performed for each of the heat-killed strains (Para probiotics), according to the steps mentioned in the previous section. At the end, the conical tubes containing the samples were centrifuged and the supernatants were analyzed by AAS to measure the Biosorption yield.

Results and Discussion

– ***Nickel resistance tests***

– ***Nickel disks***

The results of nickel-resistant disks are exhibited in Table 1. There was no growth inhibition zone around disks C and D for all of the strains, which means that they were resistant to 80 and 40 g/l nickel chloride. *L. brevis* 205 and *L. mucosae* 226 were completely resistant to all concentrations of nickel chloride. *L. plantarum* 78 exhibited a growth inhibition zone only around disk A (160 g/l), and was resistant to other concentrations. *L. casei 303* exhibited the growth inhibition zone around disks A and B but was resistant to lower concentrations.

Table 1. Nickel disk resistance results

Nickel chloride Disks (g/l)	A	B	C	D
LAB strains	160	120	80	40
L. brevis 205	R	R	R	R
L. mucosae 226	S	R	R	R
L. plantarum 78	S	R	R	R
L. casei 303	S	S	R	R

– ***MIC test***

The results of the minimum inhibitory concentration of nickel chloride are exhibited in Table 2. The lowest MIC was detected for *L. casei* 303 with a MIC of 18 g/l, and the highest MIC belonged to *L. brevis* 205 with a value of over 300 g/l. These two strains were selected for Bioremediation assays.

– ***Bioremediation assays***

The whole results of Bioremediation assays are placed in Table 3, which will be described as follows.

– ***Alive Biomass***

The results of calculated nickel Biosorption and Bioaccumulation rates are illustrated in Figure 2. As is obvious, the Biosorption rate of *L. brevis* 205 is more than 82%. Interestingly, the Biosorption rate of *L. casei* 303 is 72%, which is a very agreeable Biosorption rate for a nickel-sensitive strain.

The live biomass of *L. brevis* 205 outstrips in Biosorption in comparison to *L. casei* 303 (*P=0.01*). Also, the Bioaccumulation assay ended in a stronger nickel accumulation for *L. brevis* 205 in comparison to *L. casei* 303. Nevertheless, despite the nickel sensitivity exhibited by *L. casei* 303, it demonstrated the capability to efficiently eliminate 72% and store 34% of nickel.

Table 2. The MIC test results for the studied LAB strains.

Probiotic strains	NiCl2 (g/L)										MIC(g/L)
	289	144	72	36	18	9	4.5	2.3	1.1	0.6	
L. brevis 205	R	R	R	R	R	R	R	R	R	R	>289
L. mucosae 226	S	R	R	R	R	R	R	R	R	R	289
L. plantarum 78	S	S	S	S	R	R	R	R	R	R	18
L. casei 303	S	S	S	S	S	R	R	R	R	R	18
R: Resistant S: Sensitive											

Table 3. Biosorption, Desorption, and the Bioaccumulation rate of the alive biomass of *L. brevis* 205 and *L. casei* 303.

Strains	Biosorption	Desorption	Bioaccumulation
L. brevis 205	82.22%	42.43%	40%
L. casei 303	72%	34%	30%

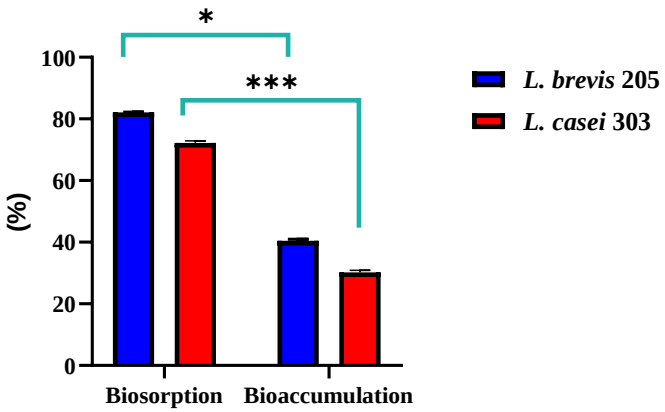

Fig. 2. Biosorption and Bioaccumulation of nickel by *L. brevis* 205 and *L. casei* 303. *L. brevis* 205 exhibited stronger nickel bioremoval and deposition than *L. casei* 303. However, Despite *L. casei* 303's nickel sensitivity, it was able to effectively remove (72%) and accumulate (34%) of nickel.

– ***Dead biomass***

Heat-killed, dead biomass of *L. brevis* 205 and *L. casei* 303 had good performance in the Biosorption test, which is illustrated in Figure 3, along with active biomass results together. The dead biomass of *L. brevis* 205 exhibited a Biosorption rate of 69%. The Biosorption rate of the dead biomass of *L. casei* 303 was 75% which even surpasses *L. brevis* 205. These results confirmed that even the dead biomass of *L. casei* 303 was almost as effective in Bioremediation as the live biomass. This indicates that the nickel sensitivity and killing of bacterial cells did not hinder the Bioremediation potential of *L. casei* 303. Indeed, the strain demonstrates exceptional efficacy in the field of nickel Bioremediation

– ***Time-dependent desorption***

During the five-hour experiment, *L. brevis* 205 consistently showed a high Biosorption rate, indicating that its cell surface held onto the nickel ions tightly without releasing them. On the other hand, *L. casei* 303 had a good Biosorption rate for the first three hours, but then experienced sharp desorption of nickel ions, meaning that its cell surface could only retain the nickel ions for three consecutive hours (Figure 4).

Nickel pollution is one of the most elaborate concerns of the industrialized world. Experts have designated bacterial biomass as an acceptable sorbent for heavy metals. LAB biomass, particularly that of probiotics, provides an affordable and safe alternative for chemical and mechanical filtration. They are nature-friendly agents and can be applied to water and edibles without side effects (Arjomandzadegan *et al*., 2014). Through the present study, we utilized probiotic LAB strains originating from healthy human microbiota. The safety and user-friendly nature of these amazing strains is one of the most important indicators that can encourage researchers to use them as food and water additives or pharmaceutical supplements.

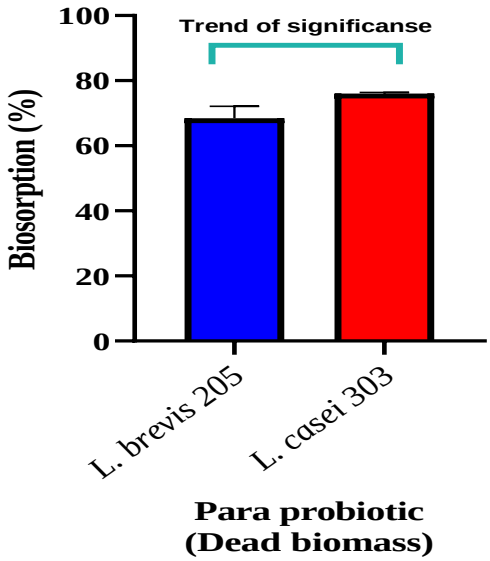

Fig. 3. Biosorption rates of the alive and dead biomass of the two strains. As it is obvious, there is a significant difference between the Biosorption of alive and the dead biomass of *L. brevis* 205, in which the alive biomass did the higher Biosorption than the dead biomass (*P=0.01*). *L. casei 303* had quite the same Biosorption rate with the alive and dead biomass. The dead biomass of *L. casei* 303, had even better Biosorption performance in comparison to the dead biomass of *L. brevis* 205, which exhibited a trend of significance (*P=0.08*).

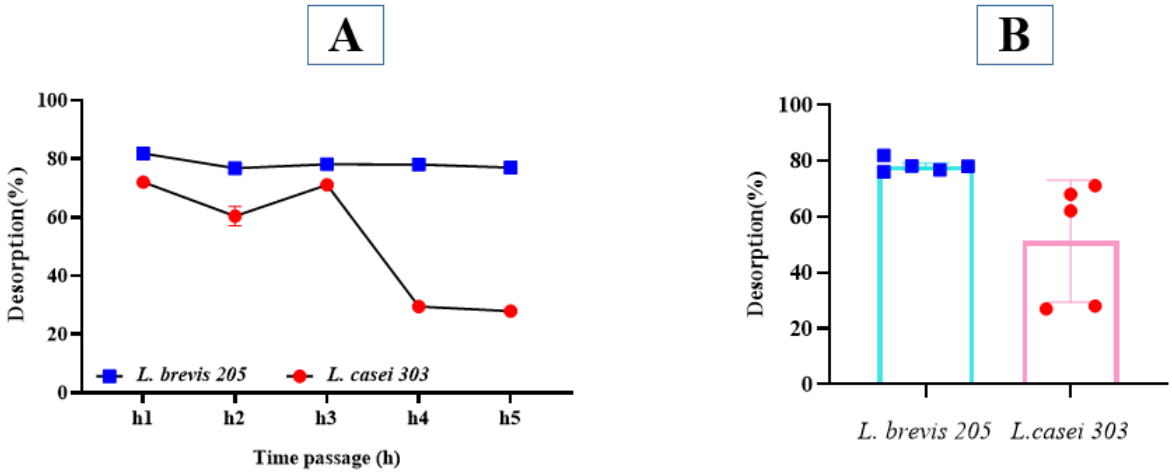

Fig. 4. Time-dependent nickel desorption for the two strains. A. *L. brevis* 205 had a consistent Biosorption rate through the one-hour intervals which means that the cell surface of this bacteria kept the nickel ions tightly and didn't release them in five hours. *L. casei* 303 also had a good Biosorption rate at the first three hours, and then the nickel ions desorbed sharply, so the cell surface could keep the nickel ions for three consecutive hours. B. The mean of desorption for the two strains. The consistent pattern of desorption in *L. brevis* 205 is illustrated in blue squares. In *L. casei* 303, the sharp release of the nickel ions from the cell surface is illustrated in 2 red dots. There was a significant difference between the mean desorption rate of the two strains (*P=0.04*).

The outcomes of our previous study validated their potential in Biosorption of heavy metals including nickel from their environment (*In vivo*) and the human gastrointestinal tract (*In vitro*) (Beglari *et al*., 2022). The Bioremediation assays of the present study showed that *L. brevis* 205 had a Biosorption rate of about 82% and the Bioaccumulation rate was 40%. Also, *L. casei* 303 had a Biosorption rate of 72% and a Bioremediation rate of 30%. Regarding other similar studies, our strains exhibited precious performance (Ameen *et al*., 2020).

There are plenty of studies covering the Bioremediation by probiotic bacteria. Belapurkar *et al*. (2016) conducted a study on the bioremediation potential of *Bacillus clausii* as a probiotic for chromium and lead resistance. Their findings indicated that *B. clausii* performed well at 525 ppm of chromium and lead (Belapurkar *et al*., 2016). Additionally, a probiotic strain, isolated from the *Stolepherous commersonni* microbiome, identified as *Bacillus siamensis*, exhibited tolerance to over 20 mg/ml of chromium, copper, and lead. This strain also demonstrated an 81% removal rate of textile dye effluent (Selta *et al*., 2022). This result is almost the same as our finding in which *L. brevis* 205 did a Biosorption of 82%.

The origin of probiotics is diverse and includes fermented foods, drinks, herbal resources, and even the sea and wastewater (Wierzba, 2015; Yi *et al*., 2017). Likewise, there is a diverse society of LAB species in the gastrointestinal tract of living creatures that are typically protective against pathogens and have numerous beneficial effects on the host's physiology and well-being (Walter, 2008). Some species are naturally resistant to chemicals and metals, making them useful for Bioremediation inside and outside the body (Monachese *et al*., 2012b). our probiotic strains also were isolated from healthy human guts with this great resistance to nickel (Beglari *et al*., 2022). To date, it was believed that to have

effective metal Biosorption, a bacterial strain must be resistant to heavy metals. That is why, metal resistance tests such as disk diffusion and MIC, have been an integral part of the strain screening for metal Biosorption in related research (Das *et al*., 2016; Gupta & Diwan, 2017; Kamika & Momba, 2013). However, due to the results of the present study, we disclosed that even a nickel-sensitive strain like *L. casei* 303, could perform an excellent nickel Biosorption. As a result, when it comes to selecting a strain for Biosorption, its resistance to the desired metal may not be a crucial factor. Based on current knowledge, this could be the first time that such a phenomenon has been reported, specifically concerning the metal resistance of bacteria and its direct correlation with the Biosorption of strains. The use of bacterial dead biomass has been the subject of recent studies to understand the amount and quality of Biosorption. In 2016, Malkoc and colleagues investigated the Biosorption rate and isotherms of Zn (II) with the live and dead biomass of bacterial strains isolated from ceramic factory waste. Out of 24 isolates tested, *Variovorax paradoxus* demonstrated the highest Biosorption rate of 92.7% and 91.3% with live and dead biomass, respectively. Also, both live and dead biomasses show an increase in Biosorption rate with higher pH and initial Zn concentration (Malkoc *et al*., 2016). In another study, Kumar Mohapatra and colleagues applied the live and dead biomass of the marine-isolated bacterium, identified as *Bacillus xiamenensis* PbRPSD202, to lead nitrate for Biosorption studies, individually. The bacterium exhibited significant resistance to a lead concentration of 2200 mg/l. Furthermore, the Biosorption rate of the studied bacterium was 99.19% and 97.18% for live and dead biomass, respectively. The authors believed that the small difference in the Biosorption rate of the live and dead biomass could be related to the metabolism-dependent pathway in the live biomass which imports additional metal ions onto the cytoplasm and accumulates inside the cell (Mohapatra *et al*., 2019). Therefore, the Biosorption rate of dead biomass could be even more plentiful than that of alive biomass (Polak-Berecka *et al*., 2017). These results confirmed our findings in which the dead biomass Biosorption of *L. casei* 303 was almost as functional as the live biomass, even higher than it. This data is precious because the dead biomass will have fewer possible side effects while used as a biosorbent in water and food detoxification projects. Furthermore, using the dead biomass of the bacteria could be a logical solution for the bacteria's probable toxicity, low cost, low energy demand, and recycling the biomass for several times usage (Ayele *et al*., 2021; da Rocha Ferreira et al., 2019).

Another crucial aspect of designating a strain for Biosorption is the time that the bacterial cell keeps the metallic ions on its surface, which is very important (Singh *et al*., 2008). In fact, desorption and resorption of bound metals from bacterial surfaces are crucial for practical biomass applications such as water treatment. The length of time that the cell can keep metal ions on its surface is a sign of the high ability of the strain in Biosorption. On the other hand, the desorption must occur at the appropriate time to collect the metal ions and reuse the biomass (Teemu *et al*., 2008). Considering this issue, we inspected one-hour intervals (of a total of five-hour Biosorption) to understand the desorption pattern of the studied strains. Our results showed that *L. brevis* 205 can consistently maintain nickel ions for up to five hours. As a result, it could be a viable

candidate for Biosorption projects without the need to recycle and reuse biomass. Also, it was observed that *L. casei* 303 retained nickel ions for a shorter period, and desorption occurred rapidly after three hours. This indicates that *L. casei* 303 has favorable Biosorption and desorption timing, making it an efficient strain for Biosorption projects. Other studies achieved likewise findings. Hossain and colleagues studied the desorption kinetics using banana peel for the Biosorption of Cu(II) from water. They reported a rapid Cu(II) adsorption of 30 minutes on the biosorbent and the ions were consistently adhered to the biosorbent for up to three hours (Hossain *et al*., 2012). Our findings confirmed their report since *L. brevis* 205 exhibited a consistent nickel absorption of five consecutive hours, which is even more than their report. *L. casei* 303 performed a three-hour nickel absorption, that not as much as *L. brevis* 205, but considering Hossain et al.'s results, had an acceptable consistency for biosorption projects.

One of the concerns of live bacterial use in the environment is gene transfer through the native bacterial society, especially antibiotic resistance genes. There are reports of antibiotic-resistance gene transfer even from dead biomass (Kittredge *et al*., 2022). It is promising to note that *Lactobacillus* strains of the present study were isolated from healthy human gut microbiota and were examined accurately for probiotic features, such as bile salt resistance, antibiotic resistance, and the plasmids' existence, and were found to have probiotic properties and no antibiotic-resistance genes (Rohani et al., 2015). So, these strains could potentially be efficient for Biosorption projects, whether using alive or dead biomass. Further research will help to better confirm the findings of this study.

Conclusion

We studied *Lactobacillus* strains for their nickel resistance and Biosorption yield. The results showed that Biosorption was independent of resistance, and the nickel-sensitive strain had a 72% Biosorption rate, indicating an effective bioremoval. This result can reduce the existing restrictions on the selection of suitable strains for nickel Bioremediation and enable the use of sensitive strains in Bioremediation projects. Therefore, Using the alive and the dead biomass of the probiotic strains of the present study in food, pharmaceutical, and Bioremediation industries can solve safety concerns, such as rare infectious reactions while applying living cells or gene transfer in the environment.

Acknowledgment

The present study has been performed as a part of the Ph.D. thesis in the Bacteriology Department of Pasteur Institute of Iran. We thank the Bacteriology department and friends for their support with this work.

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