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Adsorption of *Lactobacillus acidophilus* on attapulgite: Kinetics and thermodynamics and survival in simulated gastrointestinal conditions

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ABSTRACT

In the present study, we investigated the optimum adsorption conditions, adsorption characteristics and surviva of *Lactobacillus acidophilus* adsorbed on attapulgite (ATT). Results of single factor experiments showed that the optimum adsorption parameters were ATT concentration of 10%, initial pH of 6.5 and temperature of 35 °C. From the static experiments of adsorption characteristics, we found that the experimental data were fitted best to the pseudo-secondorder kinetics and Langmuir isotherm models, and the adsorption was a spontaneous and endothermic process. Bacteria survival test showed that the microencapsulated *L. acidophilus* stored at −18 °C for 30 days achieved the highest survival rate (83.2% ± 5.8) and obtained better protection at simulated conditions of gastric pH and at high bile salt concentrations when compared with free bacteria. The study demonstrated that microencapsulation of *L. acidophilus* in ATT was an effective technique of protection against low-temperature injury and under simulated gastrointestinal environment.

1. Introduction

Over the last 30 years, there has been an increased interest in the role of probiotic bacteria in human health. In order for these bacteria to exert positive health effects, they have to reach their site of action alive and establish themselves in certain numbers. As a guide, the International Dairy Federation has recommended that the bacteria be active and abundant (at least 10^7 CFU/g) in dairy products (Commission on Macroeconomics and Health, 2001; Iyera, Kailasapathya & Peirisb, 2004). However, some studies indicated that the bacteria might not survive in high enough numbers when incorporated into dairy products (Kailasapathy & Rybka, 1997). Now, many studies have focused on the survival of these bacteria in dairy products under different product and storage conditions (Çabuk & Tellioğlu Harsa, 2015; Pitigraisorn, Srichaisupakit, Wongpadungkiat, & Wongsasulak, 2017). In the food industry, the use of microencapsulation that controls the release of active ingredients could provide great benefits (Dubey, Shami, Rao, Yoon, & Varadan, 2009). Some studies that have been reported on microencapsulation for probiotic bacteria include chitosan (Chávarri, Marañón, Ares, Ibáñez, Marzo, & del Carmen Villarán, 2010), sweet whey (Maciel, Chaves, Grosso, & Gigante, 2014) and gellan gum (Nag, Han, & Singh, 2011). In addition, cell immobilization, which is defined as the physical localization of cells to a certain region of space with the preservation of same desired catalytic activity was also applied to stabilize and protect probiotics (Kourkoutas, Bekatorou, Banat, Marchant, & Koutinas, 2004). For example, fruits, brewery spent grains and starch-gluten-milk matrix have been reported as supports for microbial immobilization (Kourkoutas et al., 2004).

As for immobilization by adsorption, the biocatalysts are held to the surface of the carriers by some physical forces. The advantages of this method have minor influence on the conformation of the biocatalyst. So there is no need for the utilization of chemicals which could cause damage to bacterial cells and the catalytic activity could be preserved (Panesar, Kennedy, Knill, & Kosseva, 2007). Clay mineral demonstrates characteristics such as lack of toxicity and chemical reactivity, which allow fixation of cells, and the mucoadhesive capability for molecules to cross the gastrointestinal barrier (de Paiva, Morales, & Díaz, 2008). Attapulgite (ATT) is a hydrated magnesium aluminum silicate mineral with one-dimensional fibrous morphology, moderate cation exchange

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capacity, rapid hydration rate, and excellent salt resistance (Shi, Yang, Han, Wang, & Lu, 2009; Wang & Sheng, 2005). It has an important application in adsorbents and photocatalysis (Chen, Du, Zhu, & Deng, 2014; Liu, Kang, Mu, & Wang, 2014; Liu, Wang, & Wang, 2012; Stathatos, Papoulis, Aggelopoulos, Panagiotaras, & Nikolopoulou, 2012). ATT can also be used as a supporter material to immobilize probiotic bacteria. However, its adsorption for probiotic bacteria has not been reported previously in literature. Therefore, in this study, ATT was used as a new supporter for immobilization of *L. acidophilus* cells. And the survivability of adsorbed probiotic bacteria in different temperatures, simulated gastric pHs and bile salt concentrations were also studied.

2. Materials and methods

2.1. Chemicals and microorganisms

Lactobacillus acidophilus was obtained from the Institute of Microbiology of the Chinese Academy of Sciences (Beijing, China). All chemicals were analytical grade and obtained from commercial sources.

2.2. Purification of ATT

The ATT sample was purified by sedimentation method according to Ma, Xu, Guo, and You (2004). Briefly, the sample was ground and repeatedly washed with sterilized distilled-deionized (DDI) water. The mixture was stirred with the addition of DDI water and then was allowed to settle. The upper portion of the suspension was centrifuged, dried at 100 °C and ground to pass through 300 mesh sieve (48 μm). The chemical compositions of the purified ATT were 1.29% CaO, 10.47% Al_2O_3, 1.52% Na_2O, 20.41% MgO, 64.31% SiO_2, 0.13% K_2O and 0.87% Fe_2O_3.

2.3. Adsorption of L. acidophilus onto ATT

The L. *Acidophilus* culture in glycerol was propagated at 37 °C in 200 ml of deMan, Rogoda and Sharpe (MRS) medium for 72 h under anaerobic static conditions that were obtained in an anaerobic jar with an anaerobe container system sachet. The cells were separated from the culture medium by centrifugation (6000 rpm, 15 min), followed by washing twice with a sterile saline solution and centrifugation. The obtained L. *acidophilus* cells were adsorbed onto ATT and their adsorption percentage was determinated following the reporeted methods (Djukić-Vuković, Mojović, Jokić, Nikolić, & Pejin, 2013; Yee, Fein, & Daughney, 2000). A known wet weight of bacteria (W_1) was suspended in a 10 ml $NaNO_3$ solution (0.01 M, pH = 7) to which a known weight of ATT was added. The mixture prepared in this way was incubated at 30 °C with shaking at 90 rpm for a determined period of time. The separation of the unattached bacteria from the fraction containing mineral power and attached bacteria was accomplished by injecting a sucrose solution (60% by weight) into the bottom of the *L. acidophilus*-mineral suspension. The mineral powder with any adsorbed bacteria sank to the bottom of the test tube, and the unadsorbed bacteria and aqueous solution floated on top of the sucrose layer. After the sucrose separation, the suspension of unattached bacteria in the supernatant was pipetted out and its wet weight (W_2) was measured after centrifugation at 6000 rpm for 15 min. Control experiments were performed without the minerals present to determine whether adsorption occurs, and to quantify the efficiency of the separation technique. The control experiments demonstrated that 4% of the bacteria were lost during the separation procedure. Hence, 4% was subtracted from each experiment to account for separation efficiency. The adsorption percentage on the surface of ATT was calculated as shown in Eq. (1):

$$\text{Adsorption percentage } (\%) = W_1 - W_2/W_1 \times 100 - 4\% \quad (1)$$

where W_1 is the wet weight of initial bacteria including the adsorbed and unadsorbed bacteria, and W_2 means the wet weight of the unadsorbed bacteria.

2.4. Adsorption kinetics

The adsorption kinetics curves of *L. acidophilus* cells on the ATT clay were performed as follows: 1.0 g of ATT and 10 mg of bacteria were added to a 50 ml conical flask with a lid containing 25 ml of $NaNO_3$ solution (0.01 M, pH = 7). The flasks were shaken at 200 rpm in a constant temperature (35 °C). At intervals of 5 min, the unattached bacteria from *L. acidophilus*-mineral suspension were separated by using the sucrose method and the adsorption percentage was estimated as described in Section 2.3.

2.5. Adsorption isotherms

In order to obtain the adsorption isotherm of *L. acidophilus* on ATT, 1.0 g of ATT was added to 50 ml conical flasks containing 10 ml of bacteria solution with different initial concentrations (0.84–7.32 g/L) in $NaNO_3$ solution (0.01 M, pH = 7). The flasks were covered with lids and shaken at 200 rpm at temperatures of 25, 30 and 35 °C, respectively. After cultivation for 72 h, the unattached bacteria from *L. Acidophilus*-mineral suspension were separated by using the sucrose method and the adsorption percentage was determinated as described in Section 2.3.

2.6. Survival of adsorbed L. acidophilus under different temperatures

The survival of *L. acidophilus* adsorbed under the optimum parameters was determinated during 30 days of storage at 22, 5 and −18 °C, respectively. Samples were taken at 0, 10, 20 and 30 days. The bacterial count was made acoording to the method of Hrenovic, Ivankovic, and Tibljas (2009) with some modification. *L. acidophilus*-mineral complex (1 g) was placed into a tube containing 10 ml of sterile saline solution and crushed with a sterile glass rod and vigorously shaken with a mechanical shaker. The samples were serially diluted into 0.1% sterile saline solution and 0.1 ml was spread plated on MRS agar under aerobic conditions at 37 °C for 48 h. The results were expressed as log CFU/g of samples. Free cells were used as blank control. The survival rate of *L. acidophilus* was calculated by applying Eq. (2):

$$\text{Survival rate} = N_t/N_{FD} \quad (2)$$

where N_{FD} and N_t were the numbers of viable cells before and after storage. The test was done in triplicate.

2.7. Survival of adsorbed L. acidophilus in simulated gastrointestinal conditions

The milk-based medium (12% non-fat skim milk, 2% glucose, 1% yeast extract, and 0.05% cysteine) was used to simulated gastrointestinal conditions as described by Shah, Lankaputhra, Britz, and Kyle (1995). The adsorbed and free bacteria were added to the milk-based medium that had been adjusted to pH 2.0, 4.0 or 6.0 with 5 M HCl or 1 M NaOH in 10 ml aliquots. Samples of individual treatments were then incubated anaerobically at 37 °C and sampled every 60 min. Survival rates of free and adsorbed bacteria were calculated as described in Section 2.6. The resistance to bile salts