

Kinetics Characterization of Taurocholic Transport in *Lactobacillus reuteri*

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Abstract. Taurocholic acid transport in *Lactobacillus reuteri* CRL 1098 was determined. The bile acid is incorporated inside the cells by an active and saturable transport showing a typical kinetics of Michaelis-Menten with values of K_m and V_{max} of 0.35 mM and 20 mM, respectively.

Bile acids are synthesized from cholesterol in the liver, conjugated to either glycine or taurine, and then released into the intestines, where they facilitate fat absorption by the epithelial cells. These salts are modified by numerous microbial biotransformations in the gut [5], the first of which is the hydrolysis of the amide bond, releasing free amino acids and the primary bile acid. This reaction is catalyzed by the enzyme bile salt hydrolase (BSH).

Lactobacillus reuteri, an enterolactobacillus, has been proposed as a dietary adjunct because of its beneficial properties on the health of the gastrointestinal tract [3, 8] and its hypocholesterolemic effect [10]. In a previous work [9], we reported the close relationship that exists between the BSH activity and the cholesterol removal by *Lb. reuteri* CRL 1098. The BSH of this microorganism is a single, constitutive enzyme, which shows more activity in stationary-phase cultures and has an intracellular location [11]. Therefore, the conjugated bile acids have to be incorporated inside the cell for their further hydrolysis. Most studies in bile acids transport have been performed in intestinal mucosa [7] and hepatocytes [14], but scarce information is available in prokaryotes. Elkins et al. [4] have recently identified the genes encoding for bile salt hydrolase and transport in *Lb. johnsonii* 100-100.

The present work reports the uptake of taurocholic acid by *Lb. reuteri* CRL 1098 and its kinetics characterization.

Materials and Methods

Microorganism and culture conditions. The strain *Lb. reuteri* CRL 1098 used in this study was obtained from the culture collection of CERELA (San Miguel de Tucumán, Argentina). Cultures were grown in MRS broth [1] at 37°C for 16 h before use.

Taurocholic acid transport assays. Cells grown in 50 ml MRS broth at 37°C were collected by centrifugation, washed twice with 0.05 M phosphate buffer (PMB), pH 7.4, and resuspended in 5 ml ice-cold PMB. Cells were kept on ice. ¹⁴H-taurocholic acid (TCA) (0.3 mCi mmol⁻¹ 11.1 MBq mmol⁻¹) was added to 100 µg dry weight cell ml⁻¹ (amount of cells used for each assay), and the suspension was incubated at 37°C. 100-µl samples were withdrawn at intervals (0, 60, 120, 180, 240, and 300 s), filtered through 0.45-µm membrane filters (HAWP 02500, Millipore), and washed with 5 ml ice-cold PMB. Filters were dissolved in the scintillation cocktail (Hisafe3, LKB-Pharmacia, Uppsala, Sweden). Radioactivity was quantified with a scintillation counter (Wallac, LKB-Pharmacia, Uppsala, Sweden). The transport velocity was expressed as mmoles/min/mg dry weight.

To determine the effect of the growth phase, we harvested cells at both exponential (6 h) and stationary phase (16 h) by centrifugation at 12,000 g for 5 min, washed them twice with PMB, and resuspended them in acetate buffer 0.05 M, pH 5.2. The reaction mixture containing 1 ml cell suspension and 1 µl ³H-taurocholic acid (3.47 µCi/nmol) was incubated at 37°C. Samples taken each 30 s during 5 min were processed as described above.

Kinetics characterization. Cells grown in MRS broth at 37°C for 16 h were collected by centrifugation, washed twice with PMB, pH 7.4, and resuspended in acetate buffer 0.01 M (pH 5.2). Aliquots (1 ml) of the cell suspension were dispensed into Eppendorf tubes, and we added, separately, 1 µl ³H-taurocholic acid (3.47 µCi/nmol) in the following concentrations: 0.05, 0.075, 0.1, 0.2, 0.3, and 0.5 mM. Reaction mixtures were incubated at 37°C; samples were taken each 30 s during 5 min and processed as before.

Reproducibility. All results presented in this paper are the means of three independent assays. The variations were less than 10%.

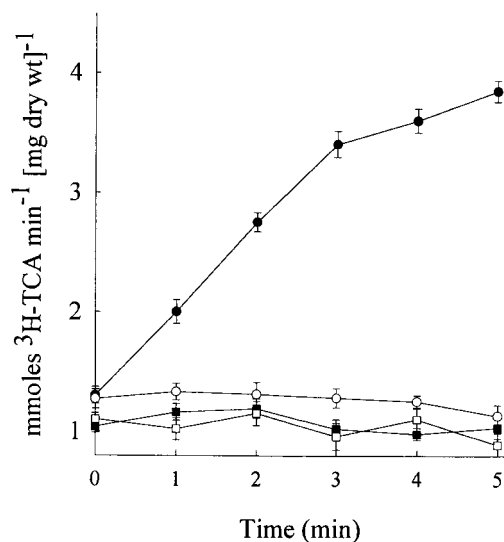


Fig. 1. ^3H -taurocholic acid transport in *Lb. reuteri* CRL 1098. (●) pH 5.2 at 37°C; (○) pH 5.2 at 0°C; (■) pH 6.8 at 37°C; (□) pH 6.8 at 0°C.

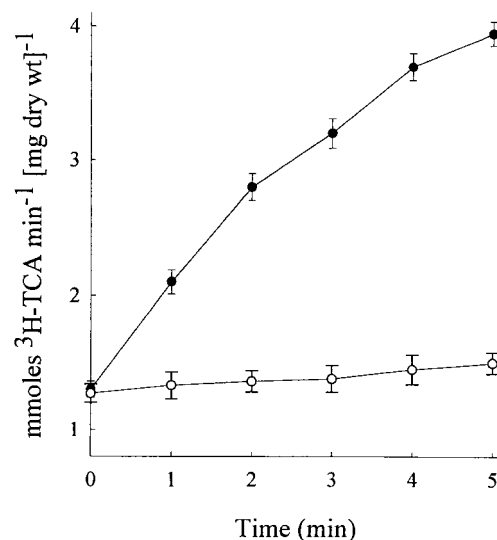


Fig. 2. ^3H -taurocholic acid transport in *Lb. reuteri* CRL 1098. Exponential (○) and stationary (●) phase cells in acetate buffer at pH 5.2 and 37°C.

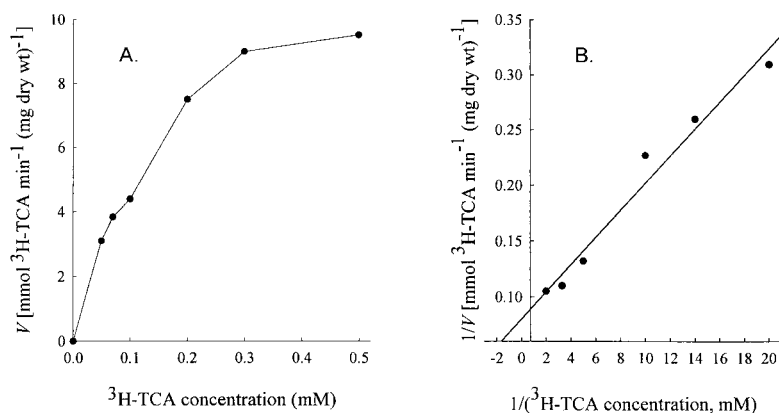


Fig. 3. ^3H -taurocholic acid transport in *Lb. reuteri* CRL 1098. (A) Rate of ^3H -TCA uptake as a function of the amount of substrate; (B) Lineweaver-Burk plot.

Results and Discussion

Figure 1 shows that ^3H -TCA is transported inside the cell at 37°C and at pH 5.2, but not at pH 6.8. Bile acid uptake was not detected at 0°C whatever the pH, indicating the absence of an unspecific adsorption of ^3H -TCA to the microbial cell.

It is interesting to note that pH 5.2, at which the ^3H -TCA uptake took place, is the optimum pH for the BSH enzyme of *Lb. reuteri* CRL 1098 [11], suggesting that both functions are closely related. This hypothesis is also supported by the fact that both BSH activity and the bile acid transport occurred in stationary-phase cells but not in cells harvested from the exponential-growth phase

(Fig. 2). These results support those reported by Elkins et al. [4], who suggest that genes encoding for bile salt hydrolase and transport in *Lb. johnsonii* would be arranged in an operon.

The ecological significance of bile acid uptake and further hydrolysis by lactobacilli is not well understood. It has been reported that lactic acid bacteria have different substrate specificity, depending on the amino acid moiety (glycine or taurine) on the C-24 position [2, 11]. According to Leer et al. [6], the protonated conjugated bile salts that enter the cell by passive diffusion may be converted to their weaker deconjugated counterparts. The latter compounds may then recapture the cotrans-

ported proton, preventing the excessive expenditure of ATP to maintain pH homeostasis. The question remains, however, what would be the factor that forces lactobacilli to hydrolyze conjugated bile salts, yielding unconjugated bile acids that are even more toxic than the conjugated substrates [12].

Figure 3a shows the incorporation of ^3H -TCA as a function of the substrate concentration. As is shown, the uptake of TCA by *Lb. reuteri* CRL 1098 is an active and saturable system, with a typical kinetics of Michaelis-Menten. According to the Lineweaver plot, a K_m value of 0.35 mM and a V_{max} of 20 mM (Figure 3b) were obtained. In contrast, Thanassi et al. [13] found that *Escherichia coli* exports deconjugated bile acids, suggesting that these molecules enter the cells passively. Results obtained in our study are the first evidence of an active transport of taurocholic acid in lactic acid bacteria.

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