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Seleno-Lactobacillus

An Organic Selenium Source

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ABSTRACT

Lactic acid bacteria are nonpathogenic bacteria commonly used in food processing. An evaluation was made of the capacity to concentrate selenium in species of Lactobacillus. A selenium concentration of 1 μg/mL in the culture medium yielded in a bacterial content of 400 μg/g dry biomass. Dialysis and TCA precipitation experiments of a native intracellular extract proved that at least 80% of the total selenium is associated with organic molecules. Seleno-cysteine was identified as the only seleno-amino acid present in the intracellular selenoproteins. This study shows that species of the lactic acid bacteria are able to concentrate selenium intracellular as seleno-cysteine, which could be applied in supplementation studies.

Index Entries: Selenium; seleno-cysteine; Lactobacillus; lactic acid bacteria, bacterial selenium supplement; selenoproteins.

INTRODUCTION

Bacterial seleno-enzymes have been characterized in *Escherichia coli*, and several *Clostridia* and *Methanococcus vanniellii*, illustrating a functional role of selenium in prokaryotes *(1)*. Strains of the eukaryotic microorganism *Saccharomyces cereviseae* incorporate selenium as selenomethionine in the protein moiety of this yeast *(2)*. In addition to inorganic compounds, such as selenate or selenite, selenium-enriched yeast and purified selenomethionine were used in supplementation studies with different results in bioavailibility *(3)*.

Lactobacilli are nonpathogenic bacteria, which are commonly applied in food processing. They can be found in dairy products, but also

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in the human body, since these species are important for the maintenance of a balanced microflora *(4)*. In this study, an evaluation was made to investigate if Lactobacilli could concentrate selenium intracellular in an organic form.

MATERIALS AND METHODS

Three species of Lactobacilli were used in this study, namely, *Lactobacillus delbrueckii ssp bulgaricus* (L.b., ATCC 11842), *Lactobacillus plantarum* (L.p., ATCC 14917), and *Lactobacillus casei ssp casei* (L.c., ATCC 393). Bacterial cultures were made in a cheese whey medium in the presence of several selenium concentrations. Selenium was added as sodium selenite (Merck). To produce labeled bacterial selenoproteins, cells were cultivated in a whey permeate medium in the presence of ^{75}Se-sodium selenite (Amersham). The culture medium was removed after centrifugation (1000*g*, 20 min), and the cells were washed repeatedly with phosphate buffer. Bacterial cells were disintegrated by ultrasonication in a Tris-HCl buffer (pH: 8.00). The cell debris was removed after centrifugation (15,000*g*, 15 min). Following lyophilization of dialysis-retentate fractions, derivatization of thiols was performed as described by Stadtman *(5)* and Allen *(6)* to yield carboxymethylated proteins. Following dialysis to remove excess of reagent the samples were hydrolysed (6N HCl, 1% phenol, 24 h at 110°C in vacuum). The HCl was removed by drying the samples in vacuum. The hydrolysates were reconstituted in 0.01*M* HCl, and thin-layer chromatography was processed. As a standard for the derivatized seleno-cysteine, a ^{14}C-carboxymethylseleno-cysteine was prepared as described by Tappel *(7)*. Autoradiography of the chromatograms was done with Hyperfilm (Amersham, 1-wk exposure time).

The selenium content of bacteria and cellular fractions was determined with flow injection hydride generation atomic absorption spectrometry (Perkin Elmer) *(8)*.

RESULTS AND DISCUSSION

Freeze-dried cells of Lactobacilli incorporated up to 400 μg/g selenium in the presence of 1 μg/mL selenium in the growth medium (Fig. 1A). Dialysis and TCA precipitation experiments proved that the majority of the intracellular selenium is bound to proteins with a mol wt above 3500 Dalton (Fig. 1B). Several ^{75}Se-selenopolypeptides were detected on the autoradiogram after SDS-PAGE in extracts of L.b. and also other lactic acid bacteria, such as *Streptococcus thermophilus* (data not shown).

Prior to carboxymethylation, intracellular protein was denaturated with reducing agents, such as dithiothreitol, guanidiniumchloride, and 2-mercaptoethanol. This treatment causes a loss of 40% of the total ^{75}Se

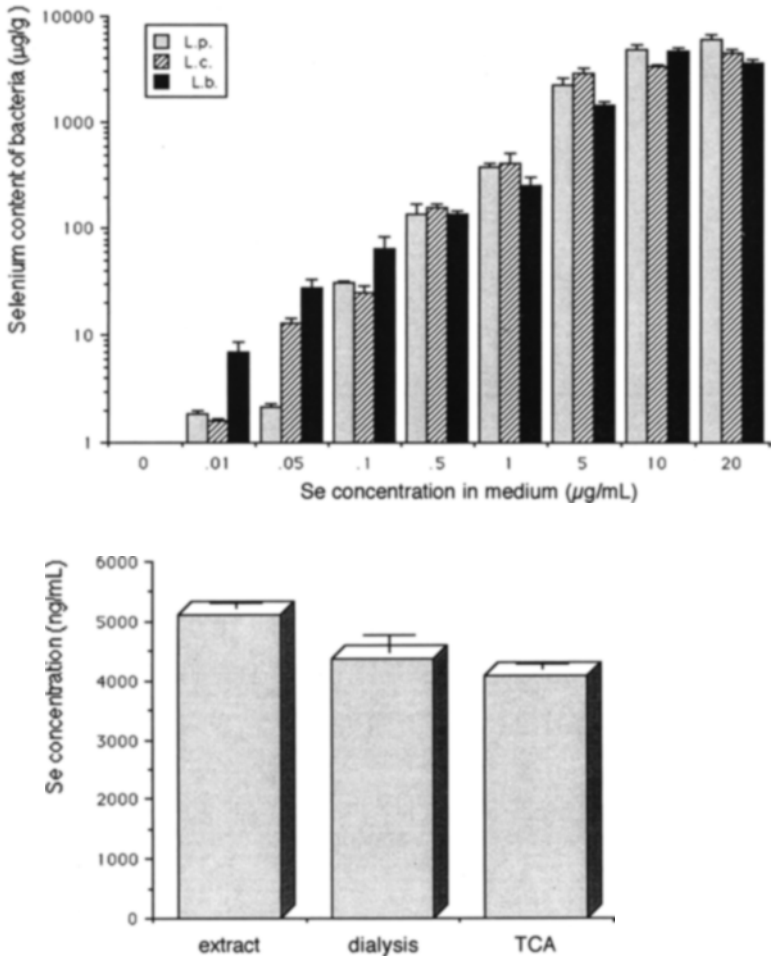

Fig. 1. Selenium concentration in whole cells of Lactobacilli and intracellular fractions. Upper: Effect of different selenium concentrations in the culture medium on the bacterial selenium concentration of three lactic acid bacteria: L.b., L.p., and L.c. The final selenium concentration in the growth medium is obtained by adding sodium selenite and is expressed as µg Se^{4+}/mL. Mean values are plotted ± SD (n = 3). For conversion of selenium to millimoles per liter, multiply by 0.01266. Lower: Selenium concentration in L.b.: a native intracellular, bacterial extract (=100%), after extensive dialysis (=86%), and in the precipitate obtained after TCA precipitation (=80%). Dialysis experiments were carried out with a 3500-Dalton mol-wt cutoff membrane (Amicon) for 48 h against Milli Q water at 4°C. The volume of the Milli Q water was 1000-fold the sample volume and was replaced each 12 h. TCA precipitation experiments were performed with a final concentration of 10% TCA at 4°C for 1 h. Denaturated proteins were precipitated by centrifugation (15,000 g). Mean values are plotted ± SD (n = 4).

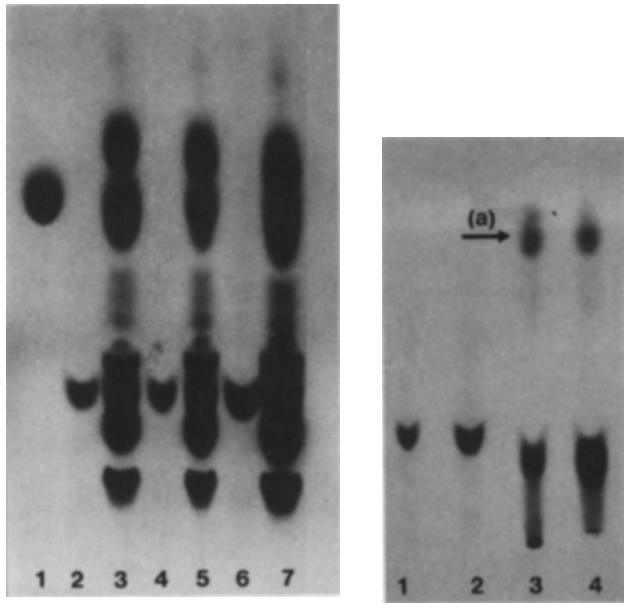

Fig. 2. Left: Ascending thin-layer cellulose chromatography (TLCC) of the ^{75}Se-bacterial protein hydrolysate with autoradiography. Lane 1: Se-methionine, 5 μg, ninhydrine visualization. Lane 2: Autoradiography of lane 3. Lane 3: ^{75}Se-protein hydrolysate, 9900 cpm spiked with Se-methionine (5 μg), ninhydrine reagent. Lane 4: Autoradiography of lane 5. Lane 5: ^{75}Se-protein hydrolysate, 9900 cpm, ninhydrine reagent. Lane 6: Autoradiography of lane 7. Lane 7: ^{75}Se-protein hydrolysate, 19800 cpm, ninhydrine reagent. Right: Autoradiography of the TLCC of the intracellular ^{75}Se-bacterial protein hydrolysate. Lane 1: ^{75}Se-protein hydrolysate, 9900 cpm. Lane 2: ^{75}Se-protein hydrolysate, 19800 cpm. Lane 3: ^{14}C-carboxymethylseleno-cysteine, 27490 cpm. Lane 4: ^{75}Se-protein hydrolysate, 9900 cpm, spiked with ^{14}C-carboxymethylseleno-cysteine, 27490 cpm. (a) Residues of the reagent: ^{14}C-Iodoacetate. Solvent composition was *tert*-butyl alcohol: methylethyl ketone: 88% formic acid:water.

after extensive dialysis, most likely because of the reduction of selenotrisulfides. However, 60% of ^{75}Se present in the intracellular proteins is incorporated as a single seleno-amino acid (Fig. 2A, B). Identical **Rf** values were found for this bacterial seleno-amino acid compared to a prepared ^{14}C-carboxymethylseleno-cysteine marker proving that selenium is present as seleno-cysteine in intracellular selenoproteins.

CONCLUSIONS

L.b. is able to incorporate selenium as seleno-cysteine into intracellular proteins, which could suggest that this bacterium metabolize Se specifically, since it was shown that the incorporation of seleno-cysteine into selenoproteins is directed by a specific UGA "sense" codon *(8)*. Up to now, seleno-cysteine-containing proteins were not used in humans or animals as a selenium supplement. Therefore, selenium-enriched lactic acid bacteria, such as Lactobacilli, are interesting to apply in selenium supplementation studies, since it would imply the addition of seleno-cysteine-containing bacterial protein to the diet.

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