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**Division:** Food Microbiology

## Probiotic Microcarrier: A Continuous Folate Producer

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## Abstract Body:

The recommended daily intake of folate (B-complex vitamin) for an adult varies between 200-400  $\mu$ g, being the intake of folate inefficient due its extremely unstable chemical forms.

The aim of this work is the creation of model to folate *in situ* production using probiotics. However, three main issues need to be overcome: (a) probiotic bacteria should be protected towards the gastric medium (encapsulation); (b) microcarriers size should be smaller than 100 µm, to avoid modifying food texture; and (c) microcarriers should adhere to gut epithelium in order to increase bacteria residence time.

Lactococcus lactis cremoris was grown in milk (30°C). Alginate-based microcarriers were produced and three layers were built using the layer-by-layer technique in that worder: poly-L-lysine; sodium alginate; chitosan. Confocal microscopy was used to confirm the consequent adhesion of the layers (poly-L-lysine/FITC; chitosan/rhodamine). After production the microcarriers where put into a 10 mL solution of KCI-HCI (pH 2 - 1 hour), at 100 rpm and then into a PBS solution (pH 7.2 - 3 hours) in order to mimic the passage through the gastrointestinal tract.

The utilization of free bacteria (LLC) in milk showed an increase of folate content in 4.73  $\mu$ g/L after 6 h. The average size of the microcarriers from 21.01  $\pm$  0.49  $\mu$ m to 39.84  $\pm$  0.79  $\mu$ m when the pH increased from 2 to 7.2. The size averages obtained were smaller than 100  $\mu$ m and showed a swelling capacity (particles duplicate their size upon passing from pH 2 to pH 7.2), being confirmed by confocal microscopy images the correct adhesion of the different layers after this experiment and the stability of the microcarriers.

Microcarriers produced through LbL showed great potential for encapsulation of probiotics, allowing their protection against harsh gastrointestinal conditions, predicting their use as a microcarrier for *in situ* folate production.

