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# Towards effective and stable probiotics

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**BACKGROUND:** Probiotics are live microorganisms, generally either lactobacilli or bifidobacteria, which when administered in adequate amounts confer a health benefit to the host [1]. Due to the growing evidence of health benefits associated with their use, probiotics are of increasing interest and represent now a significant growth area in the functional foods industry [2]. However, to be effective, orally administered probiotics should survive preparation of dosage forms and passage through acidic environment of the gastrointestinal tract (GIT). Reaching the intestine, these microorganisms should be able to establish themselves, remain viable and perform their beneficial actions. In this context, oral formulations have to protect probiotic bacteria from gastric acidity and delay their release in the small intestine in order to allow their complete release in the colon.

**OBJECTIVE:** To evaluate effects of starch formulations of lactobacilli on their survival in gastric environment and probiotic properties.

**METHODS:** Nineteen *Lactobacillus* strains belonging to the species *L. fermentum* (14 strains), *L. plantarum* (4 strains), and *L. rhamnosus* (1 strain), were isolated from dairy products and probiotics, and were used in this study. Lactobacilli were cultured in de Man, Rogosa, Sharpe (MRS) broth (Merck, Germany) under microaerobic conditions at 37°C.

Amylolytic activity of lactobacilli, cultured for 3–5 days on MRS agar supplemented with 1% soluble potato starch (SPS), was determined with iodine reagent (0.01 M $I_2$-KI solution).

Loading in starch was performed with *L. plantarum* 8PA3 bacteria ("Dry lactobacterin", Perm, Russia), which were resuspended to the concentration $10^{10}$ cells/mL in 10 mL of 0.85% NaCl solution and added to 90 mL of 2.5% SPS solution. Resulting mixture was frozen at –18°C and then lyophilized (Martin Christ Alpha 1-2 LDplus, Germany).

Atomic force microscopy (AFM) images of formulated *L. plantarum* 8PA3 cells were acquired in air by a Solver P47H atomic force microscope (NT-MDT, Moscow, Russia).

Starch swelling and dissolution was studied in simulated colonic fluid (SCF), prepared according to [3] and in distilled water (pH = 6.0) as control. Amylase from *Aspergillus oryzae* (A8220, Sigma) was added to the solutions to study the influence of amylase. The formulation form was examined visually during 14 h incubation time.

Fluorescence microscopy images were obtained with a Leica DM6000B (Germany) fluorescent microscope using Leica FW4000 software.

*L. plantarum* 8PA3 loaded in SPS were placed either in HCl solution (pH 2), or in 2% oxgall bile solution, or in 0.85% NaCl solution. Viability was tested after 2, 4 and 6 h incubation at 37°C by plating diluted aliquots onto MRS agar with subsequent counting of bacterial colony forming units

(CFU). In addition, viability was determined using LIVE/DEAD *Bac*Light bacterial viability kit L-7012 (Molecular Probes, Invitrogen) as described elsewhere [4]. Fluorescence in the stained samples was estimated with BD FACS Canto II (USA) flow cytometer or fluorescent microscope.

Nitric oxide (NO) production was assessed with DAF-FM DA and DAA fluorescent dyes as described earlier [4]. Each experiment was performed in triplicate.

**RESULTS:** In the present study we studied the probiotic composition comprising of SPS and bacteria *L. plantarum* 8PA3. We used AFM to confirm effective fixation of the cells to carbohydrate. The compositions were found to swell quickly (~5 min) in aqueous solutions either containing amylase, or not. Tested starch formulations disintegrated during the first 5-10 min of incubation in amylase solutions whereas in amylase-free probes dissolution was less intensive (after ~30 min). Amylolysis of starch excipients was less pronounced in aqueous amylase solution than in SCF, supplemented with amylase. None of 19 studied *Lactobacillus* strains hydrolyzed SPS when growing on MRS agar supplemented with it. The amount of viable *L. plantarum* 8PA3 cells formulated with SPS was high and did not change when stored for 6 months at 4°C. The bacterial viability tests also demonstrated that after 6 h treatment with 2% bile or HCl (pH 2) *L. plantarum* 8PA3 exhibited increased sensitivity (viability 14% and 0.4%, respectively). However, in similar conditions no significant differences were noticed between bacterial viability obtained for formulated with starch and non-formulated bacteria. Furthermore, we showed that loading into SPS had no effect on bacterial production of nitric oxide (NO) – a pluripotent regulatory molecule in human organism.

**CONCLUSIONS:** Overall, the results strongly support that formulation with polymeric matrices on the basis of SPS represent an appealing technology of probiotics production. It provides slow release of bacteria in target environment and does not alter their viability and NO biosynthesis. However, SPS excipient does not preserve the bacteria from harsh conditions of upper GIT. Therefore, we conclude that for oral administration the composition should be loaded in acid-resistant capsules.

Keywords: Probiotic, polymeric matrix, composition, soluble potato starch

**Conflict of interest statement:** The authors did not provide any information.

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