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Introduction

Dairy propionibacteria, commonly used as cheese ripening starters, are also consumed as probiotics in a freeze-dried form. Their probiotic potential includes modulation of the microbiota and epithelial proliferation/apoptosis equilibrium in the gut. They trigger apoptosis of colorectal cancer cells. Short chain fatty acids, propionate and acetate, their major metabolic end-products, are responsible for this programmed knock out of cancer cells, via the mitochondrial (intrinsic) death pathway. Such effects require high populations of live and metabolically active propionibacteria to reach the colon. In this context, appropriate vectors must favour probiotic efficiency. Fermented dairy products protect probiotic bacteria against digestive stresses. Emmental or fermented milks containing dairy propionibacteria may thus deliver live and metabolically active propionibacteria to the colon. Those matrices contain, however, a mixture of other microorganisms, limiting the identification of specific beneficial effects of propionibacteria. We aimed at 1) developing milk exclusively fermented by a dairy propionibacterium; 2) studying propionibacteria survival and tolerance to digestive stress in such a model dairy vector and 3) evaluating the pro-apoptotic potential of the fermented milk on cultured HT-29 human colorectal cancer cells.

Methods

Since dairy propionibacteria do not generally grow in milk, we determined their nutritional requirements with respect to carbon and nitrogen by supplementing milk ultrafiltrate with different concentrations of food grade lactate and casein hydrolysate.

Digestive stress tolerance was assessed *in vitro* towards acid challenge (pH=2) and bile salts challenge (1 g/L). Long term survival during 4°C storage was evaluated by enumerations and a live/dead staining method. Besides, the most tolerant *Propionibacterium freudenreichii* strain was used in an animal trial. Pigs were fed with the model fermented milk (1E+10 cfu/day), then *P. freudenreichii* survival was monitored in faeces and colon contents at slaughtering.

Pro-apoptotic potential of previously neutralised and sterilised fermented milk supernatants was evaluated on HT-29 cells, using usual indicators of apoptosis: cell viability assay, cell cycle analysis, activation of caspases, chromatin condensation and annexin V/7-AAD staining.

Results

Milk supplemented with 50 mM lactate and 5 g/L casein hydrolysate allowed growth of all species and subspecies of the dairy propionibacteria studied, which reached populations of at least 1E+9 cfu/mL.

In the model fermented milk, dairy propionibacteria remained viable and stress-tolerant *in vitro* during at least 15 days at 4°C. The population of the *P. freudenreichii* strain used in the *in vivo* trial reached 1E+7 cfu/g in the faeces and 1E+6 cfu/g in the colon content of pigs.

The model fermented milk was shown to induce apoptosis of HT-29 cells. Typical features of the apoptosis process were observed, including chromatin condensation, formation of apoptotic bodies, DNA laddering, ROS production, caspases activation, cell cycle arrest and emergence of a subG1 population.

Discussion

This work leads to the development of a new food grade vector containing dairy propionibacteria as the sole microorganism, allowing planning of preclinical and clinical trials in human. Such new fermented milks might be of interest in the future as a functional food to prevent colorectal cancer or to potentialize therapeutic treatments.