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Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese

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Abstract

This study aimed to compare phenotypic and genetic characteristics of *Lactobacillus rhamnosus* strains isolated at the end of the ripening of Parmigiano Reggiano cheese and to investigate an important prerequisite of probiotic interest, such as the capability to survive at low pH and in presence of bile salts. The use of API 50 CH, RAPD-PCR analysis and species-specific PCR allowed to ascertain the identity of 63 *L. rhamnosus* strains. Three *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese, *L. rhamnosus* ATCC 7469^T and the commercial strain *L.* GG were assayed to estimate the resistance to various stress factors reproducing in vitro some conditions of the gastro-intestinal environment such as low pH and different amounts of bile salts and acids. The behaviour of almost all the tested strains isolated from Parmigiano Reggiano cheese resulted analogous to that showed by *L.* GG.

Keywords: *Lactobacillus rhamnosus*; Cheese; Bile salts; Acidity; Parmigiano Reggiano

1. Introduction

During recent years, numerous studies have been undertaken to obtain scientific evidences for the beneficial effects of fermented foods containing probiotic bacteria [1,2].

Traditionally, probiotics have been utilised in dairy products such as milk or yoghurt and it has been hypothesized that milk enhances probiotic efficacy by providing lactose as a substrate [3].

At the present, a large number of dairy products are present on the market and are being promoted with health claims based on several characteristics of selected strains of lactic acid bacteria, particularly belonging to the genera *Lactobacillus* and *Bifidobacterium* [4].

To provide health benefits, the suggested concentration for probiotic bacteria is 10^6 CFU/g of a product [5]. However, some studies have ascertained low viability of probiotics in the market preparations [6,7].

Viability and survival of probiotic bacteria are the most important parameters in order to provide therapeutic functions. A number of factors have been claimed to affect the viability of probiotic bacteria in dairy foods such as yoghurt and fermented milks, including low pH and refrigerated storage [4].

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Moreover, the resistance to human gastric transit constitutes an important selection criterion for probiotic bacteria [8,9].

Micro-organisms ingested with food begin their journey to the lower intestinal tract via the mouth and are exposed during their transit through the gastrointestinal tract to successive stress factors that influence their survival [10,11]. The time reported from entrance to release from the stomach is about 90 min [12], but further digestive processes have longer residence times.

Cellular stress begins in the stomach, which has pH as low as 1.5 [13]. Bile secreted in the small intestine reduces the survival of bacteria by destroying their cell membranes, whose major components are lipids and fatty acids and these modifications may affect not only the cell permeability and viability, but also the interactions between the membrane and the environment [14,15].

Therefore before a probiotic can benefit human health it must fulfil several criteria such as the ability to tolerate acid and bile salts as well as to grow in the lower intestinal tract [16–19]. So, the first tool in the selection of a strain of probiotic interest is represented by in vitro methods aiming to ascertain the ability to survive passage through the upper gastro-intestinal tract and arrive alive at its site of action [20].

From this point of view, concrete advantages could be also obtained by using delivery vehicles with high pH values [21]. In this field, cheeses such as Parmigiano Reggiano could play the leading role, not only for their chemical and physicochemical properties but also for the particular technology of production, which is based on the use of cow's raw milk as a source of fermenting micro-organisms, and for the presence of high amounts of viable lactic acid bacteria at the moment of consumption [22].

The aim of this study was to obtain a reliable identification of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese by using API 50 CH test, RAPD-PCR and species-specific PCR and to ascertain the resistance of tested strains to bile salts and low pH.

2. Materials and methods

2.1. Isolation of lactobacilli from Parmigiano Reggiano cheese

Microbiological analyses were performed on 20 g of Parmigiano Reggiano cheese at the end of the ripening (24 months) withdrawn from 18 different producers.

Portions (20 g) from the centre of each cheese were first diluted in 180 ml of Ringer's solution and than homogenized (1 min homogenisation, 1 min break, 1 min homogenisation) in a blender (Stomacher 400, Seward Medical, London SE1 1PP, UK). Subsequent

Table 1
Phenotypic identification of 63 lactobacilli isolated from Parmigiano Reggiano cheese and of commercial strain *Lactobacillus* GG

	CO_2 production	Growth at 15 °C	Growth at 45 °C	Amygdaline	Arabinose	D-Arabitol	Arbutine	Cellobiose	Gentibiose	Gluconate	Glucose	Glycerol	Inositol	Identification
53 strains	0	53	53	53	0	0	53	53	52	53	53	0	21	*Lactobacillus rhamnosus*
10 strains	0	10	10	10	0	0	10	8	6	10	10	0	1	*Lb. paracasei* ssp. *paracasei*
Lb. GG	0	1	1	1	0	0	1	1	1	1	1	0	1	*Lb. casei*
	Lactose	Maltose	Mannitol	Melezitose	Rhamnose	Ribose	Saccharose	Salicine	Sorbitol	L-Sorbose	Trehalose	D-Turanose	Xylose	Identification
53 strains	53	41	50	53	53	42	37	53	44	23	7	24	0	*Lactobacillus rhamnosus*
10 strains	9	10	10	10	0	10	5	9	9	2	10	6	0	*Lb. Paracasei* ssp. *paracasei*
Lb. GG	0	0	1	1	0	0	0	1	1	0	1	0	0	*Lb. casei*