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Short communication

Construction of a reporter system for *Lactobacillus* sp. using the *gfpuv* gene

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Abstract

Lactobacilli are bacteria commonly associated with the gastrointestinal tract of animals and humans. They are able to produce antimicrobial substances such as bacteriocins, lactic acid and hydrogen peroxide, the factors which have been shown to be beneficial for controlling overgrowth of potentially pathogenic bacteria. For this reason lactobacilli are often applied in probiotics. The aim of present study was to construct a reporter strain based on the gfpuv expression system which can bee used as a strain with the ability to colonize the intestinal tract in future experiments.

Key words: GFP, *Lactobacillus* sp., reporter system

Introduction

Lactic acid bacteria belong to a group of Gram-positive microorganisms, which over centuries have been well known for their use in food fermentation. They are present in the environment and are an important element of intestinal microbiota of most Eukaryotic organisms (Felis et al. 2007). The key physiological feature of *Lactobacillus* sp. bacteria is their ability to produce lactic acid as the main final metabolic product. Among lactobacilli strains, many also produce bacteriocins, which are known as biologically active low molecular peptides that inhibit the growth of other bacteria, including some pathogens. Both features, production of lactic acid and bacteriocins, are the reason for which lactobacilli are an important component of many probiotic preparations regulating the composition of gut microbiota (Chin et al. 2001). The fitness of the strain as a potential probiotic depends on its ability to colonize the intestinal mucosa. This feature can be assessed by constructing a specific strain harboring a reporter gene, which can be used to check its ability to colonize the intestinal mucosa of experimental animals.

Materials and Methods

For strain construction, the following bacterial strains were used: *E. coli* DH5α and *Lactobacillus plantarum* NCDO1193, and plasmids: pBluescriptII SK(+), pGFPuv carrying the reporter gene and the pGIT32 shuttle vector with the promoter sequence of the *L. plantarum ldhL* gene. *E. coli* was grown on LB solid or liquid medium, *Lactobacillus* was grown on MRS solid or liquid medium in anaerobic conditions. Plasmid DNA from *E. coli* cells was extracted using the alkalic lysis method (Sambrook and Russell, 2001). *Lactobacilus plantarum* plasmid DNA was obtained by modifying the O'Sullivan and Klaenhammer method (O'Sullivan and Klaenhammer 1993). The

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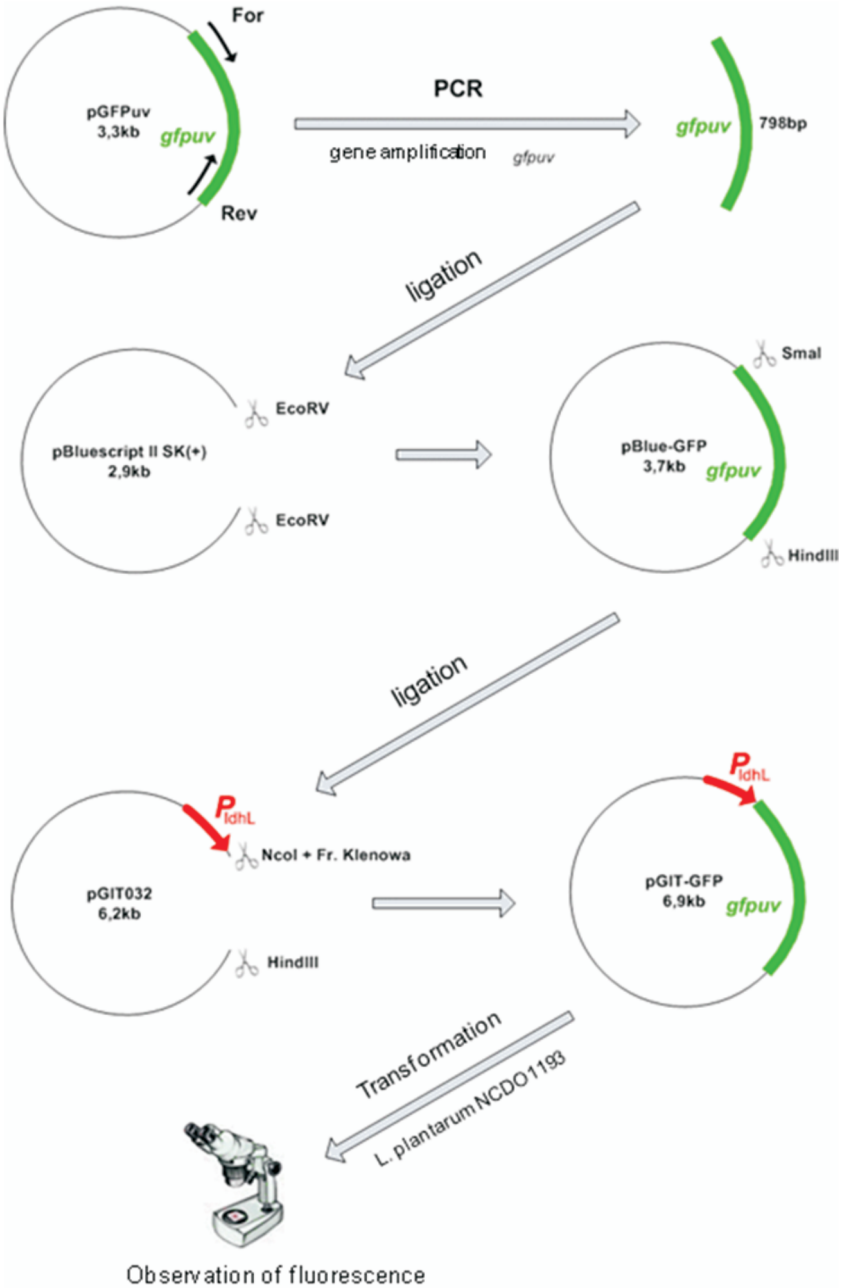

Fig. 1. Protocol of the experiment.

pGFPuv DNA fragment encoding the *gfpuv* gene was amplified by PCR, cloned into pBluescriptII SK(+), and subsequently recloned into pGIT32 (Fig. 1). The PCR protocol consisted of an initial incubation for 5 min at 94°C, followed by 25 cycles comprising: denaturation (30 sec. at 94°C), primers annealing (30 sec. at 64°C) and strand elongation (30 sec. at 72°C). Primers were as follows:

Forward (5'>3')	Reverse (3'>5')
TCC ATG GCT GCA GGT CGA CTC TAG AGG	GCA AGC TTG ACC GGC GCT CAG TTG GAA

The resulting plasmid was introduced into competent *E. coli* and *Lactobacillus plantarum* cells. The *gfpuv* gene expression was observed under a confocal microscope Olympus FV-500 (Olympus OpticalCo, Hamburg, Germany).

Results and Discussion

The PCR-amplified *gfpuv* gene at first was cloned into the pBluescriptII SK(+) plasmid and introduced into *E. coli* DH5α. Subsequently, *gfpuv* was recloned into the pGIT32 shuttle vector, resulting in the

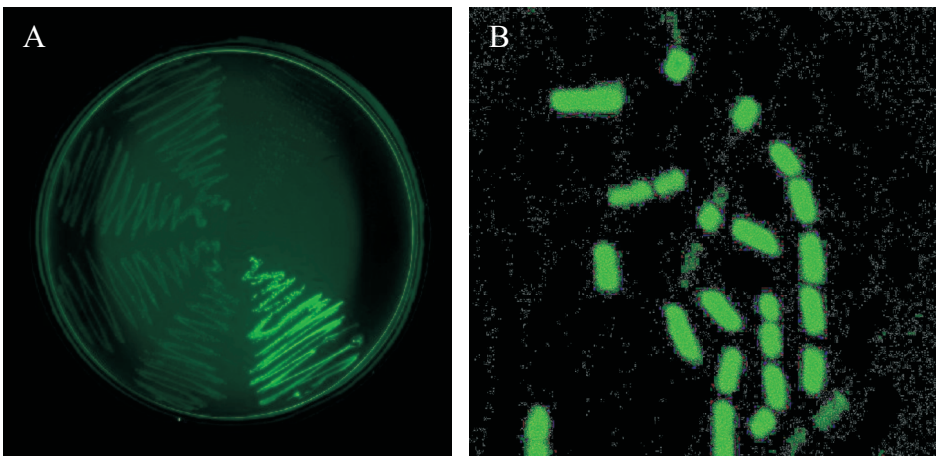

Fig. 2. *E. coli* Dh5h strain transformed with pGIT-GFP plasmid (A). *L. plantarum* strain transformed with pGIT-GFP plasmid (B). Microscope magnification: 60x.

pGIT-GFP construct, in which the *gfpuv* gene expression was from the lactate dehydrogenase *ldhL* gene promoter localized downstream. Both, *E. coli* (Fig. 2A) and *L. plantarum* cells (Fig. 2B), harboring the pGIT-GFP construct, emitted green fluorescence, derived from expression of *gfpuv* under the control of the *ldhL* promoter. Yet, the level of green fluorescence observed in the confocal microscope varied among cells, for some being very low. Literature data report that *gfp* expression is weaker in *L. plantarum* strains compared to other *Lactobacillus* strains belonging to *L. acidophilus* or *L. casei* species (Gory et al. 2001, Perez-Arellano and Perez-Martinez 2003). Therefore obtained plasmid construct can be transferred into other *Lactobacillus* species for gene expression enhancement, if necessary. It is believed that by changing the strains growth conditions and adjusting them to significantly influence GFP production, a stable gene expression can be obtained in properly selected *Lactobacillus* sp. strain.

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