Use of GFP to trace the colonization of *Lactococcus lactis* WH-C1 in the gastrointestinal tract of mice

Yanping Wang *, Jingrui Wang, Weili Dai

Keywords: *Lactococcus*  
Expression vector  
Colonization  
GFP

Abstract  
A new expression vector for *Lactococcus* was constructed using *nisI* as a selection marker and GFP as a reporter protein to explore the colonization characteristics in vivo of *Lactococcus lactis* WH-C1. By high expression of GFP, it was shown WH-C1 could pass through the stomach and survive in the gastrointestinal tract.

Humans have used lactic acid bacteria (LAB) for a long time, mainly in the food industry (De Vuyst and Leroy, 2007; Hugenholtz et al., 2002; Wisselink et al., 2002; Burgess et al., 2004; Taranto et al., 2003). Many reports have shown LAB have health benefits, including anti-tumor (Ohashi et al., 2002), cholesterol-lowering (Yanping Wang et al., 2009) and antioxidation (Verena et al., 2008) effects. Furthermore, LAB are attractive expression and delivery vehicles for recombinant proteins because they are generally regarded as safe (GRAS) by the FDA. Moreover, LAB can prevent the pathogen adhering and colonizing on the intestinal epithelial cell surface and invading the intestinal cells. Some people call this mechanism as “Adhesion Resistance”. They interact and closely cooperate with the intestinal epithelial cells by some adhesions. The metabolites of LAB could form a biological barrier and prevent the invasion of pathogenic bacteria (Biller et al., 1995; Servin, 2004; Christensen et al., 2002; Ouwehand et al., 2002). Adhesion of LAB has been claimed to be essential for the exertion of beneficial effects in the intestine. However, there are a variety of physiological characteristics among the LAB genus and not all LAB have the probiotic feature of colonization in the intestine. Therefore, screening and applying the LAB, which can colonize in the gastrointestinal tract for long time periods and simultaneously play probiotic effects in health *in situ* is a valuable and developmental tool.

Green fluorescence protein (GFP) has the advantage of being an auto-fluorescent protein that does not require a substrate and it allows for its detection in living cells and in real time (Kitts et al., 1995). In this report, we constructed a constitutive expression vector for *Lactococcus* using *nisI* as the selection marker and GFP as the reporter protein. Then, we electroporated the recombinant into *Lactococcus lactis* WH-C1 which was isolated from Kefr Grain. Finally, we fed mice with the transformants using GFP as a visible marker for tracking this strain introduced into the gastrointestinal tract and observing its colonization capability.

To construct an expression vector carrying *gfp* gene, we first amplified *nisI* gene, which was used as resistance marker, from plasmid pLEB590 using primers NisI-up and NisI-down (Table 1) (Luo and Wang, 2009). The 780 bp fragment (Fig.1A) was digested by XbaI and SacI and ligated into the original vector pMG36e. The protein NisI coded by nisin resistance gene *nisI* was a lipoprotein which could defend the toxic effects of nisin on the host itself (Takala and Saris, 2002). Although the vector pMG36e contains erythromycin resistance marker, using nisin as the selection pressure could avoid the use of antibiotics to some extent. Using primers GFP-up and GFP-down (Table 1), the 750 bp *gfp* fragment was amplified from pGFP (Fig.1B). After digestion by SacI and XbaI, it was cloned into the newly constructed pMG36e-nisI-gfp vector and resulted in pMG36e-nisI-gfp. These two constructions were both electroporated into competent *L. lactis* MG1363 and the transformants were selected on GM17 (Oxoid) plates supplemented with 40 IU/ml nisin (Sigma) at 30 °C. After confirmation by single digestion with EcoRI and PCR amplification, pMG36e-nisI-gfp was ultimately electroporated into *L. lactis* WH-C1. In Fig.3 results showed that this recombinant vector had high stability in WH-C1 (the percentage plasmid loss per cell per generation was approximately 0.1%).

* L. *lactis* WH-C1 has many good features, including producing firm texture and delicate tasting milk, strong ability to produce exopolysaccharide (Wang et al., 2007), strong acid and salt tolerance
abilities and especially adaptable to high concentrations of bile salt environment indicating its strong viability in the gastrointestinal tract (Wang et al., 2010). Based on these findings we are curious to explore the colonizing and metabolic properties of this strain in vivo. Swiss Albino mice were orally inoculated with 10^9 CFU of L. lactis WH-C1 harboring pMG36e-nisI-gfp. After 6 h, 18 h, 26 h, 42 h and 72 h, the geometric means of log_{10} concentrations of bacteria in stomach, jejunum, ileum and appendix were determined (Fig. 2). After 6 h, we found fluorescent WH-C1-GFP presenting in all samples of the bowel contents and the maximum concentration was reached after 42 h. Three days later, the amount of bacteria remained at around 10^4 CFU/g which indicated this Lactococcus strain could exist in the gastrointestinal tract for a long time.

To search for fluorescent bacteria more directly, frozen thin-sectioned gastrointestinal tract samples were observed by fluorescence microscopy (Fig.3). From the photographs we saw that Lactococcus mainly existed in the contents of gastrointestinal tract and a small number of strains adhered to the intestinal mucosa while no fluorescent bacteria existed in the controls. A few strains presented among the epithelial cells. By high expression of marker protein GFP, we first observed the exact distribution of Lactococcus in the gastrointestinal tract which indicated the expression level of GFP was sufficient to visualize the host bacteria during metabolic process in vivo. Besides, we found no fluorescent bacteria existing in the spleen or liver probably because LAB, unlike some pathogenic microorganisms, such as Salmonella, could not pass through the intestinal epithelial barrier system and then enter the internal tissues of animals.

Adhesion of LAB has been claimed to be essential for the exertion of a beneficial effect in the human intestine, and has been considered as one of the selection criteria for probiotic strains (Klaenhammer, 1982; Collins et al., 1998). As it is difficult to assess these probiotic properties in vivo, in vitro models have been developed to evaluate bacterial adherence and, in particular, human epithelial cell lines have been widely used (Hauri et al., 1985). Meanwhile, a variety of probiotic organisms have been tested for adhesive properties using Caco-2 cell line (Bianchi et al., 2004; Jacobsen et al., 1999; Tuomola and Salminen, 1998). In addition, mice models are thought to well reflect the ability of the LAB to persist in the human gut. GFP expressed by Lactococcus was successfully applied to animal testing, this will help us better understand a series of problems including the relationship between Lactococcus and intestinal epithelial cells and how to activate the immune response. The method established here undoubtedly provides a simple and fast way to detect the colonization ability of LAB. Furthermore, this work may also facilitate further studies, such as how microorganisms interact with host cells at the cellular level and the effect of microorganisms on the original intestinal flora.

**Acknowledgments**

This work was supported by a grant from the Research Fund from the Doctoral Program of Higher Education (No. 20091208110001).

**References**


**Fig. 3.** Fluorescent detection of *L. lactis* WH-C1 existing in the contents of intestinal tissues and adhered to the intestinal mucosa. A to F: the inoculated group; G the non-inoculated group; H: the fluorescent *L. lactis* cultured in vivo.


Verena, J.K., Marian, Brigitte, Stidl, Reinhard, et al., 2008. Impact of lactic acid bacteria on oxidative DNA damage in human derived colon cells. Food and Chemical Toxicology. 46, 1221–1229.


