

## Characterization of a potential probiotic strain *Lactobacillus plantarum* MY4

정환영, 정민용, 김기태<sup>1</sup>, 김천제<sup>2</sup>, 백현동

경남대학교 생명과학부, 프로코바이오텍(주)<sup>1</sup>, 건국대학교 축산가공학과<sup>2</sup>

전화 (055) 249-2717, Fax (055) 249-2995

### Abstract

The cells of *Lactobacillus plantarum* MY4 isolated from the human feces were treated for 24 h in artificial bile after incubation for 2 h in artificial gastric juice and final number of the strain was reached to around  $3.1 \times 10^8$  CFU/ml. In test of API ZYM kit,  $\beta$ -glucuronidase or  $\beta$ -glucosidase were not produced by *L. plantarum* MY4. However,  $\beta$ -galactosidase were weakly produced by it, which they would be alleviated the lactose intolerance. *L. plantarum* MY4 were resistant to antibiotics such as nisin, tetracycline, streptomycin, rifamycin, doxycycline, roxithromycin, chloramphenicol, nystatin, erythromycin, ciprofloxacin and gentamycin. *L. plantarum* MY4 was affected by alcohol concentration up to 8%, but more than 16%, their growth was not affected significantly. *L. plantarum* MY4 was shown to inhibit the growth of *Listeria monocytogenes* ATCC 19111 completely within 24 h of incubation, which indicates its bactericidal nature. Thus, *L. plantarum* MY4 show promise as a probiotic strain because of its characteristics.

### Introduction

The term of probiotics was redefined as ■ a viable mono or mixed culture of microorganisms which, applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora. The characteristics of *Lactobacillus* for probiotic use in the human, include strains of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Clostridium butyricum*, *Lactobacillus sporogenes* and *Bacillus subtilis*. The bacterium *Lactobacillus plantarum* has been of recent interest for genetic improvement due to its potential commercial applications and its GRAS(Generally Recognized As Safe) status. It is isolated from dairy products and environments, as well as from silage, sauerkraut, pickled vegetables, human mouth and intestinal tract and important in processing food for human consumption, such as fermented vegetables. Our objective is searching the industrial benefits of *L. plantarum* MY4 for probiotic use in the human intestine that are a resistance of artificial gastric juice and bile acid, an

activity of enzymes, the antibiotics sensitivity, antimicrobial activity and alcohol tolerance.

## Materials and Methods

This study was performed with strain of *Lactobacillus plantarum* MY4 isolated from human feces. *Listeria monocytogenes* ATCC 19111 used for determination of antimicrobial spectrum of activity was grown in TSA (Difco) and Listeria Selective Agar base (Unipath, UK) was used for the selective media. The viable cell counts were measured through a spreading method by plating 0.1 ml of the diluted samples appropriately with peptone 0.1% to MRS agar and incubating the plates at 37°C for 24 h. The artificial gastric juice tolerance of the *L. plantarum* MY4 was suspended (1%) in MRS broth contained 1% pepsin, adjust to pH 2.5, 7.0 with 0.1 N HCl and cultured for 180 min at 37°C. Bile acid tolerance was measured by cultivating the cells what are treated with artificial gastric juice of pH 2.5 for 120 min at 37°C in MRS broth containing 0.1% Oxgall (Difco) for 0, 4, 8, 12 and 24 h at 37°C. The stain was identified on the basis biochemical profiles according to the API CHL50 kit test (bioMereux Co., France) and Gram Stain. Its enzyme activity and antibiotic sensitivity was performed by API ZYM kit and the filter paper disc method, respectively. After *L. monocytogenes* ATCC 19111, *L. plantarum* MY4 were cultured for 12 h, they suspended until  $10^5$ – $10^6$  CFU/ml, and mixed with the same volume (1:1) in test tube (5 ml). After incubate at 0, 1, 2, 4, 8, and 24 h, they were plated on Listeria Selective Agar base. The cells were obtained by centrifugation at 8,000×g for 10 min. They suspended in a potassium phosphate buffer of 0.1 M (pH 7.0) containing ethanol 0, 4, 8, 16 and 32%, respectively. After incubating for 4 h at 37°C, viable cells were measured as its alcohol tolerance.

## Results and Discussion

Incubation of *L. plantarum* MY4 at pH 2.5 resulted in higher 90% surviving (Fig. 1). *L. plantarum* MY4 strain tested in this study is either resistance or tolerant, after 48 h incubation in MRS broth supplemented with 1% oxgall. The results show that bile exerted a slight inhibitory effect in the growth of *L. plantarum* MY4. It was lower survived than control, however, it was higher than initiate (about  $10^8$  CFU/ml). The growth in bile acid was no more or less slow, not inhibited (Fig. 2). API50 CHL fermentation system is one of the method that enables us to evaluate physiological properties of an organism. After identification using the API50 CHL kit, Strain MY4 was found to be *Lactobacillus plantarum* (data not shown). In enzyme activity result, the probiotics bacteria will not occur the jeopardy of carcinogenesis causing.

Meanwhile, the  $\beta$ -galactosidase activity of *L. plantarum* MY4 was not higher than that of *L. acidophilus* selected from Korean feces, but its activity was confirmed weakly (Table 1). It is an essential enzyme for lactobacilli to utilize lactose and has been shown to reduce the symptoms of lactose intolerance in animal or human. So, it would be alleviated the lactose intolerance in functional foods including the milk. *L. plantarum* MY4 was sensitive to rifamycin, 10  $\mu\text{g/ml}$ , doxycycline 10  $\mu\text{g/ml}$ , roxithromycin 10  $\mu\text{g/ml}$ , chloramphenicol 10  $\mu\text{g/ml}$ , erythromycin 30  $\mu\text{g/ml}$ , but resistant to nisin 100  $\mu\text{g/ml}$ , streptomycin 20  $\mu\text{g/ml}$  and tetracycline 10  $\mu\text{g/ml}$  ciprofloxacin 20  $\mu\text{g/ml}$ , nystatin 20  $\mu\text{g/ml}$ , gentamycin 20  $\mu\text{g/ml}$  (Table 2). As is shown in Fig. 3, the antimicrobial effect of *L. plantarum* MY4 was showed complete inhibition of *L. monocytogenes* ATCC 19111 before 24 h, indicating a bactericidal mode of action. Fig. 4 showed an increase in ethanol tolerance, in the rate of inactivation at 32% in *L. plantarum* MY4 following ethanol shock. Therefore, *L. plantarum* MY4 was able to survive to 32% ethanol.

## References

1. Xanthopoulos, V., Hatzikamari, M., Adamidis, T., Tsaklidou, E., Tzanetakis, N. and Litopoulou-Tzanetaki, E. 2000. Heterogeneity of *Lactobacillus plantarum* isolates from Feta cheese throughout ripening *J. Appl. Microbiol.* **88**: 1056-1064.
2. Benkerroum, N., Oubel, H., Zahar, M., Dlia, S. and Filali-Maltouf, A. 2000. Isolation of a bacteriocin-producing *Lactococcus lactis* subsp. *lactis* and application to control *Listeria monocytogenes* in Moroccan jben. *J. Appl. Microbiol.* **89**: 960-968.
3. Šušková, J., Kos, B., Matošić, S. and Besendorfer, V. 2000. The effect of bile salts on survival and morphology of a potential probiotics strain *Lactobacillus acidophilus* M92. *World J. Microbiol. Biotechnol.* **16**: 673-678.

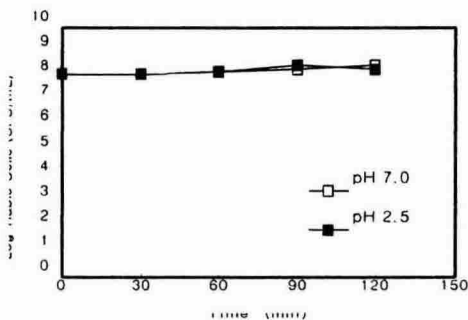


Fig. 1. Survival of *L. plantarum* MY4 in artificial gastric juice

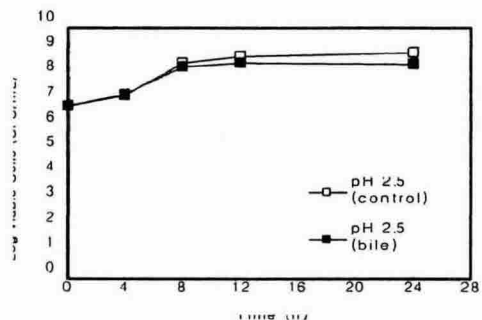


Fig. 2. Survival of *L. plantarum* MY4 in artificial bile after treated with artificial gastric juice for 2 h at 37°C

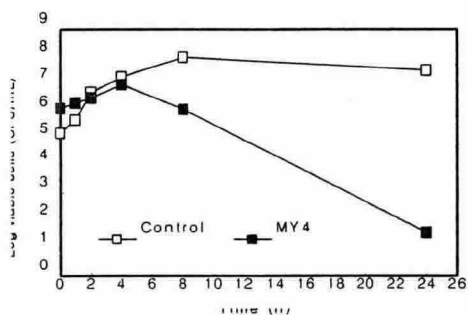


Fig. 3. Growth inhibition of *L. monocytogenes* ATCC 19111 by *L. plantarum* MY4

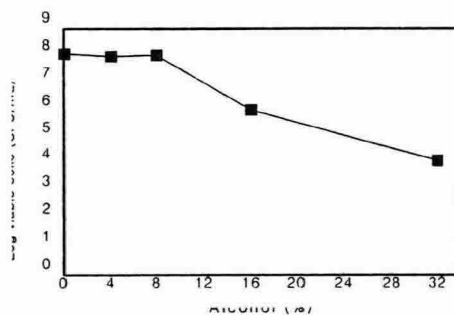


Fig. 4. Alcohol resistances of *L. plantarum* MY4

Table 1. Enzyme activities of probiotic *L. plantarum* MY4 by API ZYM kit

Enzyme	<i>L. plantarum</i> MY4
Control	0 <sup>1)</sup>
Alkaline phosphatase	2
Esterase(C <sub>4</sub> )	4
Esterase lipase(C <sub>8</sub> )	4
Lipase(C <sub>14</sub> )	2
Leucine arylamidase	5
Valine arylamidase	3
Cystine arylamidase	2
Trypsin	0
α-chymotrypsin	1
Acid phosphatase	5
Naphthol-AS-BI-phosphohydrolase	1
α-galactosidase	0
β-galactosidase	1
β-glucuronidase	0
α-glucosidase	0
β-glucosidase	0
N-acetyl-β-glucosaminidase	0
α-mannosidase	0
α-fucosidase	0

<sup>1)</sup> 0: 0 nmole, 1: 5 nmole, 2: 10 nmole, 3: 20 nmole, 4: 30 nmole, 5: > 40 nmole

Table 2. Antibiotic resistance of probiotic *L. plantarum* MY4

Antibiotics (μg/ml)	<i>L. plantarum</i> MY4
Nisin 100	+ <sup>1)</sup>
Streptomycin 20	+
Tetracycline 20	+
Rifamycin 20	-
Ciprofloxacin 20	+
Doxycycline 10	-
Roxithromycin 10	-
Nystatin 20	+
Gentamycin 20	+
Chloramphenicol 10	-
Erythromycin 30	-

<sup>1)</sup> +: growth, -: no growth