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# Pathogenicity and Single Dose Toxicity of a Potential Probiotic *Lactobacillus* spp. PSC101 in Mice

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**ABSTRACT.** This study was conducted to investigate the pathogenicity and acute single toxicity of *Lactobacillus* spp. PSC101 (PSC101) isolated from pigs and *L. acidophilus* (LA) at $2.5\times10^9$ CFU or $2.5\times10^{12}$ colony forming units (CFU) in mice for 14 days. After oral administration of the bacteria into mice, we could not find their any specific pathogenicity from the standpoints of clinical signs, and changes in body weight and body temperature, as compared with the control group during 14 days. We further investigated the toxicity of concentrated culture broth (×10) after fermentation of them for safe industrial process. As the results, we could not find any clinical signs, changes in body weight and body temperature, as compared with the control group (MRS broth) for 14 days. The results obtained in this study suggest that the potentially probiotic, PSC101, is non-toxic in mice and is therefore likely to be safe for pig use.

**Keywords:** *Lactobacillus*, Single-dose toxicity, Pathogenicity, Pig.

## INTRODUCTION

The use of antibiotics in farm animals has caused tissue residue of the antibiotics, an imbalance of normal intestinal flora, reduction of beneficial intestinal microbial populations, and generation of antibiotic resistant bacteria (Reid, 2002; Reid and Friendship, 2000). In order to overcome the above mentioned problems, the utility and development of probiotics has been increased in veterinary sectors (Abe *et al.*, 1995; Adami *et al.*, 1999; Reid and Friendship, 2000; Vanbelle *et al.*, 1990; Verstegen *et al.*, 2002).

Probiotics are defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance and have been strongly recommended as alternative for antibiotics in industrial animals (Reid and Friendship, 2000; Vanbelle *et al.*, 1990). Many investigators reported that lactic acid bacteria as a probiotic have an important role in beneficial biological functions in various animals (Chou *et al.*, 1999). Despite the ability of probiotics to improve the health and feed conversion for industrial animals (Abe *et al.*, 1995; Adami *et al.*, 1999), special probiotics for swine have still not been fully developed.

Selection criteria for novel probiotic strains include safety, functional and technological aspects (Saarela *et al.*, 2000). In the present study, PSC101 producing simultaneously active dietary enzyme such as amylase, lipase, phytase, and protease was selected on the basis of pH- and bile-resistance from the intestinal samples of pig.

The main objective of the present study, therefore, was to verify the primary safety information about PSC101 as a probiotic and further clarify their safety for swine consumption.

## MATERIALS AND METHODS

### Animal Maintenances

Sixty-five male and female ICR mice aged 8~10 weeks, bred at the Animal Production unit of Chungnam National University, were acclimated in controlled

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room (temperature: 23 ± 3°C, relative humidity: 50 ± 10%, air circulating frequency: 13~17 times/hr, artificial light: 300 Lux from 7 am to 7 pm, noise : 50 db) for 10 days before experimentation. Mice were housed in a polycarbonate cage (28 cm × 42 cm × 18 cm). Free mix (Samtako Bio Korea) and sterilized water were provided ad libitum.

### Bacterial Strains

The newly isolated potential probiotic, PSC101, was used in this study. The commercial strain *L. acidophilus* (KCTC 3146) (LA) was included as a reference strain for comparative purposes. PSC101 was isolated from intestinal samples of pigs. The strain produced amylase, lipase, phytase and protease as well as showed acid and bile tolerance.

PSC101 and LA were grown in Man-Rogosa-Sharpe (MRS) broth at 37°C under anaerobic conditions for 48 h and then centrifuged at 12,000 rpm for 15 min. After centrifugation, the supernatant was concentrated up to 10 times by vaccum evaporator for determination of toxicity on secondary metabolites or pathogenic enzymes produced for culture of the strain. The cell pellets were homogenized and re-suspended at two concentrations ($5 \times 10^9$ and $5 \times 10^{12}$ CFU/ml) after three washes in PBS.

### Experimental Designs

A total of 65 male and 65 female mice were randomly assigned to male and female 5 groups consisted of 10 and male and female 6 groups consisted of 5, respectively. To examine the pathogenicity of PSC101 strain, the highest dose of PSC101 and *L. acidophilus* that could be prepared for oral administration was $2.5 \times 10^{12}$ CFU/0.5 ml. The low dose of the strains was $2.5 \times 10^9$ CFU/0.5 ml. A single dose of the strains was administered to mice intragastrically using a polyethylene cannula attached to a disposable syringe.

To examine the toxicity of culture broth after fermentation of PSC101 and LA, the culture broth was concentrated (× 10) and administered to male and female mice.

Clinical signs were observed for 12 hr following treatment of PSC101 and LA on the day of administration and once everyday thereafter for 14 days. Body weight was measured immediately prior to dosing of PSC101 and LA and everyday after the treatment. The change of food consumption and water was measured everyday after the treatment. Following the observation period all animals were anesthetized with ether. Autopsy was conducted on every animal, and all major organs and tissues including heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined for gross lesions.

### Statistics

Body weight, body temperature, food and water consumption changes were compared using ANOVA (Dunnett's test) by SAS program (version 8.1). Differences at $P<0.05$ were considered statistically significant.

## RESULTS

### General Health Status

Throughout the experiment mice appeared healthy, inquisitive and active. No illness or death occurred. No observable difference in the animals hair lustre was noticed between the groups, and there were no signs of

**Table 1.** Survivals of male and female mice orally treated with *Lactobacillus* spp. PSC101 and *Lactobacillus acidophilus* for 14 days

| Groups | Sex | Days after treatment | | | | | | | Final survivals |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 5 | 7 | 14 | |
| Control (n=10) | male | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| PSC101-A (n=10) | male | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| PSC101-B (n=10) | male | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| LA-A (n=10) | male | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| LA-B (n=10) | male | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| Control (n=10) | female | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| PSC101-A (n=10) | female | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| PSC101-B (n=10) | female | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| LA-A (n=10) | female | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| LA-B (n=10) | female | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |

Control : Group administered by PBS (phosphate buffered saline) of pH 7.2, PSC101-A: Group administered by *Lactobacillus* spp. PSC101 ($2.5 \times 10^9$ CFU/0.5 ml), PSC101-B: Group administered by *Lactobacillus* spp. PSC101 ($2.5 \times 10^{12}$ CFU/0.5 ml), LA-A: Group administered by *Lactobacillus acidophilus* ($2.5 \times 10^9$ CFU/0.5 ml) and LA-B: Group administered by *Lactobacillus acidophilus* ($2.5 \times 10^{12}$ CFU/0.5 ml).

**Table 2.** Survivals of male and female mice orally treated with concentrated culture broth (× 10) of *Lactobacillus* spp. PSC101 and *Lactobacillus acidophilus* for 14 days

| Groups | Sex | Days after treatment | | | | | | | Final survivals |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 5 | 7 | 14 | |
| Control (n=5) | male | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| PSC101-C (n=5) | male | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| LA-C (n=5) | male | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Control (n=5) | female | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| PSC101-C (n=5) | female | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| LA-C (n=5) | female | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |

Control : Group administered by MRS broth, PSC101-C: Group administered with concentrated culture broth (× 10) by *Lactobacillus* spp. PSC101, LA-C: Group administered with concentrated culture broth (× 10) by *Lactobacillus acidophilus*.

gastrointestinal upsets including diarrhea or vomiting.

### Lethality and Clinical Signs

No death among the male and female mice treated with PSC101 and *L. acidophilus* at high ($2.5 \times 10^{12}$ CFU/0.5 ml) and low ($2.5 \times 10^{9}$ CFU/0.5 ml) bacteria was observed during the observation period of 14 days (Table 1). Meanwhile, no death among the male and female mice treated with concentrated culture broth (× 10) of PSC101 and *L. acidophilus* was observed during the observation period of 14 days (Table 2). In both tests, mice were observed for any abnormal clinical signs for 12 hr following the treatment. No bacteria-related abnormal signs were noted in mice regardless of the dose used. Observation of clinical sign was conducted everyday for 14 days.

### Body Weight Changes

During the 14 days treatment, no significant difference ($p>0.05$) in the body weight gain between the experimental groups (Table 3 and 4). No difference in net body weight gain among the different dose groups was noted in both sexes.

### Food and Water Consumption Changes

Food and water consumption changes of mice were monitored for 14 days after the treatment of animals with PSC101, LA, and concentrated culture broth (× 10) of them. The mean food consumption was slightly decreased on the first day in both male and female mice treated with the high dose ($2.5 \times 10^{12}$ CFU), low dose ($2.5 \times 10^{9}$ CFU) and concentrated culture broth (× 10) of both bacteria, but no mean food consumption were shown in animals regardless of the dose level used in the study from on the second day after treatment. There was no water consumption change in both male and female rats regardless of the dose of PSC101, LA and concentrated culture broth (× 10) of both bacteria.

### Gross Pathological Findings

At the end of the observation period all rats were sacrificed and autopsied. All major organs including heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined grossly. There were no abnormal lesions in both male and female rats regardless of the dose of PSC101 and LA

**Table 3.** Body weight changes in mice orally treated with *Lactobacillus* spp. PSC101 and *Lactobacillus acidophilus* during 14 days (Mean ± SD, g)

| Days after exposure | Male | | | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control (n=10) | PSC101-A (n=10) | PSC101-B (n=10) | LA-A (n=10) | LA-B (n=10) | Control (n=10) | PSC101-A (n=10) | PSC101-B (n=10) | LA-A (n=10) | LA-B (n=10) |
| Pre-exposure | 23.9 ± 0.8 | 24.3 ± 1.1 | 24.6 ± 0.6 | 25.1 ± 1.1 | 25.1 ± 1.1 | 24.9 ± 0.7 | 24.4 ± 0.8 | 24.3 ± 0.7 | 25.2 ± 0.6 | 25.2 ± 0.6 |
| 1 | 24.5 ± 0.6 | 24.4 ± 1.2 | 24.9 ± 1.2 | 25.3 ± 1.3 | 25.2 ± 1.0 | 24.9 ± 0.6 | 24.5 ± 0.7 | 24.6 ± 0.7 | 25.1 ± 0.6 | 25.1 ± 0.6 |
| 2 | 24.0 ± 0.4 | 24.7 ± 1.6 | 24.9 ± 0.5 | 25.4 ± 0.8 | 25.7 ± 0.9 | 25.0 ± 0.8 | 24.7 ± 1.1 | 24.5 ± 1.1 | 25.6 ± 0.7 | 25.6 ± 0.7 |
| 3 | 24.4 ± 1.2 | 25.2 ± 1.5 | 25.2 ± 1.3 | 25.9 ± 1.7 | 25.3 ± 1.4 | 25.5 ± 0.7 | 25.5 ± 0.5 | 25.4 ± 0.6 | 25.7 ± 1.1 | 25.7 ± 1.1 |
| 5 | 24.7 ± 0.3 | 25.5 ± 1.1 | 25.2 ± 1.4 | 26.1 ± 1.3 | 25.7 ± 1.3 | 25.4 ± 1.1 | 26.5 ± 0.7 | 26.3 ± 0.6 | 26.2 ± 0.7 | 25.9 ± 0.7 |
| 7 | 24.8 ± 0.6 | 25.5 ± 0.9 | 25.5 ± 1.3 | 26.3 ± 0.5 | 26.3 ± 0.9 | 25.7 ± 0.7 | 26.2 ± 0.9 | 26.4 ± 0.9 | 26.3 ± 0.5 | 26.0 ± 0.5 |
| 14 | 26.2 ± 0.6 | 26.4 ± 0.3 | 26.9 ± 1.2 | 27.2 ± 1.2 | 27.2 ± 1.2 | 26.9 ± 0.4 | 26.7 ± 0.6 | 26.5 ± 0.6 | 27.2 ± 0.7 | 27.2 ± 0.7 |

Control : Group administered by PBS (phosphate buffered saline) of pH 7.2, PSC101-A: Group administered by *Lactobacillus* spp. PSC101 ($2.5 \times 10^{9}$ CFU/0.5 ml), PSC101-B: Group administered by *Lactobacillus* spp. PSC101 ($2.5 \times 10^{12}$ CFU/0.5 ml), LA-A: Group administered by *Lactobacillus acidophilus* ($2.5 \times 10^{9}$ CFU/0.5 ml) and LA-B: Group administered by *Lactobacillus acidophilus* ($2.5 \times 10^{12}$ CFU/0.5 ml).

**Table 4.** Body weight changes in mice orally treated with concentrated culture broth (× 10) of *Lactobacillus* spp. PSC101 and *Lactobacillus acidophilus* during 14 days (Mean ± SD, g)

| Days after exposure | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Control (n=5) | PSC101-C (n=5) | LA-C (n=5) | Control (n=5) | PSC101-C (n=5) | LA-C (n=5) |
| Pre-exposure | 23.8 ± 0.5 | 24.2 ± 0.6 | 24.3 ± 0.6 | 24.3 ± 0.6 | 24.2 ± 0.9 | 24.1 ± 0.8 |
| 1 | 24.0 ± 0.6 | 24.4 ± 0.8 | 24.5 ± 1.2 | 24.4 ± 0.6 | 24.5 ± 0.8 | 24.4 ± 0.6 |
| 2 | 24.1 ± 0.4 | 24.5 ± 1.2 | 24.5 ± 0.5 | 25.2 ± 0.7 | 24.6 ± 1.1 | 24.5 ± 0.9 |
| 3 | 24.3 ± 0.6 | 25.6 ± 1.3 | 25.3 ± 0.8 | 25.5 ± 0.7 | 25.4 ± 0.6 | 25.3 ± 0.5 |
| 5 | 24.6 ± 0.5 | 25.6 ± 1.1 | 25.4 ± 0.9 | 25.4 ± 1.1 | 26.5 ± 0.6 | 26.2 ± 0.7 |
| 7 | 24.7 ± 0.8 | 25.8 ± 0.9 | 25.4 ± 0.8 | 25.5 ± 0.7 | 26.3 ± 0.8 | 26.6 ± 0.9 |
| 14 | 26.3 ± 0.9 | 26.7 ± 0.3 | 26.4 ± 1.2 | 26.8 ± 0.9 | 26.8 ± 0.7 | 26.5 ± 0.6 |

Control : Group administered by MRS broth, PSC101-C: Group administered with concentrated culture broth (× 10) by *Lactobacillus* spp. PSC101, LA-C: Group administered with concentrated culture broth (× 10) by *Lactobacillus acidophilus.*

**Table 5.** Gross pathological findings of mice orally treated with *Lactobacillus* spp. PSC101 and *Lactobacillus acidophilus* on 14 days

| Organ | Male | | | | | Female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control (n=10) | PSC101-A (n=10) | PSC101-B (n=10) | LA-A (n=10) | LA-B (n=10) | Control (n=10) | PSC101-A (n=10) | PSC101-B (n=10) | LA-A (n=10) | LA-B (n=10) |
| Adrenal Gl. L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brain | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Heart | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Liver | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kidney | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lung | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hemorrhage | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spleen | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Testis L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| (Ovary) R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stomach | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Control : Group administered by PBS (phosphate buffered saline) of pH 7.2, PSC101-A: Group administered by *Lactobacillus* spp. PSC101 ($2.5 \times 10^9$ CFU/0.5 ml), PSC101-B: Group administered by *Lactobacillus* spp. PSC101 ($2.5 \times 10^{12}$ CFU/0.5 ml), LA-A: Group administered by *Lactobacillus acidophilus* ($2.5 \times 10^9$ CFU/0.5 ml) and LA-B: Group administered by Lactobacillus acidophilus ($2.5 \times 10^{12}$ CFU/0.5 ml).

**Table 6.** Gross pathological findings of mice orally treated with concentrated culture broth (× 10) of *Lactobacillus* spp. PSC101 and *Lactobacillus acidophilus* on 14 days

| Organ | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Control (n=5) | PSC101-C (n=5) | LA-C (n=5) | Control (n=5) | PSC101-C (n=5) | PSC101-C (n=5) |
| Adrenal Gl. L | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 0 | 0 | 0 | 0 | 0 | 0 |
| Brain | 0 | 0 | 0 | 0 | 0 | 0 |
| Heart | 0 | 0 | 0 | 0 | 0 | 0 |
| Liver | 0 | 0 | 0 | 0 | 0 | 0 |
| Kidney | 0 | 0 | 0 | 0 | 0 | 0 |
| Lung | 0 | 0 | 0 | 0 | 0 | 0 |
| Hemorrhage | 0 | 0 | 0 | 0 | 0 | 0 |
| Spleen | 0 | 0 | 0 | 0 | 0 | 0 |
| Testis L | 0 | 0 | 0 | 0 | 0 | 0 |
| (Ovary) R | 0 | 0 | 0 | 0 | 0 | 0 |
| Stomach | 0 | 0 | 0 | 0 | 0 | 0 |

Control : Group administered by MRS broth, PSC101-C: Group administered with concentrated culture broth (× 10) by *Lactobacillus* spp. PSC101, LA-C: Group administered with concentrated culture broth (× 10) by *Lactobacillus acidophilus.*

used (Table 5 and 6).

## DISCUSSION

The safety of probiotic strains has been discussed in the last few years but there are still no general guidelines or specific policy requirements on this issue. Acute oral toxicity has been advocated as a fundamental test for assessing safety (Ishibashi *et al.,* 2001). For a probiotic bacteria strain to have an effect in the gut ecosystem, it has to reach a minimum population level of $10^7$ CFU/g of intestinal contents (Kaur *et al.*, 2002; Saarela *et al.,* 2000). Dosages for toxicity studies must therefore exceed this concentration, and previous studies must measuring acute toxicity for *Lactobacillus* strains evaluated daily dosages ranging from $10^{10}$ to $10^{11}$ CFU (Zhou *et al.*, 2000). Similar oral daily doses of $10^{11}$ CFU/kg have been used to measure repeated dose toxicity for a *Bifidibacterium longum* BB536 (Ishibashi *et al.*, 2001). Meanwhile, many bacteria exotoxin are excreted into medium by growing bacteria. It is necessary to confirm the toxicity of culture for industrial process.

In the present study, we investigated the acute toxicity of single oral administration with PSC101 and its concentrated culture broth (× 10) to mice as a part of the development of a new probiotic for pigs. Acute single oral toxicity of a PSC101 was examined using male and female mice in the present study, together with LA. PSC101 was administered to mice at a dose of 0, $2.5 \times 10^9$ and $2.5 \times 10^{12}$ CFU/0.5 ml intragastrically. As the results, no mortality was observed in mice treated with PSC101 at a dose of $2.5 \times 10^{12}$. This dose is the largest one which could be prepared for oral administration. Therefore, the $LD_{50}$ of PSC101, although it could not be determined precisely, is greater than $2.5 \times 10^{12}$. During the observation period of 14 days, no abnormal clinical signs were shown in animals regardless of the dose level used in the study. We also examined the acute single oral toxicity of concentrated culture broth (× 10) of PSC101 and LA. The results showed that concentrated culture broth (× 10) had no toxicity in mice.

In conclusion, PSC101 could be used as a probiotic for swine on the basis of bile and pH resistance, and non-toxic for mice. It is therefore likely to be safe for swine use. It is unlikely that PSC101 will cause adverse affects in any other animal species. However this strain further should be investigated by clinical trials in pigs.

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