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Cultural characterization of probiotic *Lactobacillus sakei* BK19

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We have selected an valuable probiotic strain; *Lactobacillus sakei* BK19 which has wide antagonic spectrum against fish pathogens. Present study investigated cultural characterization of *L. sakei* BK19 including pH tolerance, susceptibility of antibacterial agents and growth pattern with different environment such as nutritions, temperature and salinity. *L. sakei* BK19 showed significantly higher resistance at low pH (around pH 4) environment and relative high antibiotic tolerance. In the study of optimal culture condition, maltose and saccharose provided the optimal nutritional culture condition while lactose and mannitol were unable to supply its carbon source for the fermentation of *L. sakei* BK19. Moreover, *L. sakei* BK19 showed good growth at the temperature range of 15 to 45℃ and the NaCl concentration of 0 to 7%. Hence, this particular probiotic strain may be benificial both in seawater and fresh water conditions.

Key words : Probiotic, Culture condition, *Lactobacillus sakei*.

Introduction

Probiotics are usually defined as live microbial feed supplements which beneficially affects on the host animal by improving its intestinal microbial balance (Fuller, 1989). Most probiotics proposed as biological control agents in aquaculture (Conway, 1996; Ringo and Gatesoupe, 1998) belong to the lactic acid bacteria (LAB). LAB are commonly found in the gastrointestinal tract of animal and fermented foods (Bruno and Montville, 1993; Ringo and Gatesoupe, 1998). They have been shown to possess inhibitory activity towards the growth of pathogenic bacteria such as *Listeria monocytogenes* (Corsetti *et al*., 1996; Harris *et al*., 1989; Hugas *et al*., 1998), *Escherichia coli* (Choi *et al*., 1999), *Salmonella* sp. (Hudault *et al*., 1997), and *Vibrio* sp. (Olsson *et al*., 1992). In order to survive and colonize in the gastrointestinal tract probiotic bacteria should express high tolerance to acid (Joborn *et al*.,1997) and bile and have the ability to adhere to intestinal surfaces (Verschuere *et al*., 2000). However, LAB has a narrow antibacterial spectrum and inhibit only closely related species or Gram-positive microorganisms (Suma *et al*., 1998). Moreover, the most pathogens involved in aquaculture are gram negative bacteria. Therefore probiotic strain for aquaculture should have a wide antagonic spectrum, survive in low pH condition and not sensitive against chemotherapeutic agents. Previously we isolated some probiotic candidates and investigated the antagonic character against fish pathogens. Among them *L. sakei* BK19 showed the highest inhibitory effect (Yang *et al*., 2003a). The purpose of the present study was to investigate cultural characterization of *L. sakei* BK19 to give a information

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for industrial application.

Materials and Methods

Bacterial strain

We selected valuable strain for probiotics, which has wide antagonic spectrum against fish pathogens then it named *Lactobacillus sakei* BK19 (Yang *et al.*, 2003a and 2003b).

pH effects on the growth of *L. sakei* BK19

To assess survival of *L. sakei* BK19 at different initial culture pH values, MRS broth (10 *ml*) were prepared, and pH adjusted with 4 M of HCl to give a range of initial pH values from 4 to 9. One hundred *μl* of overnight culture was inoculated into 10 ml of pH-adjusted MRS broth and incubated. Each survival cell were counted by dilution method after the incubation at 32℃ for 24 h.

Antibiotic susceptibility of *L. sakei* BK19

Antibiotic susceptibility was detected by the agar disc diffusion method. One hundred *μl* of an overnight diluted to approximately 1.5×10^5 CFU/*ml* was inoculated on the Muller-Hinton Agar (MHA, Difco, USA) media. The following 12 kinds of commercial antibiotic discs (BBL) AMC; Amoxicillin, TE; Tetracycline, T; Oxytetracycline, C; Chloramphenicol, CF; Cephalothin, NA; Nalidixic acid, CIP; Ciprofloxacin, NB; Novobiocin, D; Doxycycline, SD; Sulfadiazine, N; Neomycin and E; Erythromycin were used for antibiotic sensitivity test.

The inoculated plates were kept at room temperature for 10 min, then each antibiotic discs were placed on the plates. The diameters of the clear zones around each disc were measured after the incubation at 32℃ for 24 h.

Optimal culture condition of *L. sakei* BK19

To determine the optimal growth conditions for *L. sakei* BK19, it was grown in the presence of various carbon sources added into NB broth (Difco, USA) at the final concentration of 0.1% (w/v). Sample were collected at a 4 h interval to measure (OD_{630nm}) the cell growth. For studying the effect of the incubation temperature to the bacterial growth, the bacterium was incubated in MRS broth for 48 h at 5, 15, 25, 35 and 45℃, respectively. The effect of salinity on the growth of *L. sakei* BK19 also studied at following concentrations of NaCl; 0, 1, 3, 5 and 7% (w/v) with MRS broth for 48 h at 32℃. Cell growth was monitored with spectrophotometer at 630 nm.

Results and Discussion

pH effects on the growth of *L. sakei* BK19

The effect of the initial pH of the media on growth of the *L. sakei* BK19 was examined. High resistance to low pH noted to be an important character for the probiotic strain to survive in the stomach. In this study *L. sakei* BK19 which was isolated from fish fermented foods, showed significantly high a resistance at low pH (Table 1). After 24 h of incubation, *L. sakei* BK19 was survived at pH 4.0.

Table 1. The growth of *L. sakei* BK19 at different initial culture pH values

Strain	Initial pH of the culture medium						
	4.0	4.5	5.0	6.0	7.0	8.0	9.0
BK19	+	++	+++	+++	+++	+++	+++

− : No growth; +, <10^4CFU/*ml*; ++, 10^6CFU/*ml*; +++, 10^8CFU/*ml*

Antibiotic susceptibility of *L. sakei* BK19

Inhibition zone formed by antibacterial discs were shown in Table 2. *L. sakei* BK19 was exhibit-

ed resistance to 6 antibiotic agents (Nalidixic acid, Ciprofloxacin, Novobiocin, Doxycycline, Neomycin and E: Erythromycin). Antibiotic resistance have become a serious problem in treatment of infections caused by a variety of microorganisms due to blind use of antibiotics in human and veterinary medicine. However, antibiotic sensitivity is one of the important factors on selection of probiotics. Because, probiotic strains should be able to colonize in the gastrointestinal tract in spite of antibiotic treatments. Antibiotic resistances of *Lactobacilli* have been observed (Barefoot *et al.*, 1993). Moreover, this results suggest that *L. sakei* BK19 could be able to colonize in the gastrointestinal tract in spite of antibiotic treatments with Nalidixic acid, Ciprofloxacin, Novobiocin, Doxycycline, Neomycin and E: Erythromycin.

Additionally, Amoxicillin, Oxytetracycline, Tetracycline and Doxycycline are commonly used in aquatic industry as chemotherapeutic agent. However, according to this study *L. sakei* BK19 showed high sensitivity towards fore mentioned antibacterial agents. Therefore, it has to be use carefully when treats with antibacterial agents due to effect to cell survival. Also, resistance to chemotherapeutic agent in *L. sakei* BK19 should be developed in order to use it in aquaculture even if use antibiotic.

Table 2. Antibiotic susceptibility assay of *L. sakei* BK19

Antibiotics	Inhibition zone(mm)
AMC 30	8.1
TE 30	17
T 30	17.6
C 30	26.5
CF 30	16.3
NA 30	0
CIP 5	0
NB 5	0
D 30	24.9
SD 25	0
N 30	0
E 15	0

AMC, Amoxicillin; TE, Tetracycline; T, Oxytertacycline; C, Chloramphenicol; CF, Cephalothin; NA, Nalidixic acid; CIP, Ciprofloxacin; NB, Novobiocin; D, Doxycycline; SD, Sulfadiazine; N, Neomycin; E, Erythromycin

Optimal culture condition of *L. sakei* BK19

The availability of various carbon sources on the growth and fermentation of *L. sakei* BK19 showed in Fig. 1. Glucose, saccharose and maltose were

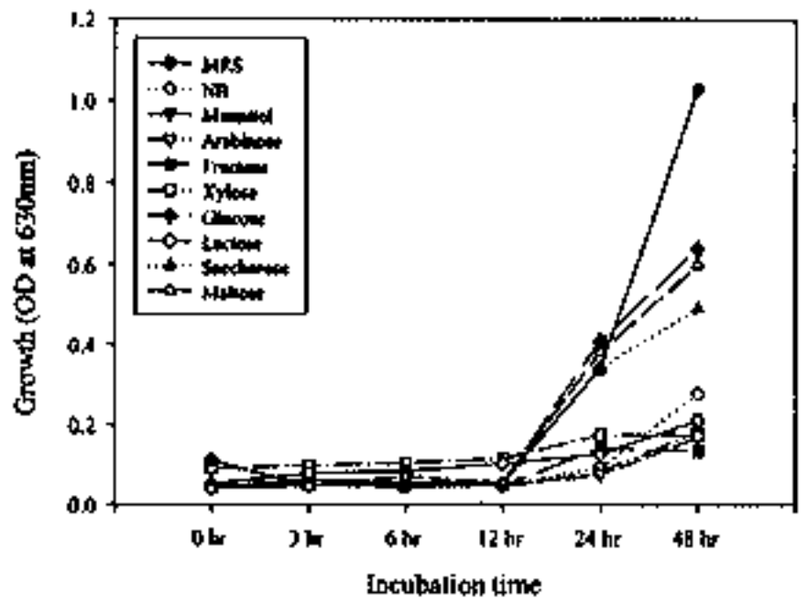

Fig. 1. Growth curve of the *L. sakei* BK19 given by different source of carbohydrate.

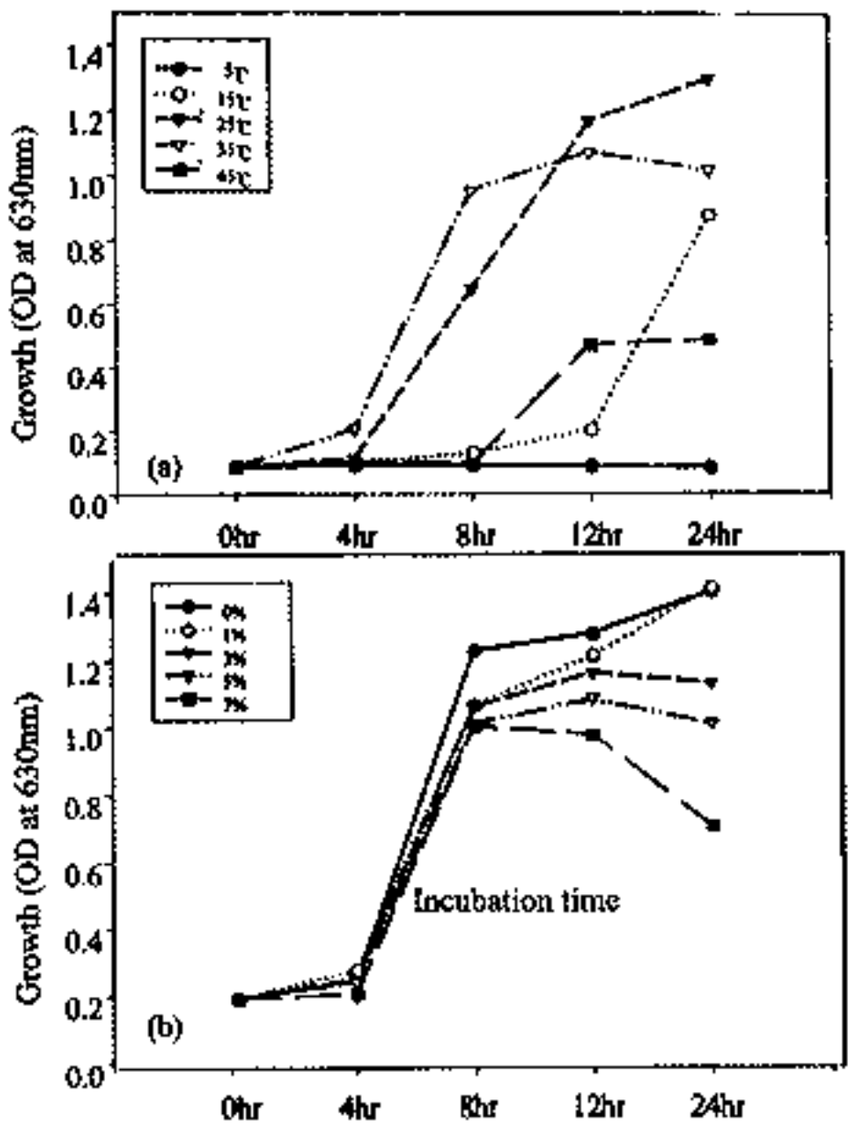

Fig. 2. The growth of *L. sakei* BK19 under different culture conditions of temperature (a), salinity (b).

good for bacterial growth as a carbon source. The glucose was reported to be better than any other carbon source for the fermentation and bacterial growth. The temperature range of *L. sakei* BK19 optimal culture was 15 to 45℃ with optimal temperature 25~35℃. *L. sakei* BK19 could grow well at 15℃ (Fig. 2a). Therefore this strain could be use in the olive flounder farm since the rearing water temperature could be around 15 to 20℃. Moreover, *L. sakei* BK19 showed well grown under 0 to 7% of NaCl concentration specially at 1% of NaCl (Fig. 2b). It has a potential that this strain to be use in aquaculture farms not only sea water but also fresh water.

However *L. sakei* BK19 did not isolate from fish intestine. Primary, probiotic character to improve host intestinal microbial balance (Fuller, 1989). This suggests that the conducted research on intestinal native probiotic strain and which sources are suitable for probiotic effect. This kinds of studies were already known as prebiotic. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth of bacteria in the colon, that can improve host health (Gibson and Roberfroid, 1995). A number of other non-digestible oligosaccharides have been developed, for which there is some evidence of their prebiotic effect (Gibson and Roberfroid, 1995; Wang. and Gibson,.1993).

This study was based on probiotic and investigating the value of nutrition. Following glucose, saccharose and maltose materials are may be useful as prebiotics. However, it is a basic information needed for research and applications in the industry. Therefore, further investigation to be carried out on the field of prebiotic concept as prebiotic methods are highly valuable in order to improve the native bacterial balance of host animals.

In conclusion, *L. sakei* BK19 strain has a high pH resistance and antibiotic tolerance. *L. sakei* BK19 used glucose, maltose and saccharose as a carbon source for their growth and fermentation but lactose and mannitol was not. Also *L. sakei* BK19 was able to grow at 15 to 45℃ and 0 to 7% of NaCl concentration. The results of this work suggests that, this strain can be use in fresh water and seawater fish farm for probiotics.

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