B-7

Isolation of useful lactic acid bacteria from jeotgal

Guysung Cho, Hyung Ki Do, Hyenkil Shin
Handong Global University

Introduction

Jeot-gal is Korean traditional fermented fish foods and is fermented by lactic acid bacteria(LAB)(Lee *et al.*, 2003). Especially normal LAB are proposed to exert health-promoting or 'probiotic' effects in humans and animals. Such effects are considered to include the inhibition of pathogenic microorganisms, the reduction of cholesterol levels and effected normal microorganism in intestinal track(Toit *et al.*, 1998). In order to select probiotic LAB, we have screened LAB from various jeotgal. The aim of this study was to characterize LAB which contain highly bile salt hydrolase activity, production of hydrogen peroxide, tolerance of low pH and tolerance of high bile salt. The isolated strains were characterized physiologically and the preliminary antimicrobial effects were tested.

Materials and methods

Serial dilution of collected jeotgal was made in quarter-strength Ringer's solution, and 100$\mu\ell$ of each dilution was spread on MRS agar plates(Toit *et al.*, 1998). The plates were incubated at 30℃ for 48h. The colonies were re-streaked onto MRS agar and were sub-cultured in MRS broth. In order to assess bile salt tolerance of bacteria, the isolated strains were incubated in MRS broth (pH 7.0) containing 0.3% (w/v) oxgall (Lim *et al.*, 2004). Also to assess pH tolerance of LAB, the strains were inoculated into MRS broth (pH 2.0)(Lim *et al.*, 2004). The isolated strains were screened by being impregnation around sterilized paper disks on the MRS agar plates. The MRS agar plates were supplemented with 0.5% (w/v) sodium salt of taurodeoxycholic acid and 0.37 g/l CaCl2 (Toit *et al.*, 1998). Bacteriocin activity was tested by spot-on-lawn method (Paik *et a.,l* 2003). The isolated strains were inoculated on MRS agar plates, incubated at 30℃ for 48h. The plates were then overlaid with the indicator microorganisms inoculated in 10 ml of 0.7% soft agar media at an approximate concentration of 10^4 cells. The clear zones of inhibition around LAB spot were examined. Finally, some species of LAB also produced hydrogen peroxide (H_2O_2)(Pascual *et a.,l* 2006). We inoculated onto a MRS agar plate with 0.25 mg/mL of 3,3V,5,5V-tetramethylbenzidine (TMB) ; 0.01 mg/mL horseradish peroxidase was added. The colonies were changed with blue color because of the

production of hydrogen peroxide from LAB.

Result and Discussion

The gram-positive, catalase-negative colonies isolated on MRS agar were identified as lactic acid bacteria. Out of 94 strains isolated from jeotgal, bile salt tolerance, low pH tolerance, bile salt hydrolase(BSH) activity, bacteriocin production and hydrogen peroxide production were carried out. Most of the strains survived in low pH and 0.3% bile salt condition. Twelve strains were highly positive for BSH activity on special plates. Seven strains were positive for hydorgen peroxide production. These data are strongly candidates of useful probiotic LAB isolated jeotgal. In near future, these probiotic LAB isolated will be identified using 16S rRNA sequencing level. Also the immune modulator or enhancer will be carried out.

References

Lee N.K., H.Wook. Kim, S.Y. Choi and H.D. Paik. 2003. Some probiotic properties of some lactic acid bacteria and yeasts isolated form jeot-gal. *Kor.J. Microbiol. Biotechnol.* 31: 297-300.

Paik H.D., K.M. Kim, J.G. Kim and N.K. Lee. 2003. Optimization for lacticin SA72 production by *Lactobacillus lactis* SA72 isolated from jeot-gal. *Kor.J. Microbiol. Biotechnol.* 31: 46-50.

du Toit M., C.M.A.P. Franz, L.M.T. Dicks, U. Schillinger, P. Haberer, B. Warlies, F. Ahrens and W.H. Holzapfel. 1998. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholsterol levels, faeces pH and faeces moisture content. *Int. J. Food Microbiol.* 40: 93-104.

Pascual L.M., M.B. Daniele, C. Pájaro and L. Barberis. 2006. *Lactobacillus* species isolated from the vagian: identification, hydrogen peroxide production and nonoxynol-9 resistance. *Contraception* 73: 78-81.

Kim H.J., S.Y. Kim and W.K. Lee. 2004. Isolation of choletserol-lowering lactic acid bacteria from human intestine for probiotic use. *J. Vet. Sci.* 5: 391-395.

Lee H.J., Y.J. Joo, C.S. Park, S.H. Kim, I.K. Hwang, J.S. Ahn and T.I. Mheen.1999. Purification and characterization of bacteriocin produced by Lactococcus lactis subsp. lactis H-559 isolated from kimchi. *J. Biosci. Bioeng.* 88: 153-159.