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The acid, bile tolerance and antimicrobial property of NIT

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ABSTRACT

The characteristics of potential probotic NIT isolated from infant feces were evaluated in vitro. The strain was examined for resistance to pH 2–4 and 1–3% bile, adhesion to Caco-2 cells, and antimicrobial activities against enteric pathogenic bacteria. NIT was shown tolerance property to bile, acid and strong antimicrobial activity against tested enteropathogens by the well-diffusion method. Furthermore, good adhesion and significant potential for decreasing adhesion of pathogens to Caco-2 cells were observed in this experiment. These results showed that NIT may be useful for improving probiotic formulae with respect to protection against enteric pathogenic infection.

1. Introduction

Specific strains of , and also, some strains have been introduced as probiotics in food products due to their growing evidence of health benefits (Alvarez-Olmos & Oberhelman, 2001; Guarner & Malagelada, 2003; Shanahan, 2002). Lactobacilli are natural inhabitants of healthy human intestinal tract and also have a long history of use in foods and fermented products. It is desirable that these bacteria have suitable general aspects (origin, identity, safety, and acid and bile resistance), technical aspects (growth properties in vitro and during processing), and functional and beneficial features (Holzapfel & Schillinger, 2002). Previous work has shown probiotic strains can possess inhibitory activity toward the growth of pathogenic bacteria, resistance to acid and bile, adherence to the intestinal epithelial cells and positive effects on the host health (Finlay & Falkow, 1989; Gorbach, 1996; Jacobsen et al., 1999).

Selection of suitable probiotic candidates is the principal basis for improving the bio-therapeutic action and functional properties of probiotic foods and pharmaceutical products. The essential characteristics for to be used as probiotics during manufacturing include the following: (i) recognition as safe (GRAS; generally recognized as safe); (ii) viability during processing and storage; (iii) antagonistic effect against pathogens; (iv) tolerance to bile acid challenge, and (v) adherence to the intestinal epithelium of the host among others (Begley, Gahan, & Hill, 2005; Lin, Hwang, Chen, & Tsen, 2006; MacFarlane & Cummings, 2002; Vesterlund, Paltta, Karp, & Ouwehand, 2005).

In order to survive in and colonize the gastrointestinal tract, probiotic bacteria should express high tolerance to acid and bile and have the ability to adhere to intestinal surfaces (Kirjavainen, Ouwehand, Isolauri, & Salminen, 1998; Lee & Salminen, 1995). Survival ability and temporary colonization of the human gastrointestinal tract have been demonstrated for some lactic acid bacteria (Alander et al., 1997; Johansson et al., 1998). However, in vivo testing is expensive and time consuming and requires approval by ethical committees. Therefore, reliable in vitro methods for selection of promising strains are required. Enterocyte-like Caco-2 cells (Pinto et al., 1983) have been successfully used for in vitro studies on the mechanism of cellular adhesion of nonpathogenic lactobacilli (Greene & Klaenhammer, 1994; Tuomola & Salminen, 1998). Also, Caco-2 cells have been used to examine the antimicrobial activity of lactobacilli (Hudault, Liévin, Bernet-Camard, & Servin, 1997) against pathogenic bacteria. Although many potentially probiotic strains are currently available for commercial use either in the form of fermented foods or pure culture in powdered, tablet or capsule form, these bacteria may not have above characteristics. It therefore appears appropriate to isolate and characterize strains with specific and well-characterized activities, not only to improve the quality and functional properties of probiotic products but also to advance both applied and fundamental science in the area of probiotics.

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The objective of the present study is to characterize the potential probiotic NIT originally isolated in infant feces. The acid, bile tolerance and antimicrobial property of this strain were evaluated. Enterocyte-like Caco-2 cells were used for testing adhesion properties and the abilities to inhibit the adhesion of pathogens. Reference pathogens strains were obtained from CCTCC (China Center for Type Culture Collection, Wuhan, China) and DSM (Deutsche Sammlung von Mikroorganismen).

2. Materials and methods

NIT used as potential probiotic was originally derived from infant feces. This strain was conserved on Key Laboratory for Molecular Design and Nutrition Engineering, Ningbo Institute of Technology, Zhejiang University. Bacterial pathogens used were CCTCC AB 206316, CCTCC M 90030, DSM 627, DSM 1447, DSM 1296. All bacterial pathogens were reactivated in LB broth (Luria–Bertani media). NIT was cultured in MRS broth, as detailed by De Man, Rogosa and Sharpe (Oxoid Ltd., Hampshire, United Kingdom). All and pathogens were cultured at 37 °C under anaerobic conditions (10% H_2, Td(Escphi39c7ozZ10ivat90030,)TJ/T1446.7968Tm(2)Tj8.1853007.9702951.3185448.1574Tm(,)-360(and)-2548Td36309TD(were)2428istured)2270(at)TJ/T1401Tf678890Td()Tj/T101Tf03.7590Td803dwothmediumthglyci-

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ability of lactobacilli to inhibit five pathogenic strains adhesion (10^7 CFU per well) to Caco-2 cells was evaluated by simultaneous addition of 10^8 CFU per well of tested lactobacilli isolate. Plates were incubated for 1 h at 37 °C, washed three times with sterile PBS. The cell-associated pathogens (extracellular plus intracellular bacteria) were lysed with 1% (v/v) Triton X-100 (Sigma) in deionized water for 5 min. This concentration of Triton X-100 did not affect bacterial viability for at least 30 min (data not shown).

4. Discussion

With the great interest on health-oriented nutritional habits, the food industry is requested to provide more and more functional foods containing healthful components. By the recently adopted definition that probiotics are live micro-organisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO., 2001), probiotic bacteria seem to be such components. However, to sustain a certain strain as a probiotic, a group of requirements should be fulfilled (Collins, Thornton, & Sullivan, 1998) and probiotic activities demonstrated in well-designed human studies. This paper presents results of some preselective studies on NIT.

In order to exert positive health effects, the lactobacilli should resist the stressful conditions of the stomach and upper intestine that contain bile (Chou & Weimer, 1999). Acidity is believed to be the most detrimental factor affecting growth and viability of lactobacilli, because their growth was down significantly below pH 4.5 (Lankaputhra & Shah, 1995; Lankaputhra, Shah, & Britz, 1996). In the present study, it was observed that NIT had certain resistance ability to acid and bile. Therefore, this stain may be expected to survive acidic conditions that exist in fermented food products or stomach and intestinal juice, which may contribute to increased shelf life.

As a functional probiotic, anti-pathogen activity is one of important properties. The antimicrobial ability of NIT against some enteropathogens was assayed in this study. The overnight culture showed strong inhibition action to selected pathogens (Table 1). Meanwhile, treated supernatant without peroxide and lactic acid also had week anti-pathogen activity. It was suggested that NIT produced bacteriocins to inhibit the test pathogens. Some authors have reported that production of bacteriocins by lactobacilli is relatively common, which may contribute to their colonization of habitats and their competitive edge over other bacteria (Garriga, Hugas, Aymerich, & Monfort, 1993). The antimicrobial activity of lactic acid bacteria may be due to a number of factors. Among these are decreased pH levels, competition for substrates and the production of substances with a bactericidal or bacteriostatic action, including bacteriocins (Parente & Ricciardi, 1999). In fact, the drop in pH arising from the production of lactic acid can be enough to inhibit certain strains. This is because the non-dissociated form of lactic acid triggers a lowering of the internal pH of the cell that causes a collapse in the electrochemical proton gradient in sensitive bacteria, hence having a bacteriostatic or bactericidal effect (O'KeeVe & Hill, 1999).

Although antagonistic effects are essential, the ability of probiotic bacteria to adhere to the intestinal epithelium is a prerequisite for probiotic micro-organisms to be effective. Thus, the ability to adhere to epithelial cells and mucosal surfaces has been suggested to be an important property of many probiotic bacterial strains (Collado, Gueimonde, Hernandez, Sanz, & Salminen, 2005; Ouwehand, Isolauri, Kirjavainen, & Salminen, 1999). The binding ability of our isolates was evaluated using the human colon carcinoma cell line Caco-2 as a cellular model. Caco-2 has been used frequently because it exhibits, in vitro, the characteristics of a mature enterocyte (Pinto et al., 1983). Because association with, and invasion of, the cultured cell lines have been reported to mimic the in vivo conditions of adhesion and infection of pathogenic bacteria (Kerneis et al., 1992; Mounier, Vasselon, Hellio, Lesourd, & Sansonetti, 1992). In this study, we demonstrated that NIT had a strong adhesion property to Caco-2 cells (Fig. 3). Some studies demonstrated that the ability of some bacterial strains to adhere and colonize the intestinal cell in vivo or the cultured intestinal cell in vitro is similar (Finlay & Falkow, 1989). However, it must be considered that Caco-2 cells were only used as a model to study adhesion because of the different morphological, physicochemical and environmental conditions surrounding the epithelial cells in both types of experiences.

Recent studies have explained the role of lactobacilli in the prevention and treatment of gastrointestinal disorders (Coconier et al., 1998; Hudault et al., 1997). One of the important factors is the competitive inhibition of enteropathogen attachment to epithelial cells by lactobacilli. So, we investigated the competitive inhibition of adherence of pathogenic bacteria to Caco-2 cells by adhering cells. The can strongly inhibit the adhesion of most selected pathogens (Table 2

no obvious effect on the adhesion of (> 0.05). However, the mechanisms by which lactobacilli inhibit pathogen adhesion to human cell lines in vitro are not fully understood. Steric hindrance rather than blockage of specific receptors may be involved (Bernet, Brassart, Neeser, & Servin, 1993). Additional experiments are needed to determine the precise mechanism of inhibition observed in our study.

In conclusion, NIT isolated from infant feces in this study presented interesting probiotic characteristics, especially greater resistance to acid and bile conditions, as well as good adhesion capacity to Caco-2 cells. This stain also showed greater enteropathogen growth inhibiting activity and interference with pathogens adhesion to Caco-2 cells. These characteristics may enable them to establish themselves in the intestinal tract and to compete with other bacterial groups. Further studies are needed to characterize the antimicrobial factors, and assay in vivo.

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References

Alander, M., Korpela, R., Saxelin, M., Vilpponen-Salmela, T., Mattila-Sandholm, T., & Wright, A. (1997). Recovery of GG from human colonic biopsies. , 361–364.

Alvarez-Olmos, M. I., & Oberhelman, R. A. (2001). Probiotic agents and infectious diseases: A modern perspective on a traditional therapy. , 1567–1575.

Begley, M., Gahan, C. G., & Hill, C. (2005). The interaction between bacteria and bile. , 625–651.

Bernet, M. F., Brassart, D., Neeser, J. R., & Servin, A. L. (1993). Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen–cell interactions. , 4121–4128.

Chou, L. S., & Weimer, B. (1999). Isolation and characterization of acid and bile-tolerant isolates from strains of . , 23–31.

Claire, L. Vernazza, Glenn, R. Gibson, & Robert, A. (2006). Carbohydrate preference, acid tolerance and bile tolerance in five strains of Bifidobacterium. , 846–853.

Coconier, M. H., Klaenhammer, T. R., Kernéis, S., Bernet, M. F., & Servin, A. L. (1992). Protein-mediated adhesion of BG2FO4 on human enterocyte and mucus-secreting cell lines in culture. , 2034–2039.

Coconier, M. H., Lievin, V., Hemery, E., & Servin, A. L. (1998). Antagonistic activity against infection in vitro and in vivo by the human strain LB. , 4573–4580.

Collado, M. C., Gueimonde, M., Hernandez, M., Sanz, Y., & Salminen, S. (2005). Adhesion of selected strains to human intestinal mucus and its role in enteropathogen exclusion. , 2672–2678.

Collins, J. K., Thornton, G., & Sullivan, G. O. (1998). Selection of probiotic strains for human applications. , 487–490.

FAO/WHO. (2001). Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina.

Finlay, B. B., & Falkow, S. (1989). Common themes in microbial pathogenicity. , 210–230.

Garriga, M., Hugas, M., Aymerich, T., & Monfort, J. M. (1993). Bacteriocinogenic activity of lactobacilli from fermented sausages. , 14–148.

Gorbach, S. L. (1996). The discovery of GG. , 2–4.

Greene, J. D., & Klaenhammer, T. R. (1994). Factors involved in adherence of lactobacilli to human Caco-2 cells. , 4487–4494.

Guarner, F., & Malagelada, J. R. (2003). Gut flora in health and disease. , 512–519.

Hechard, Y., Dherbomez, M., Cenatiempo, Y., & Lettllier, F. (1990). Antagonism of lactic acid bacteria from goats' milk against pathogenic strains assessed by the 'sandwich method'. , 185–188.

Holzapfel, W. H., & Schillinger, U. (2002). Introduction to pre- and probiotics. , 109–116.

Hudault, S., Liévin, V., Bernet-Camard, M. F., & Servin, A. L. (1997). Antagonistic activity exerted in vitro and in vivo by (strain GG) against C5 infection. , 513–518.

Jacobsen, C. N., Nielsen, V. R., Hayford, A. E., Moller, P. L., Michaelsen, K. F., Paerregaard, A., et al. (1999). Screening of probiotic activities of forty-seven strains of spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. , 4949–4956.

Johansson, M. L., Nobaek, S., Berggren, A., Nyman, M., Björck, I., Ahrne, S., et al. (1998). Survival of DSM 9843 (299v) and effect on the short-fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats. , 29–38.

Kerneis, S., Chauvière, G., Darfeuille-Michaud, A., Fourei, V., Coconnier, M. H., Joly, B., et al. (1992). Expression of receptors of enterotoxigenic during enterocytic differentiation of human intestinal epithelial cells, Caco-2 and HT-29 in culture. , 2572–25801Tf7.156(A.,)-4lon235d,Aoly,84(L2)-323