# **Development of Probiotic Starter Culture/Food Adjunct Using Selected Bifidobacteria\***

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### Introduction

Bifidobacteria are Gram positive, non acid fast, non spore forming, non motile organism. They are rod shaped but enormously variable in appearance. Bifidobacteria are anaerobic organism but some species can tolerate oxygen only in the presence of carbon dioxide. The organims was first isolated from facces of breast fed infant. However, they are also found in adult human intestine, vagina and mouth as well as in the alimentary tract of various kinds of animals. Bifidobacteria are known as a probiotic organism. They have been known to produce several prophylactic and therapeutic effects on human as well as animals. These properties include improvement of intestinal microflora, alleviation of lactose intolerance, reduction of serum cholesterol levels, and antitumor and anticarcinogenic activities.

## **Materials and Methods**

Bifidobacteria were isolated from faeces of breast-fed infants and identified using biochemical test and organic acids production. Their antibiotic susceptibility test was conducted against broad range of antibiotics. Their antibacterial activity was conducted against selected food-borne pathogens using double-layered assay. Survival of bifidobacteria in environment simulated to gastrointestinal tract was conducted by exposure to low pH and high concentration of bile. The adhesion of probiotic microorganims onto Caco2 and HT29 cell lines was conducted in vitro. Symbiotic relationship of several probiotic microorganims and their inhibitory activity against selected food-borne pathogen was studied using chemostat culture.

## **Results and Discussion**

Bifidobacteria were found dominant in the faeces of breast fed infant. Other bacteria were present in small number. Some strains were found to be able to survive in low pH and in high concentration of bile. The antibacterial activity against selected pathogens was varied among the strains tested. The substances responsible for the inhibitory activity were found to be organic acids as no inhibition observed in buffered medium. The adhesion study of bifidobacteria to human intestinal epithelial cell lines was not encouraging as only one strain was able to adhere to HT29 cell lines. The presence of symbiotic relationship among probiotic microorganisms was proven in chemostat culture. Combination of two-probiotic microorganism showed better inhibitory activity against E. coli as compared to monoculture of probiotic microorganism.

#### Conclusions

This study was designed to select the right strains of bifidobacteria to be used as food adjunct based on several characteristics. However, none of the strains tested fulfill all the requirements outlined in this study. Therefore, combinations of two or more strains of probiotic are strongly recommended to produce superior probiotic products or food adjuncts.

#### Benefits from the study

Information generated from this study could be used by dairy industries and health food manufacturers in the production of a wide range of products.

Literature cited in the text None.

# Project Publications in Refereed Journals

- Shuhaimi, M., Ali. A.M., Saleh, N.M., Yazid, A.M. 2001. Utilisation of enterobacterial repetitive intergenic consensus (ERIC) sequence-based PCR to fingerprint the genomes of *Bifidobacterium* isolates and other probiotic bacteria. *Biotechnology Letters*. 23(9): 731-736.
- Shuhaimi, M., Yazid, A.M., Ali, A.M., Ghazali, M.H., Zaitun, H. and Nur Atiqah, N.A. 1999. Antibacterial activity, Antimicrobial Susceptibility and Adherence Properties of Bifidobacterium infantis G4. Pakistan Journal of Biological Sciences, 2(4): 1231-1235.
- Shuhaimi, M., Yazid, A.M., Shuhaimi, M., Ali, A.M., Zaiton, H. and Ghazali, M.H. 1999. Acid Adaptation of *Bifidobacteria* Isolated from infant stool to simulated pH of human stomach. *Pakistan Journal of Biological Sciences.* 2(4): 1203-106.
- Yazid, A.M., Ali, A.M., Kalaivaani, V. and Shuhaimi. 1998. Properties of *Bifidobacterium* spp.: antibacterial, growth in milk and survival during low temperature storage. *Asian Pacific Journal of Molecular Biology and Biotechnology*, 6(2): 153-159.
- Yazid, A.M., Ali, A.M., Shuhaimi, M., Kalaivaani, V., Rokiah, M.Y. and Reezal, A. 2000. Antimicrobial susceptibility of bifidobacteria. *Letters in Applied Microbiology.* 31: 57-62.
- Yazid, A.M., Rezaei Sabet, M., Abdullah,
  S., Nur Atiqah, A. and A.B. Fatimah.
  1999. Probiotic Microorganisms:
  Potential Use in the Reduction of Serum
  Cholesterol level. *Pakistan Journal of Biological Sciences.* 2(4): 1663-1667.
- Yazid, A.M., Salina, A.B., Shuhaimi, M., Osman, H. and Normah, J. 1999.
  Inhibitory effects of probiotic bacteria against selected food-borne pathogens. *Pakistan Journal of Biological Science*. 2(3): 660-663.
- Yazid, A.M., Shuhaimi, M., Ali, A.M., Kalaivaani, V., Ghazali, M.H., Normah, J.

and Reezal, A. 1999. Survival of bifidobacteria in simulated gastric pH and growth in the presence of bile. Asian Pacific Journal of Molecular Biology and Biotechnology. 7(2): 1-11.

- Yazid, A.M., Shuhaimi, M., Ali, A.M., Raha, A.R., Ernie-eileen, R.R., Rowina, H. and Abdullah, N.A.N. 1999. Antimicrobial susceptibility and plasmids profiles of bifidobacteria isolated from infant stool. *Pakistan Journal of Biological Science*. 2(2): 555-558.
- Yazid, A.M., Shuhaimi, M., Ali, A.M., Zaiton, H., Ghazali, M.H. and Reezal, A. 1999. Inhibitory activity, antimicrobial susceptibility and adherence properties of *Bifidobacterium infantis* G4 isolated from infant stools. *Pakistan Journal of Biological Science*. 2(4): 1231-1235.
- Yazid, A.M., Shuhaimi, M., Rezaei Sabet, M. and Raizawanis, A.R. 1999. Relationship between bile tolerence and deconjugation activity of *Bifidobacterium* spp. *Pakistan Journal of Biological Science*. 2(3): 759-762.

## Project Publications in Conference Proceedings

- Abu Bakar, F., Cha, Y.L., Saari. and Yazid, A.M. 2000. Biogenic amines production and microbiological quality of *cincaluk* during fermentation. In: Proceedings of the Malaysian Science & Technology Congress 2000 and Asia Pacific Symposium on Food Science & Nutrition 2000. September 18-20, 2000. Kota Kinabalu, Sabah, Malaysia. Pp 147.
- Anis, S.M.H., Salina, A..S., Yazid, A.M., Shuhaimi, M. and Normah, J. 2001. inhibition of pathogenic micro-organisms by probiotic cultures. In: Proceedings of the *Conference on Functional Food-Latest Development*. Renaissance Palm Garden, Putrajaya. Malaysia. April 10-11 2001.
- Cheng, L.L., Manap, M.Y. and Shuhaimi. 1997. Carbohydrate metabolism and end products analysis of selected Bifidobacterium spp. In: Proceedings of the National Conference on Food Industry 2000: Technology and Opportunities. Kuala Lumpur. May 5-7.
- Fandi, K.G., Ghazali, H.M., Yazid, A.M. and Raha, A.R. 2000. Properties of fructose-6-phosphate phosphoketolase purified from Bifidobacterium asteroids. In: Proceedings of the 100<sup>th</sup> General Meeting ASM, Los Angeles Convention Centre, L.A., Ca., USA.

- Ismail, M.M., Ghazali, H.M., Manap, M.Y and Hashim, D.M. 1997. Lipase-catalysed transesterifcation of palm kernel olein and anhydrous milk fat. In: Proceedings of the 9<sup>th</sup>. National Biotechnology Seminar, Pulau Pinang.
- Kalaivani, V., Manap, M.Y. and Shuhaimi,
  M. 1997. Selection of probiotic starter culture as a food adjunct using *Bifidobacterium* spp. In: Proceedings of the National Conference on Food Industry 2000: Technology and Opportunities. Kuala Lumpur. May 5-7.
- Koh, C.C., Nor Aini, I., Dzulkifly, M.H. and Yazid, A.M. 2001. A low fat non-dairy synbiotic soft serve ice cream mix formulation. In: Proceedings of the *Conference on Functional Food-Latest Development*. Renaissance Palm Garden, Putrajaya. Malaysia. April 10-11 2001.
- Liew, M.Y.B., Ghazali, H.M., Long, K.,
  Lai, O.M and Manap, M.Y. 1998. Effect of incubation period and glucose concentration on the production of mycelium-bound lipase from *Rhizomucor miehei*. In: Proceedings of the 10<sup>th</sup>.
  National Biotechnology Seminar, Shah Alam. Pp. 369-372.
- Lim, L.C., Normah, J. and Yazid, A.M.
  2001. Symbiotic relationships of probiotic cultures in chemostat system. In:
  Proceedings of the Conference on Functional Food-Latest Development.
  Renaissance Palm Garden, Putrajaya.
  Malaysia. April 10-11 2001.
- Masran, A., Manap, M.Y. and Shuhaimi, M. 1997. Antibacterial activity of selected *Bifidobacterium* spp and *Lactobacillus* spp against *Escherichia coli* and *Listeria monocytogenes*. In: Proceedings of the National Conference on Food Industry 2000: Technology and Opportunities. Kuala Lumpur. May 5-7.
- Nur Atiqah Ng Abdullah., Mohd. Yazid Abd. Manap., Shuhaimi Mustafa and Abdul Manaf Ali. 2000. Antimicrobial Susceptibility and antibacteria activity of bifidobacteria isolated from breast fed infant stool. In: Proceedings of the 3<sup>rd</sup> Asian Conference on Food Safety and Nutrition – Improving Nutrition and Food Safety for the New Millennium. October 3-6, 2000. Beijing, China. Pp. 74.
- Rezaei Sabet, M., Yazid A.M., Sipat, A., Ali, A.M., Abdul-Rahim, M. and Nur Atiqah, A. 2000. Cholesterol-lowering ability of *Bifidobacterium* spp in cholesterol fed rats. In: Proceedings of the Malaysian Science & Technology

An update of the abstract published in UPM Research Report 1993.

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- Shuhaimi Mustafa, Mohd Yazid Manap, Abdul Manaf Ali and Norehan Mohd.
  Saleh. 2001. Cloning and expression of bile salt hydrolase (bsh) gene from Bifidobacterium longum into E. coli. In: Proceedings of the 13<sup>th</sup>. National Biotechnology Seminar. 'Towards Commercialization of Malaysian Biotechnology'. 10-13<sup>th</sup> Nov 2001, The Bay View Beach Resort, Penang, Malaysia.
- Shuhaimi Mustaffa, Abdul M. Ali., Mohd.
  Yazid Manap and Khatijah Yusoff. 2000.
  Randomly Amplified Polymorphic DNA (RAPD) analysis of *Bifidobacterium* strain. In: Proceedings of the Molecular Biology and Biotechnology in the 21<sup>st</sup> Century: The need for a quantum leap.
  The 10<sup>th</sup> Scientific meeting of MSMBB.
  May 14-16, 2000. Hyatt Regency Saujana Subang, Selangor, Malaysia. Pp. 40.
- Shuhaimi, M., Yazid, A.M., Ali, A.M and Saleh, N.M. 2001. Characterisation of *Bifidobacterium* species isolated from infant stool. In: Proceedings of the International Conference of Intestinal Bacteriology. Keidanren Kaiken, Tokyo, Japan. July 5-6, 2001.
- Yazid A.M. and Shuhaimi, M. 2001. Probiotic, prebiotic and gut health. In: Proceedings of the Conference on Functional Food-Latest Development. Paper presented at the Seminar on Functional Foods. Renaissance Palm Garden, Putrajaya. Malaysia. April 10-11 2001.
- Yazid, A.M., Shuhaimi, M. and Kalaivaani, V. 1997. Development of probiotic starter culture: Bile and low pH tolerance of selected *Bifidobacterium* spp. In: Proceedings of the 3<sup>rd</sup> Symposium on Trends in Biotechnology and 7<sup>th</sup> Scientific Meeting of MSMBB. UPM Serdang. May 15-17.

#### Graduate Research

- Kalaivaani N. 1997. Starter Culture Technology [MSc]. Universiti Putra Malaysia.
- Normah Jusoh. 1998. Food Biotechnology [MSc]. Universiti Putra Malaysia.
- Shihaimi Mustaffa. 1998. Food Biotechnology [MSc]. Universiti Putra Malaysia.