CHOLESTEROL-REDUCING ACTIVITY OF *LACTOBACILLUS* SPECIES FROM CHICKEN

Y.W. Ho, R. Kalavathy, N. Abdullah and S. Jalaludin

Institute of Bioscience
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Keywords: *Lactobacillus*, cholesterol, chicken, probiotic, bile salt hydrolase.

Introduction

Probiotics (direct-fed microbials), which included *Lactobacillus* cultures, increase the natural defence mechanism of chickens and have been used as an alternative to antibiotics in poultry production. Probiotic-fed chickens not only grow faster, consume less feed, have less mortality rate, but also have lower total serum cholesterol level (Jin et al. 1998). Several hypotheses have been put forth to explain the hypocholesterolemic effect of *Lactobacillus*. These are: i) by direct assimilation of cholesterol by *Lactobacillus*, ii) by co-precipitation of cholesterol with bile acids as a result of bile salt hydrolase enzyme released by *Lactobacillus*, and iii) by both assimilation and co-precipitation. The objectives of the present study were to determine the ability of *Lactobacillus* to reduce cholesterol and to establish the mechanism(s) involved in the reduction of cholesterol.

Materials and Methods

A preliminary study was conducted to assess the ability of 12 *Lactobacillus* isolates (the same isolates as those described by Jin et al. 1996) to reduce cholesterol. The media used were i) MRS (acted as control), ii) MRS + cholesterol (MRSC), and iii) MRS + bile salt + cholesterol (MRSBC). From this preliminary study, three *Lactobacillus* isolates viz., *L. acidophilus* I 16, *L. acidophilus* I 26 and *L. brevis* C 10, were selected for further studies on the mechanism(s) involved in the reduction of cholesterol. For the study on co-precipitation of cholesterol with bile salt as a result of bile salt hydrolase enzyme released by *Lactobacillus*, the three isolates were cultured in MRS agar incorporated with 0.5% sodium salt of taurodeoxycholic acid (conjugated bile salt). Bile salt hydrolase active *Lactobacillus* isolates produced opaque white colonies on the agar due to precipitates formed from the bile salt hydrolase activity on the conjugated bile salt. The ability of the *Lactobacillus* isolates to deconjugate bile salt was also assessed by determining the free cholic acid liberated when the isolates were incubated in MRS + bile salt (MRSB) medium. For the study on the assimilation of cholesterol by the isolates, the media used were MRSC and MRSBC. Cholesterol was extracted from the bacterial cells (Rudel and Morris, 1973) and the cholesterol assimilated in the bacterial cells using Filipin stain (Klinkner et al. 1997)

Results and Discussion

All the 12 *Lactobacillus* isolates were capable of reducing cholesterol, but the percentage of cholesterol reduction varied considerably among the isolates. *Lactobacillus brevis* C 10 showed the best ability to reduce cholesterol (73.91% in MRSC and 85.41% in MRSBC) followed by *L. acidophilus* I 26 (64.39% in MRSC and 45.24% in MRSBC), but *L. brevis* I 218 had the least ability (34. 95% in MRSC and 43.39% in MRSBC). Some isolates reduced significantly ($P< 0.05$) more cholesterol in the presence of bile (MRSBC), while others reduced cholesterol more effectively in the absence of bile (MRSC). The percentages of cholesterol assimilated in the cells of *L. acidophilus* I 16, *L. acidophilus* I 26, and *L. brevis* C 10 grown in MRSC medium were 52.72%, 49.49% and 59.61% respectively, and in MRSBC medium were 37.07%, 36.43% and 63.52% respectively. These results showed that *L. acidophilus* I 16 and *L. acidophilus* I 26 assimilated more cholesterol in the absence of bile salt whereas *L. brevis* C 10 assimilated more cholesterol in the presence of bile salt. The cells of the three *Lactobacillus* isolates incubated in MRS medium (control) and stained with Filipin stain did not fluoresce, but those incubated in MRSC and MRSBC media fluoresced with an intense yellow colour, indicating the presence of cholesterol in the cells. This showed that cholesterol was not an inherent part of the cell and the cholesterol present in the cells of isolates incubated in MRSC and MRSBC media was probably assimilated and incorporated into the cell wall or membrane by the isolates during growth in the media. of the three *Lactobacillus* isolates, only *L. brevis* C 10 showed strong bile salt hydrolase activity. The enzyme deconjugated the bile salt which then co-precipitated with the cholesterol forming white opaque precipitates around the bacterial colonies. The analysis of bile salt hydrolase activity (by measuring the amount of cholic acid liberated) of the isolates incubated in MRSB medium also showed that the most rapid deconjugation of the bile salt was by *L. brevis* C 10. It released 0.86 μmol ml^{-1} free cholic acid, whereas *L.acidophilus* I 16 and *L. acidophilus* I 26 released 0.418 and 0.425 μmol ml^{-1} free cholic acid, respectively.

The results from the present study showed that for *L. brevis* C 10, which had high bile salt hydrolase activity and was able to decojugate bile salt effectively, reduction of cholesterol was mainly by co-precipitation of cholesterol with deconjugated bile salt, and assimilation of cholesterol played a smaller role. For *L. acidophilus* I 16 and *L. acidophilus* I 26, which had low bile salt hydrolase activity, reduction of cholesterol was mainly by assimilation of cholesterol by binding the cholesterol to the bacterial cell wall or membrane, and co-precipitation of cholesterol with deconjugated bile salt played a minor role.

Conclusions

All 12 *Lactobacillus* isolates studied reduced cholesterol to varying degrees. Some isolates reduced more cholesterol in the presence of bile salt while others reduced more in the absence of bile salt. The mechanisms involved in the reduction of cholesterol were assimilation of cholesterol by the isolates and co-precipitation of cholesterol with deconjugated bile salt.

References

Jin, L. Z., Ho, Y. W., Abdullah, N. and Jalaludin, S. 1998. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. *Poultry Science*. 77: 1259-1265.

Jin, L. Z., Ho, Y. W., Ali, M., Abdullah, N., ong, B. K. and Jalaludin, S. 1996. Adhesion of *Lactobacillus* isolates to intestinal epithelial cells of chicken. *Letters in Applied Microbiology*. 22: 229-232.

Klinkner, A. M., Bugelski, P. J., Waites, C. R., Louden, C., Hart, T. K. and Kerns, W. D. 1997. A novel technique for mapping the lipid composition of atherosclerotic fatty streaks by en face fluorescence microscopy. *The Journal of Histochemistry and Cytochemistry*. 45: 743-753.

Rudel, L. L. and Morris, M. D. 1973. Determination of cholesterol using o-phthalaldehyde. *Journal of Lipid Research*. 14: 364-366.

Supported by IRPA Grant 01-02-04-0292