International Food Research Journal 24(2): 819-824 (April 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Evaluating the anti-obesity potential of *Lactobacillus fermentum* 4B1, a probiotic strain isolated from balao-balao, a traditional Philippine fermented food

[1,2]Balolong, M.P., [2]Bautista, R.L.S., [2]Ecarma, N.C.A., [3]Balolong Jr., E.C., [2]Hallare, A.V. and [4*]Elegado, F.B.

[1]School of Bio-Resources Science, Dankook University, Cheonan, Korea
[2]College of Arts and Sciences, University of the Philippines Manila, Philippines
[3]Institute of Biomedical Science and Technology, Konkuk University, Seoul, Korea
[4]National Institutes of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Philippines

Article history

Received: 2 November 2015
Received in revised form: 29 March 2016
Accepted: 16 April 2016

Keywords

Lactobacillus fermentum
4B1
HFD
Adiposity
Obesity
Probiotics

Abstract

This paper examined the anti-obesity potential of orally-administered *Lactobacillus fermentum* strain 4B1, a probiotic candidate isolated from balao-balao (fermented shrimp) in Central Luzon (Philippines), using histological data and changes in body weight. Parameters associated with obesity including adipose cell area, adipose tissue weight and adipose cell number were obtained and compared between groups either receiving the *Lf4B1* treatment or the anti-obesity drug Orlistat (Xenical®) with those not receiving any treatment. Obesity was induced using a high fat diet to achieve a 34% difference of mean body weight compared to the group fed with a normal diet. The supplementation received was either *L. fermentum* 4B1 (5.0×10^8 cfu/ml at 50 ml/kg) or Orlistat (12 mg/kg) administered orally for 21 days. Adipose tissues from the abdominal region were extracted after treatment and subjected to histological examination. Changes in body weights and adipose tissue weights were also taken. Results showed that orally administered *L. fermentum* 4B1 significantly reduced adipose cell areas, significantly lowered body weights and significantly lowered adipose tissue weights compared to the groups which received no treatment. Interestingly, the anti-obesity effects of *L. fermentum* 4B1 and Orlistat were not significantly different, suggesting comparable outcomes in preventing weight gain and adiposity associated with obesity. Various studies utilizing different probiotic strains have been widely documented emphasizing the strain specificity of probiotic microbes in exerting their beneficial effects. This study described the potential benefits of a locally-isolated strain that may be utilized as a starter culture for the development of functional foods with promising anti-obesity activity.

Introduction

Obesity is a preventable health concern caused by abnormal or excessive fat accumulation presenting a risk to one's health. In 2014, more than 1.9 billion adults, 18 years and older, were overweight (WHO, 2014). About 42 million children under the age of 5 were overweight or obese during the previous year. In the Philippines, a 2011 survey by the Food and Nutrition Research Institute (FNRI) showed that 22.3% of Filipino adults are overweight and 6.1% are obese. Obesity has been previously shown to be associated not only with body weight gain (West and York 1998), but also with enlargement of adipose tissue cells, increase in adipose fat pad weight, and increase in adipose cell number (Ronakinen *et al*., 2015). Currently, available pharmacological treatments offer limited efficacy in producing sustained long-term weight loss, thereby prompting further research to discover new drug therapies that are safe and can be used to reduce the prevalence of obesity.

Probiotics are defined as viable microbial dietary supplements that exert beneficial effects on host health (Fuller, 1989). Probiotics have attracted public attention because of their potential effectiveness for both the prevention and the treatment of immune diseases (Borchers *et al.*, 2009). In addition, some probiotics have been demonstrated to have an anti-obesity property by regulating lipid and glucose metabolism (Kang *et al*., 2013). In recent years culture-based approaches in LAB isolation have become more targeted for detection of bacteriocin-producers and those that have potential as probiotics.

*Corresponding author.
Email: *fbelegado@hotmail.com*

In the Philippines, LAB isolates from fermented foods were screened for bacteriocin production and a PCR-based assay was used to detect specific bacteriocin-encoding genes (Banaay *et al.*, 2013). Acid and bile tolerance were also determined. Among all the isolates tested, *Lactobacillus fermentum* 4B1 has been identified as most promising for the development of new probiotic food products, hence it was chosen for subsequent biomedical application assays.

This study demonstrated the potential of orally-administered *L. fermentum* 4B1, a strain locally isolated from native fermented rice and shrimp, in reducing obesity in mice as compared to a commonly prescribed weight-loss drug Orlistat (Xenical®). Obesity-associated parameters such as body weight gain and adiposity were utilized to determine the treatment effects.

Materials and Methods

Preparation of L. fermentum *4B1 (Lf4B1) for oral administration*

Lf4B1 was obtained from the Food, Feeds and Specialty Products Laboratory, BIOTECH, University of the Philippines Los Baños. Stock cultures were sub-cultured twice and were later statically grown in Man Rogosa and Sharpe (MRS; BD Difco) broth for 24 h at 37°C. Cells were then harvested using centrifugation at 1500 x g, 10 min, washed twice with physiological saline and resuspended to a final concentration of 5 x 10^8 cfu/ml.

Animals and experimental design

The protocols involving animals were adapted from the approved guidelines of the Philippine Association for Laboratory Animal Science Code of Practice for the Care and Use of Laboratory Animals in the Philippines (PALAS 2002). Animals were carefully monitored for any signs of pains or distress during the entire duration of the experiment. Fifty healthy four-week-old female ICR mice weighing 10-12 g each were obtained from the Bureau of Animal Industry (BAI), Philippines. ICR mice have been used as an animal model to study obesity and its prevention (Nakahara *et al*., 2013; Lee *et al*., 2013). The mice were housed in plastic cages on a 12-h light/dark cycle and were fed with mouse pellets and tap water ad libitum. Fifteen mice were then randomly assigned to receive the normal diet while 35 mice received the high-fat diet (condensed milk diet). Condensed milk diet was prepared based on previous protocols (West *et al*., 1992; Cleary *et al*., 2004). This diet was maintained for 49 days until obesity was observed i.e., 20-30% increase in body weight compared to the normal diet group (Wang and Liao, 2012). Animals were then grouped based on the treatment administered for 21 days as follows, each group having 5 mice for analysis: a) normal diet/no treatment; b) normal diet/*Lf4B1* (5.0 x 108 cfu/ml at 50 ml/kg); c) high-fat diet/no treatment; d) high-fat diet/ *Lf4B1* (5.0 x 10^8 cfu/ml at 50 ml/kg); e) high-fat diet/Orlistat (12 mg/kg). The drug Orlistat (Xenical®) was commercially obtained from local drugstores.

Measurement of body weight gain, adipocyte area and adipose tissue weight

An observer blinded to the assigned study groups obtained the data on body weight gain, adipocyte cell size and adipose tissue weight. The body weights of every mouse in the experiment were measured on both the 1st and 21st days of treatment, and the percent weight gain of the treatment groups was compared. Adipocyte areas were measured from adipocyte tissues fixed in 10% formalin solution and stained with hematoxylin and eosin. Cell areas were measured using a standard photomicroscope with a graduated ocular. Likewise, the intra-abdominal fat pad of each mouse were precisely dissected and weighed.

Statistical analyses

The statistical significance was determined using ANOVA followed by Tukey's HSD (body weight gain) and Tamhane's T2 (for adipose weight and for cross-sectional area). A P value of <0.05 was considered significant in all cases. All values were expressed as mean + standard deviation.

Results

The high-fat diet (HFD) feeding induced significant weight gain in ICR mice after a 49-day pre-treatment period compared to the mice on the normal diet (ND). The HFD group showed approximately 35% higher body weight compared to the ND group, classified the HFD group as obese. The two groups were then used to analyze the effects of *Lb*4B1 supplementation on various obesity-associated parameters.

Effects of Lb*4B1 feeding on body weight gain*

The supplementation of *Lb*4B1 in mice belonging to either the HFD or the ND group significantly controlled body weight gain (Figures 1 and 2). Mice on a normal diet that received *Lb*4B1 recorded a weight gain of 3.66 ± 0.13% while mice that did not receive the supplement recorded a weight gain of

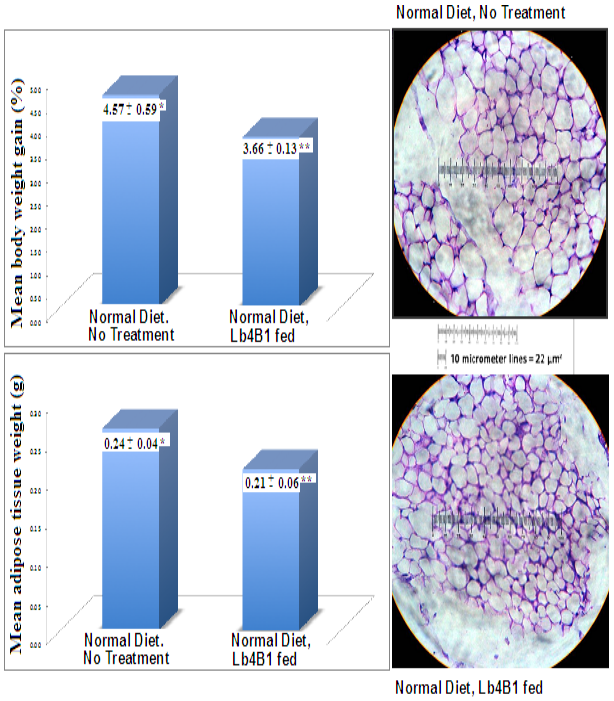

Figure 1. Effect of *Lactobacillus fermentum* 4B1 supplementation on body weights, adipose weights and fat accumulation among mice with normal diet (values having the same superscript (*) are not significantly different at $p < 0.05$), representative adipocyte area shown stained with eosin and hematoxylin (µm², 200x)

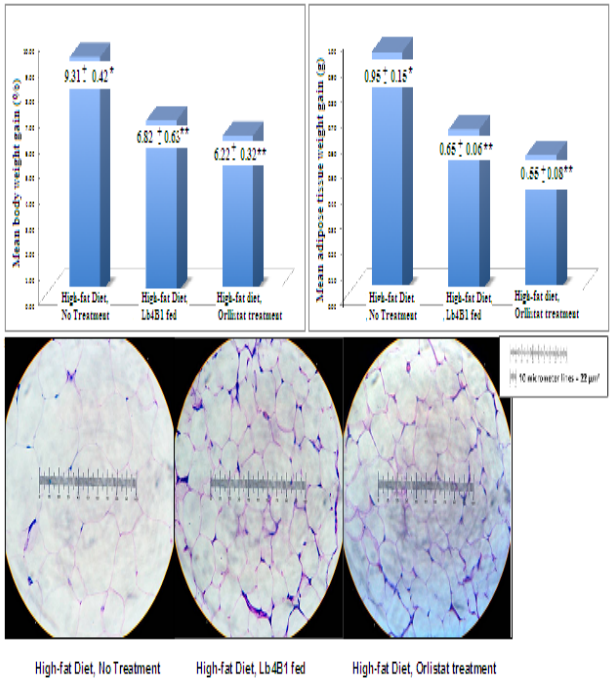

Figure 2. Effect of *Lactobacillus fermentum* 4B1 supplementation on body weights, adipose weights and fat accumulation among mice with high-fat diet (values having the same superscript (*) are not significantly different at $p < 0.05$), representative adipocyte area stained with eosin and hematoxylin (µm², 200x)

4.57 ± 0.59%. This difference observed though was not significant (p = 0.912) suggesting similar effects with or without the probiotic supplementation. But for the obese mice induced by a HFD, the difference in body weight gain was significant between mice receiving *Lb*4B1 (p = 0.000) or Orlistat (p = 0.000) compared with mice not receiving any supplement. The *Lb*4B1 supplementation significantly lowered body weight gain for HFD fed with Lb4B1 with only 6.82 ± 0.63% increase compared to the obese mice not receiving Lb4B1 with 9.31 ± 0.42% increase. Interestingly, the difference in body weight gain was not significant between mice receiving *Lb*4B1 compared with mice receiving Orlistat (p = 0.444) suggesting possible similar effects on body weight. Mice given the Orlistat treatment recorded a gain of 6.22 ± 0.32% in body weight.

Effects of Lb*4B1 feeding on intra-abdominal adipose tissue weight*

The supplementation of *Lb*4B1 in mice belonging to either the HFD or the ND group significantly resulted to lowered intra-abdominal tissue weight (Figures 1 and 2). Mice on a normal diet that received Lb4B1 recorded an intra-abdominal adipose weight of 0.21 ± 0.06 grams while mice that did not receive the supplement recorded 0.26 ± 0.04 grams. This difference observed though was also not significant (p = 0.900) suggesting comparable effects on adipose weight with or without the probiotic supplementation. However, in obese mice induced by a HFD, the difference in intra-abdominal tissue weight was significant between mice receiving *Lb4*B1 (p = 0.000) or Orlistat (p = 0.000) compared with mice not receiving any supplement. The *Lb4*B1 supplementation significantly lowered intra-abdominal adipose tissue weight for HFD fed with *Lb4*B1 with only 0.65 ± 0.06 g increase compared to the obese mice not receiving Lb4B1 with 0.95 ± 0.15 g increase. Interestingly, the difference in intra-abdominal adipose tissue weight was not significant between mice receiving *Lb*4B1 compared with mice receiving Orlistat (p = 0.706) suggesting comparable effects on intra-abdominal adipose tissue weight. Mice given the Orlistat treatment recorded a gain of 0.55 ± 0.08 g in intra-abdominal adipose tissue weight.

Effects of Lb*4B1 feeding on intra-abdominal adipose cell area*

The supplementation of *Lb*4B1 in mice belonging to either the HFD or the ND group significantly resulted to smaller intra-abdominal adipose cross-sectional cell area (or cell size) (Figures 1 and 2). Mice on a normal diet that received *Lb*4B1 recorded an intra-abdominal adipose cross-sectional cell area

(or cell size) of 327.3 μm^2 ± 116.6 μm^2 while mice that did not receive the supplement recorded 786.1 μm2 ± 128.9 μm^2. This difference was significant (p = 0.029) suggesting divergent effects on intra-abdominal adipose cross-sectional cell area (or cell size) with or without the probiotic supplementation. However, in obese mice induced by a HFD, the difference in intra-abdominal adipose cross-sectional cell area (or cell size) was significant between mice receiving *Lb*4B1 (p = 0.000) or Orlistat (p = 0.000) compared with mice not receiving any supplement. The *Lb*4B1 supplementation significantly lowered intra-abdominal adipose cross-sectional cell area (or cell size) for HFD fed with *Lb*4B1 with only 1987.9 μm^2 ± 359.5 μm^2 increase compared to the obese mice not receiving *Lb*4B1 with 6214.7 μm^2 ± 683.0 μm^2 increase. Interestingly, the difference in intra-abdominal adipose cross-sectional cell area (or cell size) was not significant between mice receiving *Lb*4B1 compared with mice receiving Orlistat (p = 0.182) suggesting comparable effects on intra-abdominal adipose cross-sectional cell area (or cell size). Mice given the Orlistat treatment recorded in increase of 1689.6 μm^2 ± 354.7 μm^2 in intra-abdominal adipose cross-sectional cell area (or cell size).

Discussion

The Philippines is home to a distinct array of lactic acid-fermented indigenous fermented specialties varying from region to region. Recently, Banaay and colleagues (2013) described, through a survey, the various untapped locally-isolated strains awaiting discovery of their possible uses in health and functional food development. However, much is still to be established about the said existing probiotic strains. Here we describe the potential of a locally isolated probiotic strains to alleviate the problems associated with diet-induced obesity.

It was previously emphasized that the regulation of body weight and visceral fat through dietary modification, rather than medication, is safer and more cost-effective (Miyoshi *et al.*, 2014). Here we present a probiotic candidate, *Lactobacillus fermentum* 4B1 or *Lb*4B1, with anti-obesity potential isolated from a traditional fermented food in the Philippines known as balao-balao. *Lactobacillus fermentum* has been previously reported to confer health benefits to both animals and humans. The strain M3 (Mikelsaar and Zilmer, 2009) has been shown to have antimicrobial as well as physiologically effective antioxidant properties when consumed and has now been successfully marketed in Finland. *Lactobacillus fermentum* CECT5716 was described recently to enhance the effects of influenza virus vaccination and to improve antibody responses to influenza virus vaccination in humans (Olivares *et al*., 2007). Likewise, *L. fermentum* LC272 from raw milk was tapped for vitamin K production (Lim *et al*., 2011) while *L. fermentum* M1-16 displayed significant reductions in hepatic cholesterol and lipid deposition (Xie *et al*., 2011). *Lactobacillus fermentum* I5007 on the otherhand, when given to young piglets improved their health and growth (Cai *et al*., 2014) while *L. fermentum* Lee has been shown to prevent constipation (Yu *et al*., 2015). Thus, our findings on *Lb*4B1 hope to add up to the list of benefits from this LAB species.

In *Lb*4B1-fed mice receiving the normal diet (ND), body weight gain and intra-abdominal adipose tissue weight did not differ significantly compared to the placebo group. A reported anti-obesity effect (Million *et al.*, 2013) is characterized by a consistent absence of significant weight-gain effect in lean individuals. In addition, anti-obesity property can also be characterized by its influence on intra-abdominal adipose tissue weight and intra-abdominal adipose cell area. This suggests that *Lb*4B1 has the potential to help prevent obesity-associated indications in lean hosts.

In *Lb*4B1-fed mice receiving the high-fat diet (HFD), body weight gain, intra-abdominal adipose and intra-abdominal adipose cross-sectional cell area differ significantly compared to the placebo group. Similar to our findings, probiotic supplementation using various lactobacillus (LAB) strains has been shown recently to help lower body weight in diet-induced obese mice models. *Lactobacillus curvatus* HY7601 and *L. plantarum* KY1032 supplementation to HFD mice resulted to an average body weight with 11% lower compared the placebo group (Park *et al.*, 2013). Other strains of *L. plantarum* such as K21 (Wu *et al.*, 2015) and FH185 (Park *et al*., 2015) have been also implicated to possess anti-obesity activities. High sucrose fed mice, when given *L. gasseri* BNR 17 also helped reduce body weight gain (Kang *et al*., 2013). These findings including ours support the claim that probiotic supplementation is indeed beneficial to health.

In addition, oral administration of our strain *Lb*4B1 resulted to significantly lower intra-abdominal tissue weight and significantly smaller intra-abdominal adipose cross-sectional cell area in HFD mice compared to the placebo group. This observation is parallel to previously reported probiotic strains owing to their anti-obesity potential such as *Lactobacillus gasseri* BNR17 (Kang *et al*.,

2013) and *L. plantarum* FH185 (Park *et al*., 2015). Like *Lb*4B1, the mentioned strains significantly suppressed the increase of fat mass in adipose tissue. Since HFD increased the size of adipocytes, administration of probiotic strains resulted to reduction of its size. Previous reports also mentioned that fat digestion and absorption in the small intestine may be affected by the gut microbiota, imbalances in the gut microflora could be considered to be environmental factors involved in the development of obesity and its associated metabolic disorders (Xie *et al*., 2011; Million *et al*., 2013). Modulation of the intestinal microbiota by supplementation with certain LAB strains may have caused body weight reduction.

In Orlistat-treated mice receiving the high-fat diet (HFD), body weight gain, Intra-abdominal adipose and intra-abdominal adipose cross-sectional cell area also differ significantly compared to the placebo group. Orlistat acts by binding covalently to the serine residue of the active site of gastric and pancreatic lipases (Guerciolini, 2001), thus, when administered with fat-containing foods, orlistat partially inhibits hydrolysis of triglycerides, thus reducing the subsequent absorption of monoacylglycerides and free fatty acids. Interestingly, comparing *Lb*4B1-fed and Orlistat-treated mice receiving the high-fat diet (HFD), body weight gain, intra-abdominal adipose and intra-abdominal adipose cross-sectional cell area did not differ significantly suggesting similar effects. Previous report mentioned that fermentation activities of LAB strains may reduce fat storage through the inhibition of dietary fat absorption (Xie *et al.*, 2011). This mechanism of LAB somehow points to be similar on how orlistat works.

The exact mechanism of action by which *L. fermentum* 4B1 exerts its potential anti-obesity effects is not yet known, and cannot be explained completely by the experimental data obtained. The mechanism of other probiotics however has been described (Kang *et al.*, 2013; Miyoshi *et al*., 2014). For instance, conjugated linoleic acid produced by *Lactobacillus rhamnousus* PL60 and *L. plantarum* PL62 (Lee *et al.*, 2006; Lee *et al*., 2007) were attributed to the anti-obesity effect in diet-induced obese mice. Bifidobacterium breve B-3 and *L. paracasei* ssp. *paracasei* F19 were described to stimulate the intestinal production of angiopoietin-like 4 preventing the accumulation of fat in the adipose tissue by the inhibition of lipoprotein lipase (Aronsson *et al*., 2010; Kondo *et al*., 2010). In addition, it has become apparent that obesity may also be associated with low-grade inflammation of visceral adipose tissue (Bastard *et al.*, 2006). In another note, *L. gasseri* SBT2055 prevented body weight gain and visceral fat accumulation in mice, and this was associated with a significant inhibition in gene expression of pro-inflammatory CCL2, and a downward tendency in CCR2 and TNF-a, in the adipose tissue (Miyoshi *et al*., 2014). These mechanisms somehow hint the immunomodulatory effects of probiotic administration as well.

Recently, proposed mechanisms linking the microbiota to fat content and weight include differential effects of bacteria on the efficiency of energy extraction from the diet, and changes in host metabolism of absorbed calories (Million *et al*., 2013). It will be interesting to discover how *Lb*4B1 reduces weight gain and manages obesity-associated symptoms like adipose cell sizes and weight increase and how it can help regulate other beneficial flora in the gut.

In conclusion, this is the first report describing the potential property of *Lactobacillus fermentum* 4B1, a locally-isolated probiotic strain from balao-balao. This probiotic can help alleviate certain diet-induced metabolic disorders such as obesity. Our findings provide additional support to previous and recently reported health claims of the species *L. fermentum.* And more importantly, the similar effects of *Lb*4B1 compared to Orlistat, stressed the importance of its further development as a practical and safe functional food to preventing obesity.

References

Aronsson, L., Huang, Y., Parini, P, Korach-Andre, M., Hakansson, J., Gustafsson, J.A., Pettersson, S., Arulampalam, V. and Rafter, J. 2010. Decreased fat storage by *Lactobacillus paracasei* is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). PLoS One 5(9): pii:e13087.

Banaay, C.G.B., Balolong, M.P. and Elegado, F.B. 2013. Lactic acid bacteria in traditional Philippine fermented foods. In: Kongo, J.M. (ed). Lactic Acid Bacteria-R and D for Food, Health and Livestock Purposes. p 571-588. Rijeka, Croatia: InTech.

Bastard, J., Maachi, M., Lagathu, C, Kim, M.J., Caron, M., Vidal, H., Capeau, J. and Feve, B. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. European Cytokine Network 17(1): 4–12.

Borchers, A. T., Selmi, C., Meyers, F. J., Keen, C. L. and Gershwin, M. E. 2009. Probiotics and immunity. Journal of Gastroenterology 44: 26–46.

Cai, C.J., Cai, P.P., Hou, C.L., Zeng, X.F. and Qiao, S.Y. 2014. Administration of *Lactobacillus fermentum* I5007 to young piglets improved their health and growth. Journal of Animal and Feed Sciences 23: 222–227.

Cleary, M.P., Grande, J.P. and Maihle, N.J. 2004. Effect of high fat diet on body weight and mammary tumor

latency in MMTV-TGF-alpha mice. International Journal of Obesity and Related Metabolic Disorder 28: 956–962.

Fuller, R. 1989. Probiotics in man and animals. Journal of Applied Bacteriology 66: 365-378.

Guerciolini, R., Radu-Radulescu, L., Boldrin, M., Dallas, J. and Moore, R. 2001. Comparative evaluation of fecal fat excretion induced by Orlistat and chitosan. Obesity Research 9: 364-367.

Kang, J. H., Yun, S. I., Park, M. H., Park, J. H., Jeong, S. Y. and Kang, H. O. 2013. Anti-obesity effect of *Lactobacillus gasseri* BNR17 in high sucrose diet induced obese mice. PLoS One 8(1): e54617.

Kondo, S., Xiao, J., Satoh, T., Odamaki, T., Takahashi, S., Sugahara, H., Yaeshima, T., Iwatsuki, K., Kamei, A. and Abe, K. 2010. Antiobesity effects of *Bifidobacterium breve* strain b-3 supplementation in a mouse model with high-fat diet-induced obesity. Bioscience Biotechnology Biochemistry 74: 1656–1661.

Lee, H., Park, J., Seok, S., Baek, M., Kim, D., Lee, K., Paek, K., Lee, Y. and Park, J.H. 2006. Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochimica et Biophysica Acta 1761: 736-744.

Lee, K., Paek, K., Lee, H.Y., Park, J.H. and Lee, Y. 2007. Anti-obesity effect of trans-10, cis-12-conjugated linoleic acid-producing *Lactobacillus plantarum* PL62 on diet-induced obese mice. Journal of Applied Microbiology 103: 1140-1146.

Lee, S.I., Lee, Y.K., Kim, S.D., Lim, J.H., Suh, J.W. and Lee, I. A. 2013. Anti-obesity effect of soybean curd residue fermented by genus *Aspergillus*. Journal of the Korea Academia-Industrial Cooperation Society. 14(1): 5800-5808.

Lim, S., Kim, K. and Do, J. 2011. Physiological characteristics and production of Vitamin K2 by *Lactobacillus fermentum* LC272 isolated from raw milk. Korean Journal for Food Science of Animal Resources 31(4): 513-5210.

Mikelsaar, M. and Zilmer, M. 2009. *Lactobacillus fermentum* ME-3- an antimicrobial and antioxidative probiotic. Microbial Ecology in Health and Disease 21(1): 1-27.

Million, M., Angelakis, E., Maraninchi, M., Henry, M., Giorgi, R., Valero, R., Vialettes, B. and Raoult, D. 2013. Correlation between body mass index and gut concentrations of *Lactobacillus reuteri, Bifidobacterium animalis, Methanobrevibacter smithii* and *Escherichia coli*. International Journal of Obesity 37: 1460-1466.

Miyoshi, M., Akihiro O., Satoshi H. and Yukio K. 2014. Anti-obesity effect of *Lactobacillus gasseri* SBT2055 accompanied by inhibition of pro-inflammatory gene expression in the visceral adipose tissue in diet-induced obese mice. European Journal of Nutrition 53: 599–606.

Nakahara, K., Bannai, M., Maruyama, K., Suzuki, Y., Okame, R. and Murakami, N. 2013. Characterization of a novel genetically obese mouse model demonstrating early onset hyperphagia and hyperleptinemia. American Journal of Physiology, Endocrinology and Metabolism 305(3): e451-63.

Olivares, M., Diaz-Ropero, M.P., Sierra, S., Lara-Villoslada, F., Fonolla, J., Navas, M., Rodriguez, J.M. and Xaus, J. 2007. Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination. Nutrition 23: 254-260.

Philippine Association for Laboratory Animal Science (PALAS). Code of practice for the care and use of laboratory animals in the Philippines. 2nd Edition. Philippines. 2002.

Park, D.Y., Ahn, Y.T., Park, S.H., Huh, C.S., Yoo, S.R., Yu, R., Sung, M.K., McGregor, R.A. and Choi, M.S. 2013 Supplementation of *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 in diet-induced obese mice is associated with gut microbial changes and reduction in obesity. PLoS ONE 8: e59470.

Park, S.Y., Cho, S.A., Lee, M.K. and Lim, S.D. 2015. Effect of *Lactobacillus plantarum* FH185 on the reduction of adipocyte size and gut microbial changes in mice with diet-induced obesity. Korean Journal for Food Science of Animal Resources 35(2): 171-178.

Ronkainen, J., Huusko, T.J., Soininen, R., Mondini, E., Cinti, F., Mäkelä, K.A., Kovalainen, M., Herzig, K.H., Järvelin, M.R., Sebert, S., Savolainen, M.J. and Salonurmi, T. 2015. Fat mass- and obesity-associated gene Fto affects the dietary response in mouse white adipose tissue. Science Report 18(5): 9233. doi: 10.1038/srep09233.

Wang, C. Y. and Liao, J. K. 2012. A mouse model of diet-induced obesity and insulin resistance. Methods in Molecular Biology 821: 421–433.

West, D.B., Boozer, C.N., Moody, D.L. and Atkinson, R.L. 1992. Obesity induced by a high-fat diet in nine strains of inbred mice. American Journal of Physiology 262: 1025-1032.

West, D.B. and York, B. 1998. Dietary fat, genetic predisposition, and obesity: lessons from animal models. American Journal of Clinical Nutrition 67 (suppl): 505S-512S.

World Health Organization. 2014. The Problem with overweight and obesity. *http://www.who.int.*

Wu, C.C., Weng, W.L., Lai, W.L., Tsai, H.P., Liu, W.H., Lee, M.H. and Tsai, Y.C. 2015. Effect of *Lactobacillus plantarum* strain K21 on high-fat diet-fed obese mice. Evidence-Based Complementary and Alternative Medicine Ecam 2015: 391767.

Yu, Q., Suo, H., Du, M. Zhao, X., Li, J., Li, G., Song, J. and Liu, Z. 2015. Preventive effect of *Lactobacillus fermentum* Lee on activated carbon-induced constipation in mice. Experimental and Therapeutic Medicine 9: 272-278.

Xie, N., Cui, Y.,Yin, Y., Zhao, X., Yang, J., Wang, Z., Fu, N., Tang, Y., Wang, W., Liu, X., Wang, C. and Lu, F. 2011. Effects of two *Lactobacillus* strains on lipid metabolism and intestinal microflora in rats fed a high-cholesterol diet. BMC Complementary and Alternative Medicine 11: 53-58.