World Journal of Dairy & Food Sciences 9 (2): 86-94, 2014
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2014.9.2.1134

Effect of *Moringa oleifera* Leaves Extract as a Growth Factor on Viability of Some Encapsulated Probiotic Bacteria

[1]Abeer E. A. Amer, [2]Baraka A. Abd El-Salam, [3]Aida S. Salem

[1]Dairy Microbiology Department, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt
[2]Dairy Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt
[3]Dairy Technology Departments, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt

Abstract: *Moringa oleifera* is one of the most useful tropical trees. Its leaves contain all of the essential amino acids, vitamins B and minerals. *Moringa oleifera* leaves extract (MOLE) was added with different concentrations (4, 8 and 12% v/v) to growth medium to study the growth of probiotic bacteria before microencapsulation. The highest probiotic growth was recorded with 8% (v/v) MOLE. The effect of microencapsulation of studied probiotic bacteria with 8% (v/v) *Moringa oleifera* leaves extract (MOLE) on the changes in survival rate during exposure to artificial gastrointestinal conditions was studied. Non-encapsulated cells were significantly destroyed when exposed to low pH (2) or high taurocholic acid (3%). Encapsulated cells with 8% (v/v) MOLE exhibited higher resistance to artificial gastrointestinal conditions than encapsulated cells without MOLE. *Lb. jonsonii* and *B. adolescentis* encapsulated 8% MOLE showed the highest acid-bile tolerant and their morphology were investigated by transmission electron microscopy(TEM).The microencapsulation with 8% (v/v) MOLE effectively protected the microorganisms from artificial gastrointestinal conditions.

Key words: Artificial gastrointestinal conditions · Microencapsulation · *Moringa oleifera* · Probiotic bacteria · TEM

INTRODUCTION

Moringa oleifera is referred to as a “miracle tree” or a “wonder tree” [1] of significant socio economic importance because of its several nutritional, pharmacological [2, 3] and industrial applications [4, 5].The leaves of this plant contain high amount of vitamin B complex, calcium, potassium, iron and protein. Also, they contain all of the essential amino acids in a good proportion [6]. Modern consumers expect their food to be healthy and to prevent illness as they are increasingly interested in their personnel health [7]. This explains the reason for a rising interest in a probiotic health based products. Probiotic live bacteria are recognized as good or friendly bacteria and thought to be reducing potentially harmful bacteria from the intestine. Therefore, these live bacterial micro-organisms can improve microbial balances in intestine and exert positive health effects on the host. Probiotic is a term that means “for life” and defined as “Live microorganism that beneficially affects the host health by improving its microbial balance” [8]. More recently probiotics have been defined as “Live microorganisms that when administrated in adequate amounts, confer a health on the host [9]. Several factors have been reported to affect the viability of probiotics in fermented dairy products, including pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of associative culture organisms, concentration of lactic and acetic acids and even whey protein concentration [10, 11, 12] and in the gastrointestinal tract of the host.

Viability loss of probiotics in food products and acidic bile condition of gastrointestinal tract has always encouraged researches to find new efficient methods of

Corresponding Author: Abeer E.A. Amer, Dairy Microbiology Department, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.

viability improvement. Various approaches have been attempted so as to increase the resistance of probiotic bacteria against adverse conditions like use of micronutrients and encapsulation. Providing probiotic living cells with a physical barrier against adverse conditions is an approach currently receiving major interest [7]. The technology designed to improve delivery of probiotic bacteria into the human gastrointestinal tract by providing protection to the sensitive bacterial microorganisms may be referred as microencapsulation. It is currently receiving a considerable interest for improving the viability of probiotic bacteria [13]. Microencapsulation is entrapping a substance by a material such that it is released at controlled and required rate under suitable conditions. Microencapsulation is a process by which live cells are packaged within a shell material, which confer them protection by preventing their direct exposure to unfavorable environment, but permits diffusion of nutrients in and out of the matrix, thereby supporting the viability of the cells [14].

The aims of this study were to evaluate the influence of *Moringa oleifera* leaves extract as a growth factor on probiotc bacterial growth before encapsulation and determine of resulted encapsulated probiotc bacteria resistance against artificial gastrointestinal conditions.

MATERIALS AND METHODS

Probiotic Bacterial Strains and Growth Conditions: *Bifidobacterium adolescentis* ATCC15704 and *Lactobacillus casei* DSMZ 20011 were obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. *Bifidobacterium lactis* Bb.12 was obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark. Finally, *Lactobacillus jonsonii* ATCC 33200 was purchased from American Type Culture Collection, Manassas, VA. These four bacterial strains were subjected for probiotic criteria investigations in previous study [15, 16] and selected for their high ability to withstand environmental conditions similar to those of the human digestion tract. The probiotic organisms were maintained individually and grown in MRS broth at 37°C using a 1% inoculum. MRS broth containing *Bifidobacterium* spp. was supplemented with filter sterilized 0.05% (w/v) L-cysteine-hydrochloride and 0.075% agar to create a more favorable anaerobic environment. Before use, the probiotic organisms were activated by growing 3 times successively in MRS broth at 37 °C for 24h.

Preparation of *Moringa oleifera* Leaves Extract: Fresh leaves of *Moringa oleifera* were obtained from Horticultural Research Institute, Agricultural Research Center, Giza, Egypt. The leaves extract of *Moringa oleifera* was obtained by squeezing the leaves using blender and then filtered through cheesecloth. The supernatant was heated on the water bath for 1 minute.

Chemical Analysis of *Moringa oleifera* Leaves Extract (MOLE): The chemical analysis of *Moringa oleifera* leaves extract was performed. The vitamins of Riboflavin (B2), Thiamin (B1) and Nicotinic acid (B3) were determined according to Batifoulier *et al*. [17]. The amino acids (Arginine, Histidine, Lysine, Tryptophan, Theonine, Metheonine, Lucine, Isolucine, Valine and Phenylanaline) were analyzed using an amino acid analyzer [18]. Also, the minerals (Calcium, Magnesium, Phosphrous, Potassium, Copper, Iron and Sulphur) were determined according to Vzaviniene and Tautkus [19].

The Effect of *Moringa oleifera* Leaves Extract on Probiotic Bacterial Strains Growth: The MRS broth for lactobacilli and MRS broth supplemented with filter sterilized 0.05% (w/v) L-Cysteine hydrochloride and 0.075% agar for bifiddobacteria were fortified with 0, 4, 8 and 12% of (v/v) of *Moringa oleifera* leaves extract to know the best concentration for high probiotic bacterial strains growth.

Encapsulation of Probiotic Bacteria: The encapsulation of probiotic bacteria with or without *Moringa oleifera* leaves extract was performed using the methods of Shah and Ravula [20] and Sultana *et al*. [21]. The cultures were grown separately in MRS broth (lactobacilli) or MRSCA (MRS +0.05 % L-cysteine-hydrochloride and 0.075% agar) broth (bifidobacteria) supplemented with 0 or 8% (v/v) of *Moringa oleifera* leaves extract at 37°C for 24h. The cells were harvested by centrifugation at 2000g for 10 min at 4°C. The cells were washed twice and then resuspended in normal saline. The final cell concentration was adjusted to 10^{11} CFU/ml. 100 ml of 3% (w/v) sodium alginate was prepared in a volumetric flask and sterilized by autoclaving. The sterile encapsulating material was mixed with 25 ml of washed and concentrated (10^{11} CFU/ml) probiotic organisms. The mixture was dropped into oil containing Tween 80 (0.02%). After the dropping was completed, the mixture was stirred vigorously till it was emulsified and appeared creamy. A solution of 0.1 M calcium chloride was then added quickly along the side of

the beaker, the phase separation of oil /water emulsion occurred. The mixture was allowed to stand for 30 min for the calcium-alginate beads to separate and settle at the bottom of the calcium chloride layer. The oil layer was drained and the capsules were removed from the aqueous phase and refrigerated at 4°C for 10 h and then lyophilized. The encapsulated probiotic bacterial count before and after lyophilization was determined.

Acid Resistance: Acid resistance of encapsulated probiotic bacteria with or without *Moringa oleifera* leaves extract and control consisting of free probiotic bacteria was carried out according to the method of Liong and Shah [22]. Briefly, MRS broth was adjusted to pH 2 with 5M HCl. Approximately 10^{10} CFU/ml of each probiotic bacteria was inoculated into the acidified MRS broth and incubated at 37°C for up to 2 h and samples were taken at 30 min intervals for enumeration. Serial dilutions were performed and vortexed for 30 s individually before inoculation to MRS or MRSCA agar plates. Plates were incubated at 37°C for 72h in an anaerobic jar with an anaerobic gas generating kit (Oxoid Ltd.). For the enumeration of microencapsulated probiotic organisms, the bacteria were released from the capsules by sequestering calcium ions with phosphate buffer (0.1 M, pH 7.0) followed by gentle shaking at room temperature for 15 min. Acid tolerance was determined by comparing the final plate count after 2h with the initial plate count at 0 h. All acid resistance tests were repeated 3 times.

Bile Resistance: Bile resistance of encapsulated probiotic bacteria with (8%) or without *Moringa oleifera* leaves extract and free probiotic bacteria was carried out according to the method of Liong and Shah [22]. Taurocholic acid (Sigma) was used to study the bile resistance of probiotic bacteria because it is a conjugated bile salt commonly found in the intestinal tract [23]. Briefly, MRS or MRSCA broth containing 3% (w/v) taurocholic acid was adjusted to pH 5.8 using 5M HCl. Approximately 10^{10} CFU/ml of each probiotic bacteria was inoculated into the MRS or MRSCA broth with bile salts and incubated at 37°C for up to 8h. Although the concentration of bile acid and pH varies largely between individuals, the level used in this study is within the physiological concentrations found in the human duodenum [23]. Bacterial growth was observed by monitoring viable cell counts at 0, 4 and 8h on MRS or MRSCA agar. The bile tolerance of each strain was determined by comparing the count after 4 and 8 h of exposure to bile salts with the initial count at 0 h. All experiments were repeated 3 times.

Microscopic Observation: The morphology of encapsulated *Lb. jonsonii* and *B. adolescentis* with 8% *Moringa oleifera* leaves extract was observed by transmission electron microscopy (TEM) (JEOL, Jem-2100 electron microscope-Japan [24].

Statistical Analysis: The mean values and standard deviations were determined for all obtained data. Differences between samples were determined by T-test and were considered to be significant when $P \leq 0.05$ [25].

RESULTS AND DISCUSSION

Chemical analysis of *Moringa oleifera* Leaves Extract (MOLE): Table 1 presents the chemical analysis of *Moringa oleifera* leaves extract (MOLE). It is obvious that 100 ml of MOLE contained 406.6, 149.8, 342.4, 107, 310.3, 117.7, 117.7, 492.2, 229.6 and 374.5mg arginine, histidine, lysine, tryptophan, phenylanaline, methionine, threonine, leucine, isoleucine and valine, respectively. These results are in generally harmony with those reported by Kumalaningsih *et al*. [26], who stated that all of the essential amino acids can be found in Moringa leaves extract. The levels of B1, B2 and B3 in MOLE were found to be 0.09, 0.05 and 0.8 mg per 100ml, respectively. Gopalan *et al.* [27] found the same values of these vitamins. Similar values of minerals content showed in Table 1 were obtained by Maroyi [28].

Table 1: Chemical analysis of *Moringa oleifera* leaves extract (MOLE).

Component	Per 100 ml
Amino acids content(mg)	
Arginine	406.6
Histidine	149.8
Lysine	342.4
Tryptophan	107
Phenylanaline	310.3
Methionine	117.7
Threonine	117.7
Leucine	492.2
Isoleucine	229.6
Valine	374.5
Vitamins content(mg)	
B1	0.09
B2	0.05
B3	0.8
Minerals content(mg)	
Calcium	440.0
Magnesium	24.0
Phosphorous	70.0
Potassium	259.0
Copper	1.1
Iron	0.7
Zn	0.16

Effect of *Moringa oleifera* Leaves Extract (MOLE) on Probiotic Bacterial Strains Growth: The effect of *Moringa oleifera* leaves extract (MOLE) at different concentrations on growth of studied probiotic bacteria is presented in Fig. 1. The growth of all studied probiotic bacteria was affected by (MOLE). The obtained data revealed that increasing the concentration of (MOLE) from 0 to 8% led to increase the probiotic bacterial growth at 37°C for 24h of incubation time. Also, it could be noticed that all studied probiotic bacteria did not grow well at 12% (MOLE) and when the incubation time was lengthened up to 48h compared with the growth at 8% (MOLE) and 24h of incubation time. Therefore, the optimum growth was recorded for all probiotic bacteria at 8% (MOLE) at 37°C for 24h of incubation time. *Lb. jonsonii* and *B. adolescentis* exhibited a higher growth than those of *Lb. casei* and *B. lactis*, respectively. These outcomes are similar to that obtained by Van Tienen *et al*. [29], who found that the addition of *M. oleifera* enhanced the survival of probiotic bacteria in yogurt. The growth improvement of bacteria by *Moringa oleifera* has been explained by Kumalaningsih *et al*. [26], who reported that the presence of essential amino acids in the *Moringa oleifera* leaves improved the growth of the organisms.

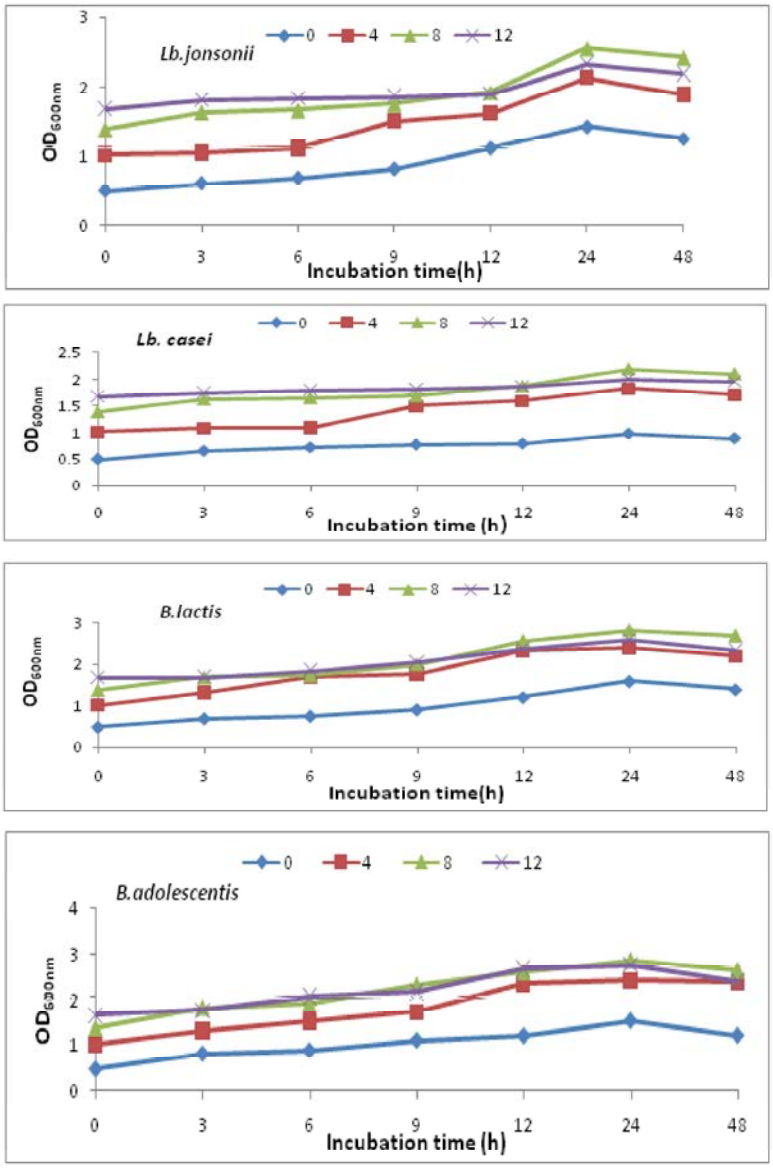

Fig 1: The effect of *Moringa oleifera* leaves extract on probiotic bacterial growth

Count of Free and Encapsulated Probiotic Bacteria Before and After Lyophilization: Fig. 2 shows count of free and encapsulated probiotic bacteria without or with 8% (MOLE) before and after lyophilization. It could be seen from the results that, there was no significant differences between the count of encapsulated probiotic bacteria with 8% (MOLE) before and after lyophilization. Also, the reduction level after lyophilization for count of encapsulated probiotic bacteria without (MOLE) was lower, 0.36 to 1.5 Log, than those of free cells, 1.7 to 2.8 Log. Ding and Shah [30] stated that the Protection of probiotics by microencapsulation in alginate beads is one of the methods of improving their viability. The Freeze drying has been traditionally used for preserving starter culture and probiotic bacteria [31, 32]. This is because the low temperatures used during freeze drying are expected to be less injurious to labile biological organisms than drying at ambient or higher temperatures [33].

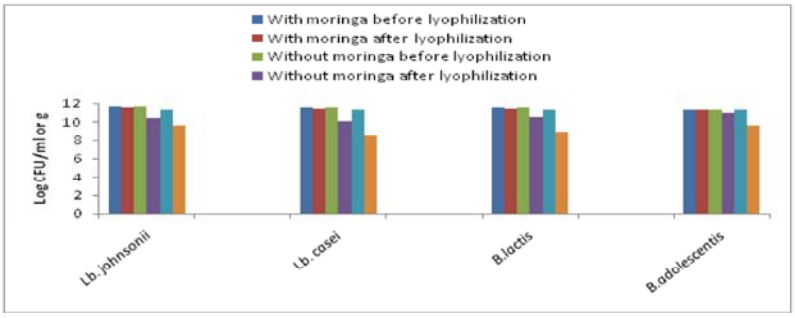

Fig 2: Count of free and encapsulated Probiotic bacterial before and after lyophilization

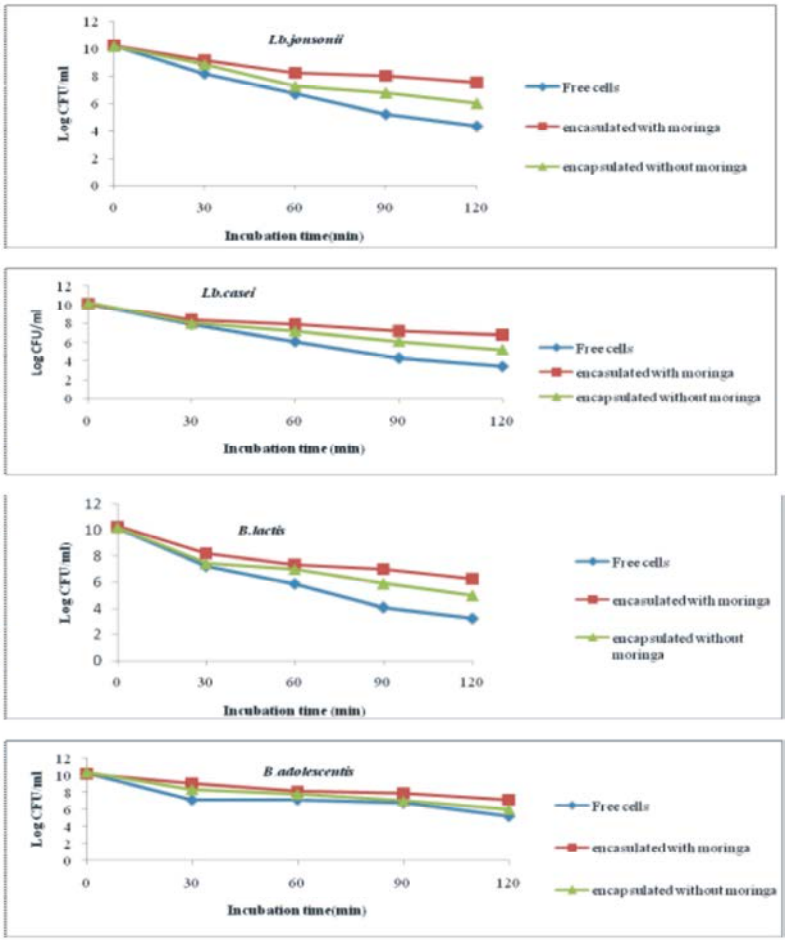

Fig 3: Effect of low pH (2.0) on viability of free and encapsulated probiotic bacteria.

Acid Resistance: The survival of free and encapsulated probiotic bacteria without or with 8% (MOLE) under *in vitro* acid conditions is given in Fig. 3. Free cells were drastically reduced from an average of 10.18 Log CFU/ml at zero time to an average of 4.06 Log CFU/ml at the end of acidic condition (pH 2) exposure period (60 min.) at 37ºC. After 60 min. of incubation, viability of free *Lb. jonsonii* (4.32 Log CFU/ml) was higher than *Lb. casei* (3.44 Log CFU/ml). Also, free *B. adolescentis* was more acid- tolerant than *B. lactis,* with 5.2 and 3.28 Log CFU/ml surviving after 60 min. of incubation at pH 2, respectively. Higher survival of encapsulated probiotic bacteria without or with 8% (MOLE) at acidic pH compared to free probiotic cells. Also, it could be noticed that an encapsulated probiotic bacteria with 8% (MOLE) had a more resistance than encapsulated probiotic bacteria without (MOLE) after 60 min. of acidic condition exposure. At 60 min. of exposure, all an encapsulated probiotic bacteria without (MOLE) survived at average of 5.55 Log CFU/ml. It is estimated that 10^7 CFU/ml of live probiotic cells are needed to confer health benefits to the consumer [34]. Thus, encapsulated probiotic bacteria without (MOLE) after 60min. of acidic condition exposure would not confer health benefits. *Lb. jonsonii* and *B. adolescentis* encapsulated with 8% (MOLE) were the most resistance to acidic condition, with 7.5 and 7.11 Log CFU/ml surviving after 60 min incubation at pH 2, respectively. These results are in agreement with those obtained by Lee *et al*. [35] and Le-Tien *et al*. [36], who reported that an encapsulation of lactobacilli in alginate improved survival in simulated gastric fluid, while the free cells were destroyed completely [37]. Also, higher survival of immobilized bifidobacteria at acidic pH of GIT was observed [38, 39]. However, our results are in contrast with those obtained by Sultana *et al.* [21], Hansen *et al.* [40] and Trindade and Grosso [41], who reported that microencapsulation in alginate bead did not effectively protect the microorganisms from low pH.

Bile Resistance: The effect of the taurocholic acid (3%) on the viability of free and encapsulated probiotic bacteria without or with 8% (MOLE) is presented in Fig. 4. All free

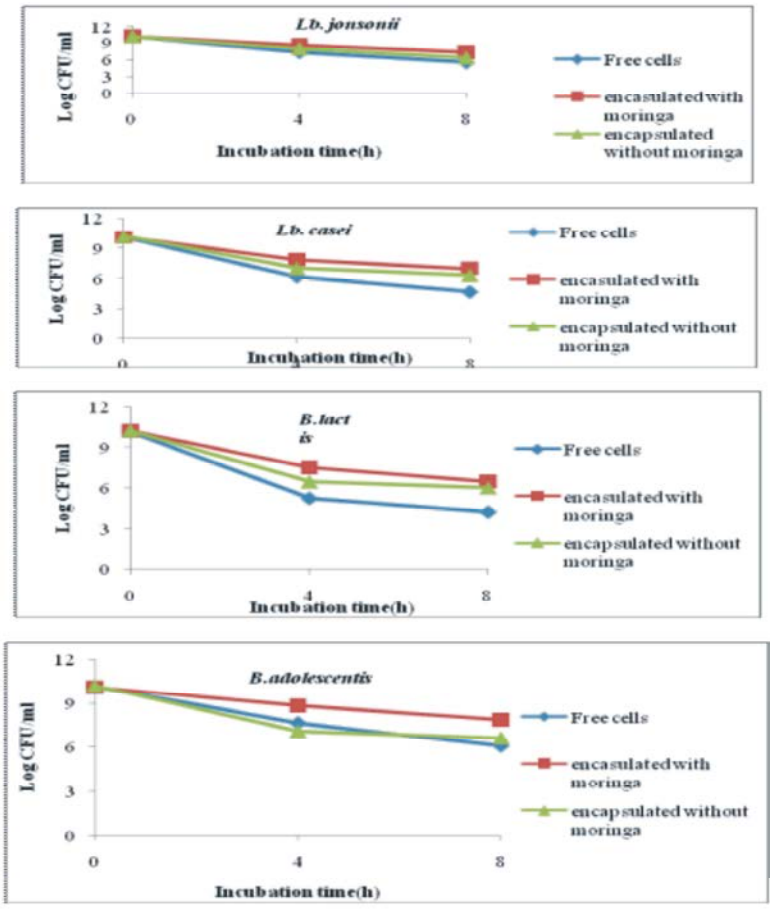

Fig 4: Effect of taurocholic acid (3%) on viability of free and encapsulated probiotic bacteria.

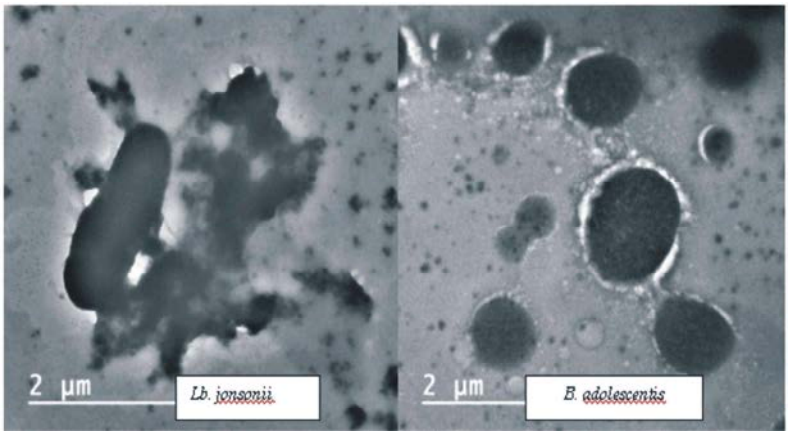

Fig 5: Morphology of encapsulated probiotic bacteria with 8% MOLE.

probiotic bacteria showed a significant loss of viability when exposed to 3% (w/v) taurocholic acid. The initial average viable count was 10.16 Log CFU/ml but this level of viability was reduced to an average 6.6 log CFU/ml after 4h of exposure and the average viable number was further reduced to 5.1 Log CFU/ml after 8h. The strains most susceptible to taurocholic acid were *Lb. casei* and *B. lactis*. The viability of encapsulated probiotic bacteria without or with 8% (MOLE) was higher after 8h of 3% taurocholic acid exposure compared with that of free probiotic bacteria. Also, encapsulated probiotic bacteria with 8% (MOLE) exhibited a good bile tolerance compared with encapsulated probiotic bacteria without (MOLE). The highest survival of cells after 8h of 3% taurocholic acid exposure was obtained by *Lb. jonsonii* and *B. adolescentis* encapsulated with 8% (MOLE). These results concur with other studies that used similar concentration of bile salts of 3% and shown that encapsulated probiotic bacteria can survive better than free probiotic cells [42, 43]. In contrast, Trindade and Grosso [41] also reported that immobilization of *B. bifidum* and *L. acidophilus* in Ca-alginate beads was not effective in protecting the cells from 2% and 4% bile salt.

Microscopic Observation: The morphology of the best acid-bile tolerant encapsulated probiotic bacteria with 8% MOLE, *Lb. jonsonii* and *B. adolescentis*, was investigated using TEM and shown in Fig 5. Microcapsules of *B. adolescentis* were approximately spherical but the rod shape was observed for *Lb. jonsonii* capsule. Bacteria are in direct contact with the alginate and may be attached. Similar observations were obtained by Chan and Zhang [24] and Allan-Wojtas *et al*. [44].

CONCLUSION

It can be concluded that the addition of *M. oleifera* leaves extract increased the growth of probiotic bacteria. Microencapsulation of probiotic bacteria with 8% (v/v) MOLE exhibited higher resistance to artificial gastrointestinal conditions than encapsulated cells without MOLE.

REFERENCES

1. Kasolo, J.N., G.S. Bimenya, L. Ojok, J. Ochieng and J.W. Ogwal-Okeng, 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J. Med. Plants Res., 4: 753-757.
2. Caceres, A., O. Cabrera, O. Morales, P. Mollinedo and P. Mendia, 1991. Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. J. Ethnopharmacol., 33: 213-216.
3. Caceres, A., A. Saravia, L. Zabala and E. Leon, 1992. Pharmacological properties of *Moringa oleifera.* 2: screening for antispasmodic, anti-inflammatory and diuretic activity. J. Ethnopharmacol, 36: 233-237.
4. Makkar, H. and K. Becker, 1997. Nutrients and anti-quality factors in different morphological parts of the *Moringa oleifera* tree. J. Agric. Sci. Cambridge, 128: 311-322.
5. Foidl, N., H. Makkar and K. Becker, 2001. In The Miracle Tree: The Multiple Uses of Moringa(Ed, J, F.) Wageningen, Netherlands. pp: 45-76.
6. Mishra, S.P., P. Singh and S. Singh, 2012. Processing of *Moringa oleifera* Leaves for Human Consumption. Bulletin Environ. Pharm. & Life Sci., 2:28-31.

7. Kailasapathy, K., 2009. Encapsulation technologies for functional foods and nutraceutical product development. CAB Reviews Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 4: 6-7.
8. Fuller, R., 1989. Probiotics in man and animals. J. Appl. Microbiol., 66: 365-378.
9. FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization, 2002. Guidelines for the evaluation of Probiotics in Food. London, Ontario, Canada
10. Kailasapathy, K., 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. Lwt-Food Sci. & Technol., 39: 1221-1227.
11. Anal, A.K. and H. Singh, 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci. & Technol., 18: 240-251.
12. Sabikhi, L., R. Babu, D. Thompkinson and S. Kapila, 2010. Resistance of Microencapsulated Lactobacillus acidophilus LA1 to Processing Treatments and Simulated Gut Conditions. Food and Bioprocess Technol., 3: 586-593.
13. Vivek, K.B., 2013. USE of encapsulated probiotics in dairy based foods. Int. J. Food, Agric. and Veter. Sci., 3(188): 199-206.
14. Talwalkar, A., C.W. Miller, K. Kailasapathy and M.H. Nguyen, 2004.Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yoghurt. Int. J. Food Sci. & Technol., 39: 605-611.
15. Abd El-Salam, B.A., Nagwa I. Sultan, E.O. Fayed and M.A. Zedan, 2004. *In vitro* study on probiotic criteria of some lactobacilli. Egyptian J. Dairy Sci., 32: 17-29.
16. Fayed, E.O., Nagwa I. Sultan, M.A. Zedan and Baraka A. Abd El-Salam, 2008. Selection of bifidobacterial strains for use as probiotic adjuncts. Egyptian J. Dairy Sci., 36: 163-173.
17. Batifoulier, F., M.A. Verncy, C. Besson, C. Demigne and C. Remesy, 2005. Determination of thiamine and its phosphate esters in rat tissues analyzed as thiochromes on a RP-amide C16 column. J. Chromatography B, 816: 67-72.
18. Moyo, B., P.J. Masika, A. Hugo and V. Muchenje, 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African J. Biotechnol., 10: 12925-12933.
19. Vzaviniene, D. and S. Tautkus, 2007. Determination of calcium in mineral waters by flame atomic absorption spectrometry. Chemija, 18: 34-37.
20. Shah, N.P. and R.I. Ravula, 2000. Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. Aust. J. Dairy Technol., 55: 41-5.
21. Sultana, K., G. Godward, N. Reynolds, R. Arumugaswamy, P. Peiris and K. Kailasapathy, 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int. J. Food Microbiol., 62: 47-55.
22. Liong, M.T. and N.P. Shah, 2004. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J. Dairy Sci., 88: 55-60.
23. Floch, M.H., 2002. Bile salts, intestinal microflora and enterohepatic circulation. Digest. Liver Dis., 34: 54-7.
24. Chan, E.S. and Z. Zhang, 2002. Encapsulation of probiotic bacteria *Lactobacillus acidophilus* by direct compression. Trans I Chem. E, 80: 79:82.
25. Snedecor, G.W. and G.W. Cochran, 1989. Statistical Methods. 8thEd. Iowa State Univ. Press, Ames, Iowa, USA.
26. Kumalaningsih, S., M. Padaga, D. Suprayogi and R.P. Vitta, 2011. Encapsulation of *Lactobacillus* sp. with *Moringa oleifera* leaves extract for food supplement. Int. Res. J. Agric. Sci. Soil Sci., 1: 273-277
27. Gopalan, C., B.V. Rama and S.C. Balasubramanian, 1971. Revised and updated by B.S. Narasinga, Y.G. Deosthale and K.C. Pant, 1989. Nutritive value of Indian foods. National Institute of Nutrition, Hyderabad, India.
28. Maroyi, A., 2006. The utilization of *Moringa Oleifera* in Zimbabwe: A Sustainable livelihood approach J. Sustain. Develop.in Africa, 8: 172-185.
29. Van Tienen, A., Y.M. Hullegie, R. Hummelen, J. Hemsworth, J. Changalucha and G. Reid, 2011. Development of a locally sustainable functional food for people living with HIV in Sub-Saharan Africa: laboratory testing and sensory Evaluation, 2: 193-198.
30. Ding, W.K. and N.P. Shah, 2009. Effect of various encapsulating materials on the stability of probiotic bacteria. J. Food Sci., 74: M53-M61.

31. Gardiner, G.E., E. O'Sullivan, J. Kelly, M.A.E. Auty, G.F. Fitzgerald, J.K. Collins, R.P. Rossa and C. Stanton, 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl. Environ. Microbiol, 66: 2605-12.
32. Kurtmann, L., C.U. Carlsen, J. Risbo and L.H. Skibsted, 2009. Storage stability of freeze-dried *Lactobacillus acidophilus* (La-5) in relation to water activity and presence of oxygen and ascorbate. Cryobiology, 58: 175-80.
33. Frank, F., 1998. Freeze-drying of bioproducts: putting principle into practice. Eur. J. Pharm. Biopharm., 45: 221-9.
34. Ouwehand, A.C. and S.J. Salminen, 1998. The health effects of cultured milk products with viable and non-viable bacteria. Int. Dairy J., 8: 749-58.
35. Lee, J.S., D.S. Cha and H.J. Park, 2004. Survival of freeze-dried *Lactobacillus bulgaricus* KFRI 3673 in chitosan-coated calcium alginate microparticles. J. Agric. & Food Chem., 52: 7300-7305
36. Le-Tien, C., M. Millette, M.A. Mateescu and M. Lacroix, 2004. Modified alginate and chitosan for lactic acid bacteria immobilization. Biotechnol. Appl. Biochem, 39: 347-354.
37. Goderska, K., M. Zybals and Z. Czarnecki, 2003. Characterization of microencapsulated *Lactobacillus rhamnosus* LR7 strain. Polish J. Food & Nutrition Sci., 53: 21-24.
38. Guerin, D., J.C. Vuillemard and M. Subirade, 2003. Protection of bifidobacteria encapsulated in polysaccharide-protein gel against gastric juice and bile. J. Food Protect., 66: 2067-2084.
39. Picot, A. and C. Lacroix, 2004. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. Int. Dairy J., 14: 505-515.
40. Hansen, L.T., P.M. Allan-Wojtas, Y.L. Jin and A.T. Paulson, 2002. Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. Food Microbiol., 19:35-45.
41. Trindade, C.S.F. and C.R.F. Grosso, 2000. The effect of the immobilization of *Lactobacillus acidophilus* and *Bifidobacterium lactis* in alginate on their tolerance to gastrointestinal secretions. Milchwissenschaft, 55: 496-499.
42. Chandramouli, V., K. Kailasapathy, P. Peiris and M. Jones, 2004. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. J. Microbiol. Methods, 56: 27-35.
43. Kailasapathy, K., 2005. Survival of free and encapsulated probiotic bacteria and effect on the sensory properties of yoghurt. Food Sci. Technol., 1: 1-2.
44. Allan-Wojtas, P., L. Truelstrup Hansen and A.T. Paulson, 2008. Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. LWT, 41: 101-108.