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Effect of Protein Hydrolysates Added to Date Juice on the Growth of Lactic Acid Bacteria

[1]Zineb. Derradji, [1]Baida. Djeghri- Hocine, [2]Abdeltif Amrane, [1]Hadda.Melouka, [3]Reda Djabbar

1 Laboratoire de Biochimie et de Microbiologie Appliquée, Département de Biochimie, Faculté des Sciences, Université Badji- Mokhtar BP12, 23000 Annaba, Algérie.
2 Ecole Nationale de Chimie de Rennes, CNRS, UMR 6226, Rue du General Leclerc, CS 50837, 35708 Rennes Cedex 7, France, Université Européenne de Bretagne, France
3Université des Sciences et de la Technologie Houari Boumediene, BP32 EL ALIA 16111 BAB EZZOUAR ALGER.

Address For Correspondence:
Zineb. Derradji, Laboratoire de Biochimie et de Microbiologie Appliquée, Département de Biochimie, Faculté des Sciences, Université Badji- Mokhtar BP12, 23000 Annaba, Algérie.

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ABSTRACT

The culture of LAB requires culture media that are rich in sugar, nitrogenous substances and growth factors, which are often based on yeast extract, peptones or meat extracts, casein hydrolysates and bacterial autolysates, substrates expensive and are not produced locally. Previous studies have shown that some lactic strains can grow on protein crops based media, which can replace peptones, as well as MRS and M17 meat extracts. The aim of this study was to evaluate the potential use of protein crops, namely ; juice of lentil, chickpeas and beans which are produced in Algeria, associated with date juice in the formulation of optimum medium for lactic acid bacteria growth. The growth of four lactic strains cultivated on such formulated media was determined by the ecometric method on solid medium and by measuring the optical density of the biomass produced in liquid medium. Bacteria growth was compared to that recorded on a control medium, MRS (standard laboratory medium). In solid media, the results show that the prepared media cover the complex nutritional needs of the majority of the strains tested. The growth is significant because it registered on 4-5 segments. The viable count in the optimized culture media based on juice of lentils, chickpeas, and / or beans added to date juice were significantly appreciable with 60% compared than those registered on control medium and with a low cost in spite of the complex nutritional requirements of lactic acid bacteria. This preliminary work demonstrates the interesting potential of the protein crops associated with date juice in the nutrition of the lactic flora. thus protein hydrolysates added to date juice are good alternative sources of nitrogen that lead to a better growth of the strain and can be used for large-scale production.

KEYWORDS: nutritional requirements - lactic acid bacteria - date juice – peptones - protein crops

INTRODUCTION

Lactic acid bacteria (LAB) and humans share a long and intricate history. Well known are the first food fermentations reported in ancient times that contributed to the preservation and quality improvement of raw plant, meat and milk substrates. Douillard and de Vos [13]. Furthermore many probiotics have been claimed and efficiently proven to cure or reduce various health problems including lactose intolerance, cholesterol reduction immunomodulation in gastric illnesses and diarrhoea, food allergy, antimutagenic and antimicrobial activities. Patel *et al*. [12]. The growth of LAB requires a complex supply, both qualitatively and quantitatively, in nutrient intake because their nutritional requirements are large and vary from one strain to another, Ashraf and Shah. It is not still easy to find an economic culture medium containing the elements necessary for survival and proliferation of these strains.

Generally culture media specific for LAB are based on animal resource, such as peptones, yeast extract, meat extract or milk [8,7]. But studies are relatively scarce concerning the growth of lactic acid bacteria on vegetal substrates, [9,3,21]. Among vegetal substrates used in this work Lentil which is the most ancient cultivated crops among the legumes, it is commonly used for human nutrition, animal feed and soil fertility Alihan and Munqez. We have also used beans and cheackpeas which are produced in algeria for there protein and sugar content. In the current time, food industries focuses on the strategy to select and employ folate producing probiotic strains, to produce fermented products with elevated amount of natural folate without increasing production cost and inherent tendency to provide desire health benefits [11]. For this purpose we have chosen these pulses which have a high folic acid content.

Our study fits to evaluate the potential use of a local and cheap vegetal juice, to replace, at least partially, the usual complex and expensive nitrogen supplements for the growth of LAB strains.

Knowledge:

Several studies have focused on the formulation of specific culture media for LAB. Djeghri-Hocine *et al*. [5,6] showed that it is possible to grow some lactic strains on protein crops based media ; other studies have also indicated that is possible to grow these strains on date juice based media, namely at high sugars content (60 to 70% of total weight), such as glucose, fructose and sucrose Boudjelal *et al*. [2] Their work confirmed that protein crops and sugars contained in date juice could replace peptones and meat extract of MRS and M17, expensive substrates which are not produced locally. But no studies have concern combination of both.

MATERIALS AND METHODS

Microorganisms and culture conditions:

The microorganisms used in this study were the following (Table 1)

Table 1: Strains used

Strain	Germs	Product / Identified by
Lb1 (CHTD27)	*Lactobacillus brevis*	Laboratory of Microorganisms biology and Biotechnology, Essenia Oran,(Algéria)
Lb2 (BH14)	*Lactobacillus plantarum*	
Lb3 (LH100)	*Lactobacillus delbrueckii subsp. Lactis*	Rhodia® (France).
Lb4	*Lactobacillus rhamnosus*	Isolated from the feces of healthy and breastfed infants

All strains used in this study were maintained on whole milk at -18 ° C. They were revived on MRS broth by an incubation of 18 h at 37 ° C.

2.2 Extraction of date juice sugar:

The method used was adapted from Nancib *et al*. [9]. The dates were cleaned manually then the seeds and discards were removed. Tap water was added at a ratio of two parts of water to one part of dates (by weight). The preparation was heated at 80 ° C for 2 h with continuous stirring. The mixture was centrifuged at 5000 rpm for 30min to separate the cellulosic debris, while the supernatant was used essentially as the carbon source in the fermentation medium [9].

2.3 Growth media:

We have used 3 protein crops, which are Algerian varieties produced by < The Institute of field crops > in Guelma, Algeria

- Chickpeas (*Cicer arietinum*), variety Flip 90/13.
- Lentils (*Lentil culinaris*) NEL/45.
- Beans (*Vicia fava*), variety of Sidi Aich.

Preparation of culture media:

Chickpeas and lentils were cleaned manually and, the beans were manually skinned. All samples were ground in a semi-industrial mill for wheat. The flours obtained were sieved and stored in glass jars at 4 ° C.

Plant juice preparation:

50 g of flour were dissolved in 450ml of distilled water, a pH 9 for lentil and beans flour and, at pH 6, 50 for chickpeas flour. The mixture was stirred during 30 min, and then enzymatically hydrolyzed at pH 6 by papaine (50mg/450ml), before stirred during 24 h at 70°C and then centrifuged at 4000 rpm for 20 min. The juice was used to prepare two types of culture media: (1) medium A: plant juice solidified by adding agar 15 g/L, (2) Medium B: it contained only 10 ml of juice (lentils, beans or chickpeas), (3) medium C: 10 ml of plant

juice + 0,2 ml of date juice. Before sterilization (121° C for 20 min), the pH was adjusted to 6,4. Experiments were also carried out on MRS media (Difco) for comparison.

Cultivation on solid media by ecometric method:

From broth cultures, each strain was inoculated on 5 sections of the box and then incubated for 24 to 72 hours at the chosen temperature in a CO_2-enriched atmosphere. The procedure performed on each medium was repeated 3 times.

Cultivation on broth media added with date juice:

The inoculum was prepared by transferring one colony into 10 ml of MRS broth, and incubated for 16 h at 37°C.The culture adjusted to an OD_{600} of 0,60 was used to inoculate growth media.

Test Methods:

Solid medium:

The growth of the strains on solid media is estimated following the multiplication of the germ. If the germ multiplied on 5 segments, growth is considered optimal; on 2-3 segments, growth is small; and absent if no colony is developed [1].

Liquid medium:

The biomass evaluation is determined by measuring the optical density (OD) at 600 nm.

Analysis:

The proteins and sugars content of chickpeas, lentils and beans were identified by thin layer chromatography (TLC); for this purpose a precoated layers based on silica-gel were used. The developing reagent for proteins was composed of butanol, acetic acid and water in a volumetric ratio of (70, 18, and 12). For sugars, chloroform, acetic acid and water in a volumetric ratio of (3, 3.5, and 0.5 were used). Visualization of proteins proceeded by spraying the developer of the amino acids, 1% ninhydrine, in acetone and then heating the plates to 80°C for visualization. The spots were identified by comparing the frontal report (RF) with a control. Revelation of the soluble sugars was carried out by thymol prepared in alcohol and sulfuric acid (0.5 g of thymol, 95 ml of ethanol 96° and 5 ml of sulfuric acid), after placing the plates in the incubator at 80 ° C for 15 minutes spots appear pink coloring.

RESULTS AND DISCUSSION

Table 2: Solid medium Evaluation of growth of lactobacilli on solid media.

Strains	Medium	Culture time (h)			Appearance of the colonies
		24	48	72	
Lb1	Lentils 5/5	++	++	++	Whitish colonies, shiny, curved with a regular contour and relatively large. (Φ≈2mm).
	Beans 5/5	++	++	++	Whitish colonies, bright, domed with a regular contour and relatively large. (Φ≈2mm).
	Chickpeas 5/5	++	++	++	Whitish, bright and flat colonies, with a white center, a regular contour and relatively large. (Φ≈2mm).
	MRS 5/5	+	++	++	Whitish colonies, shiny, fine and domed with a regular contour.
Lb2	Lentils 5/5	++	++	++	Whitish, flat colonies, bright and domed with a regular contour (Φ≈1mm).
	Beans 5/5	+	++	++	Transparent and flat colonies with a regular contour (Φ≈1mm). A decrease in the density is remarkable.
	Chickpeas 5/5	++	++	++	Milky colonies and flat with regular contour. (Φ<1mm).
	MRS 5/5	+	++	++	Bright colonies, whitish, flat and convex With a regular contour (Φ≈1mm).
Lb3	Lentils 5/5	+	++	++	Relatively whitish colonies and very flat.
	Beans 2/5	-	+	+	Transparent colonies and very flat.
	Chickpeas 5/5	+	++	++	Slightly whitish colonies and very flat.
	MRS 3/5	+	+	++	Transparent and very flat colonies with a sail growth.
Lb4	Lentil 5/5	++	++	++	Large, whitish circular colonies, bulging and shiny.
	Beans 5/5	+	++	++	Very fine colonies (Φ<1mm) and transparent. A thick crop.
	Chickpeas 5/5	++	++	++	Big, whitish, flat and shiny colonies with a regular contour and a centre more white (Φ ≈ 2-3 mm).
	MRS 5/5	++	++	++	Big, whitish and shiny domed colonies with a regular contour.

Diameter of colony:

These results (Table 2) clearly show that the prepared media cover the complex nutritional needs of the majority of the strains tested. Growth on these media is often optimal and quite comparable to that obtained on the control medium. The growth is significant because it is registered on 4-5 segments. On the 3 types of media tested cultures were dense, the sizes and colors of the colonies were variable (table 2). Their rate of onset was variable too, depending on the strains and media, but their bacterial density was nearly identical after 72 hours of incubation. However it should be noted that the strains Lb1 and Lb4 grew almost similarly on the 3 types of culture media prepared. The comparison between the different media confirms that: Culture media based on

lentil and beans have a good potential and almost equivalent for the multiplication of all strains except Lb3; this strain appears to be more demanding regarding nutritional and especially growth factors requirements. These results are consistent with a previous work showing that

LAB have a fastidious nutritional requirements which may vary among species and even among strains; it is therefore impossible to formulate single medium appropriate for all strains. Vera Pingitoreal. (2009). The chickpeas based medium seems to be suitable to the majority of strains, since growth was observed on the 5 segments. The MRS medium allows optimal growth for all strains tested. Generally, the colonies are whitish, shiny, thin and curved with regular contour. Only Lb3 strain gives fine transparent colonies. This is consistent with the literature indicating that MRS is the medium of choice for the cultivation of lactobacilli strains. Altaf *et al*. (2007).

On all media tested, Lb3 strain is characterized by a slow but dense growth, with usually transparent and fine or ultrafine colonies.

Liquid media:

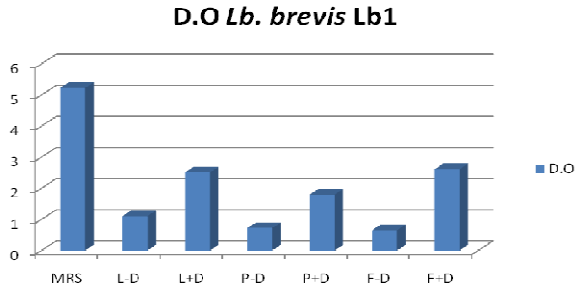

Fig. a: Evaluation of growth of *Lb. brevis* (Lb1) on broth media added with date juice.

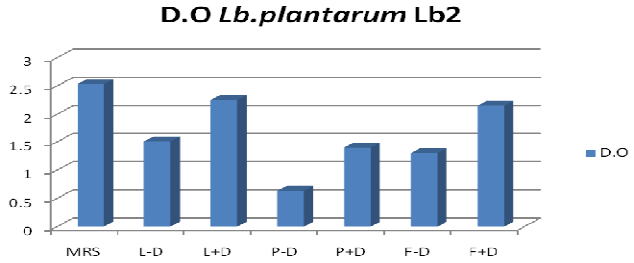

Fig. b: Evaluation of growth of *Lb.plantarum* (Lb2) on broth media added with date juice.

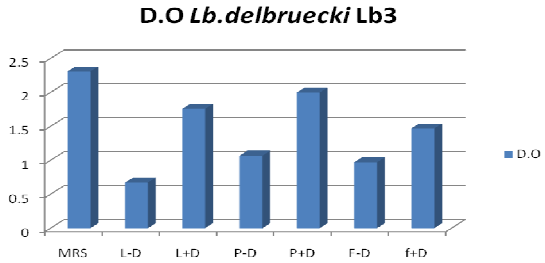

Fig. c: Evaluation of growth of *Lb.delbruecki* (Lb3) on broth media added with date juice.

The results of the culture of the four lactobacilli tested on liquid media confirm that nutritional requirements vary from one strain to another:

It can be noted that the two strains: Lb1 and Lb2 have a certain preference for lentils and beans associated with date juice based media (Figure a et b) this can be explained by the richness of these hydrolysates in minerals including manganese; which has important biological effects such as the structure and function of enzymes and detoxification of cells grown in the presence of O_2. In addition, the presence of Mn^{2+} in the culture medium can significantly stimulate the growth of lactobacilli during fermentation. Leroy *et al*. [23]. Magnesium Mg2+ is another essential element for the growth and metabolic activities of LAB. Mg2+stimulated the growth and improved LAB survival. It was shown that Mg2+ is the only essential oligoelement for the growth of *L.*

delbrueckii ssp. *Lactis.* Hebert *et al*. The addition of Mg2+ and Mn2+ to minimal medium of *L. plantarum* assured its growth. Wegkamp *et al*. [19]. Potassium has also an important role in the regulation of intracellular pH. As for sodium, Na^+ and cl^- were found to be essential components in minimal growth media for *L. Plantarum.* Wegkamp *et al*. [19]. The enzymatic activity of acid phosphatase can be enhanced by both Ca2+ and Mg2+ with a higher effect due to Ca2+. Tham *et al*. Therefore, metal ions play an important role in the growth and metabolic activity of LAB and could be further used to optimize and control the enzymatic activity. Saeed *et al*.

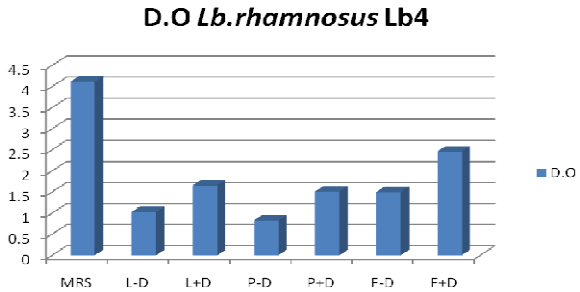

Fig. d: Evaluation of growth of *Lb.rhamnosus* (Lb4) on broth media added with date juice.

Lb1 strain also requires for its growth the presence of certain vitamins such as thiamine; vitamin B1 and B9 folic acid found in large quantities in the two protein crops lentils and beans (Table3).

For the Lb3 strain, it can be noticed that more biomass was produced on lentils and chickpeas based media (Figure c) compared to Lb4 strain that grew well on a medium containing beans (Figure d), contrarily to its growth on other media.

According to the results growth varies depending on media composition.

The media tested have an average protein content of 23 %, with values decreasing in the following order : beans > lentils > chickpeas, 6,4 and 3 mg/ml according to the assays performed in the laboratory by the method of Bradford. Moreover, these media are rich in essential amino acid crucial to the growth of lactic strains as aspartate, histidine, lysine, (Separated by chromatography). LAB with there proteolytic activities broke down proteins by peptidases and proteinases, to make peptide and amino acids available for bacterial growth. Liu *et al*..Thus, it is evident that vitamin requirements of LAB represent many differences among strains, and some vitamins can replace each other; however, individual strains required from one to four vitamins for normal growth. Saeed *et al*. All species of lactobacilli have an absolute requirement for pantothenic acid (vitamin B5) and riboflavin (B2). Both vitamins in addition to others are present in the tested media (Table 3). It is also to be noted that these hydrolysates have a very important concentration of sugar (energy source) (Table 3), such as sucrose, maltose, arabinose, galactose, glucose, xylose, fructose, lactose (sugars identified by thin layer chromatography).

The addition of date juice promotes the growth of these strains due to the large amount of total sugars contained in dates juice, that are needed for the fermentative metabolism of these strains, confirming previous findings of [2] who have shown that the biomass as well as the production of lactic acid increase after the addition of total sugars contained in the date juice. Sugars are the main source of carbon and energy that form essential components in LAB media for normal growth and functionality [16,18].

Table 3: Average nutritional values per 100g of protein crops used.

Average nutritional values 100g	Lentil cooked	Beans cooked	Chickpea cooked
Magnesium	36 mg	43 mg	48 mg
Potassium	369 mg	268 mg	291 mg
Manganese	0,494 mg	0,421mg	1,03 mg
Thiamine B1	0,169mg	0,097mg	0,116mg
Folic acid B9	181 µg	104 µg	172 µg
total sugar	20,13 g	19,65 g	27,42 g

***Conclusion**:*

Our study focused on the nutrition of lactic acid bacteria, whose nutritional requirements are complex. We have tested the growth of four lactobacilli on three media composed exclusively of hydrolyzed protein crops added to date juice. Bacteria growth was compared to that recorded in the control medium MRS. This comparison allowed to show that the 3 hydrolyzed proteins partially cover the nutritional requirements of the majority of the strains tested, although in agreement with wealth of these vegetal substrates are rich in starch and rare sugars (sucrose and the alpha-galactosides, raffinose, stachyose and verbascose) in protein, vitamins and

minerals. This positive impact should be most likely related to the breakdown of long chains of macromolecules on the one hand, and to the release of growth factors essential to the development of these bacteria on the other hand.

This preliminary work demonstrates the interesting potential of the protein crops and date juice in the nutrition of the lactic flora. It should be continued by optimizing the composition of the culture media by testing other strains and studying more accurately the parameters of the solubilization and the hydrolysis of the tested protein crops.

REFERENCES

[1] Bonaparte, C., G. Klein, W. Kneifel and G. Reute, 2001. Développement d'un milieu sélectif pour le dénombrement des bifidobactéries dans les laits fermentés. Le Lait, 81(1-2) : 227–235.
[2] Boudjelal, A., N. Nancib, 2001. Production d'acide lactique par Lactobacillus Rhamnosus sur milieu à base de jus de dattes. Production et valorisation – biomasse p 41-46.
[3] Chalampopoulos, D., S. Pandiella, C. Webb, 2002. Growth studies of potentially probiotic lactic acid bacteria in cereal –based substrates. Journal of applied microbiology, 9(2): 851-859.
[4] De Roissart, H., F.M. Luquet, 1994. Les bactéries lactiques aspects fondamentaux et technologiques. Lorica-Uriage.
[5] Djeghri-Hocine, B., M. Boukhemis, N. Zidoune, A. Amrane, 2007. Growth of lactic acid bacteria on oilseed crop pea- and chickpea-based media. World Journal of Microbiol. Biotechnol, 23(6): 765-769.
[6] Djeghri-Hocine, B., M. Boukhemis, N. Zidoune and A. Amrane, 2006. Horse Bean Extract for the Supplementation of Lactic Acid Bacteria Culture Media. Journal of Food Technol, 4(4): 299-302.
[7] Sakai, T., K. Oishi, T. Asahara, T. Takada, N. Yuki, K. Matsumoto, K. Nomoto and A. Kushiro, 2010. M-RTLV agar, a novel selective medium to distinguish Lactobacillus casei and Lactobacillus paracasei from Lactobacillus rhamnosus. International Journal of Food Microbiology, 139(3): 60–154.
[8] Lee, H.M. and Y. Lee, 2008. A differential medium for lactic acid-producing bacteria in a mixed culture. Journal of Letters in Applied Microbiology, 46: 676–681.
[9] Nancib, N., A. Nancib, A. Boudjelal, C. Benslimane, F. Blanchard and J. Boudrant, 2001. The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *rhamnosus*. Journal of Bioresource Technol, 78: 149-153.
[10] Cokkizgin, A. and J.Y. Shtaya, 2013. Lentil: Origin, Cultivation Techniques, Utilization and Advances in Transformation. North American center of Agricultural Science, 1(1): 55-62.
[11] Rossi, M., A. Amaretti and S. Raimondi, 2011. Folate production by probiotic bacteria. Journal of Nutrients, 3(1): 118-134.
[12] Patel, A., N. Shah and J.B. Prajapati, 2013. Biosynthesis of vitamins and enzymes in fermented foods by lactic acid bacteria and related genera - A promising approach. Journal of Food science and Thechnology, 5(2): 85-91.
[13] Douillard, F.P. and M. De Vos Willem, 2014. Functional genomics of lactic acid bacteria : From food to health. Journal of Microbial Cell Factories, 13(1): S8.
[14] Ashraf, R. and N.P. Shah, 2011. Selective and differential enumerations of Lactobacillus delbrueckii subsp. Bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium spp. in yoghurt: Int. J. Food Microbiol, 149: 194-208.
[15] Pingitore, E.V., E.M. Hebert, F. Sesma and M.E. Nader-Macias, 2009. Influence of Vitamins and Osmolites on Growth and Bacteriocin Production by *Lactobacillus sali- varius* CRL 1328 in a Chemically Defined Medium. Canadian Journal of Microbiology, 55(3): 304-310.
[16] Wright, A.V. and L. Axelsson, 2011. Lactic Acid Bacteria: An Introduction In: S. Lahtinne, S. Salminen, A. Von Wright and A. Ouwehand, Eds., Lactic Acid Bacteria: Microbiological and Functional Aspects, CRC Press, London, 1-17.
[17] Liu, M., J.R. Bayjanov, B. Renckens, A. Nauta and R. J. Siezen, 2010. The Proteolytic System of Lactic Acid Bacteria Revisited: A Genomic Comparison. Journal of BMC Genomics, 11: 1-15.
[18] O' Donnell, M.M., B.M. Forde, B. Neville, P.R. Ross and P.W. O' Toole, 2011. Carbohydrate Catabolic Flexibility in the Mammalian Intestinal Commensal *Lactobacillus ruminis* Revealed by Fermentation Studies Aligned to Genome Annotations. Journal of Microbial Cell Factories, 10(l): 1-11.
[19] Wegkamp, A., B. Teusink, W.M. De Vos and E.J. Smid, 2010. Development of a Minimal Growth Medium for *Lactoba- cillus plantarum*. Journal of Letters in Applied Microbiology, 50: 57-64.
[20] Hébert, E.M., R.R. Raya and G.S. Giori, 2004. Nutritional Requirements of *Lactobacillusdelbrueckii* subsp. *lactis* in a Chemically Defined Medium. Journal of Currunt Microbiology, 49(5): 341-345.

[21] Altaf, M.D., B.J. Naveena and G. Reddy, 2007. Use of Inexpen sive Nitrogen Sources and Starch for L (+) Lactic Acid Production in Anaerobic Submerged Fermentation. Journal of Bioresource Technology, 98(3): 498-503.
[22] Hayek, S. and S. Ibrahim, 2013. Current Limitations and Challenges with Lactic Acid Bacteria. Journal of Food and Nutrition Sciences, 4: 73-87.
[23] Leroy, F., J. Verluyten and L. De Vuyst, 2006. Functional Meat Starter Cultures for Improved Sausage Fermentation. International Journal of Food Microbiology, 106(3): 270-285.
[24] Tham, S., C. Chang, H. Huang, Y. Lee, T. Huang and C.C. Chang, 2010. Biochemical Characterization of an Acid Phosphatase from *Thermus thermophilus*. Journal of Bioscience, Bio- technology, and Biochemistry, 74(4): 727-735.