American Journal of Food Science and Nutrition Research
2019; 6(1): 1-5
http://www.openscienceonline.com/journal/fsnr
ISSN: 2381-621X (Print); ISSN: 2381-6228 (Online)

Determination of Tryptophan and Glutamic Acid During Fermentation of Kiwi-based Milk by Different Combinations of *Saccharomyces boulardii* and *Lactobacilli*

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To cite this article

Ahmed Hassan Mousa, Gang Wang, Hao Zhang. Determination of Tryptophan and Glutamic Acid During Fermentation of Kiwi-based Milk by Different Combinations of *Saccharomyces boulardii* and *Lactobacilli*. *American Journal of Food Science and Nutrition Research.* Vol. 6, No. 1, 2019, pp. 1-5.

Received: January 31, 2019; **Accepted**: February 28, 2019; **Published**: March 6, 2019

Abstract

The aim of the present study was to evaluate the synergy interactions between *Saccharomyces boulardii* and preselected strains of *lactobacilli* during fermentation of skimmed milk fortified with kiwi juice (4% v/v) regarding tryptophan and glutamic acid production. The low capacity of *S. boulardii* in internalizing different sources of nitrogen produced marginal amounts of ≤0.66 mg/L (tryptophan) and ≤8.26 mg/L (glutamic acid) whether the milk was free or fortified with kiwi juice. The distinct production of tryptophan and glutamic acid were observed when the formulations were inoculated with *lactobacilli* and *S. boulardii* together. And so, the increased production was greater as much as ≤5.40 mg/L (tryptophan) and ≤12.09 mg/L (glutamic acid) when *Lb. casei* 20975 had a chance to grow in the milk with and without added kiwi juice where the *S. boulardii* was present. *Lactobacilli* strains could exercise its proteolytic system through cleavage of the higher molecular weight nitrogenous compounds. Indicating the presence of *S. boulardii* in the formulations where *Lb. plantarum* JXJ (6-12) or *Lb. fermentum* F16 were grown, the produced tryptophan and glutamic acid were at rapid rate and much more than those observed for the formulations inoculated with *S. boulardii* and *Lb. plantarum* RS (35-11), *Lb. casei* LCS, or *Lb. fermentum* F9.

Keywords

Saccharomyces boulardii, Kiwi Juice, Fermented Milk, Tryptophan, Glutamic Acid

1. Introduction

Fermented dairy products are products that can be produced through lactose fermentation by microorganisms especially by lactic acid bacteria "LAB" [1]. *Lactobacillus* is by far the largest genus of the LAB, and more than 125 species and subspecies names are currently recognized [2]. The key property in defining LAB is that these bacteria produce lactic acid as the major or sole fermentation product [3]. *Lactobacillus* species (e.g., *casei*, *plantarum*, *fermentum*) are considered to be a ubiquitous and metabolic versatile bacterium that can be used largely as culture or in mixtures in traditional dairy products. The claim for functional foods is increasing quickly due to growing up awareness of these foodstuffs importance. Thus, production of fermented dairy products contained lactic acid bacteria, such as *Lactobacillus* strains, fruits fortified, have developed in the last decade

[1],[4]. Most fermented dairy products are produced using bacteria rather than yeasts. Kefir is an exception and is the product of a mixed fermentation with yeasts and bacteria [4]. *Saccharomyces boulardii* disclosed its synergistic effect on the *lactobacilli* growth in the dairy products as probiotic functional foods [5]. So far the active use of yeasts as dietary adjuncts for *lactobacilli* has been limited, despite the occurrences of yeasts as an integral part of the microflora of many dairy-related products. In the context of yeasts, *S. boulardii* possesses fermentative functions that contribute to the *lactobacilli* metabolism in fruit-based milk and vice versa. These frequent occurrences of *S. boulardii* indicate its ability to survive and metabolize milk constituents and sugars derived from the fruits substantially [6]. *S. boulardii* cells could adapt at the low pH condition, besides their beneficial characteristics in suppressing the pathogenic microorganism [7]. In addition, the proliferation of *lactobacilli* together with *Saccharomyces* synergistically in dairy products has been reported based on their metabolic activity [1, 8], physiochemical characteristics of the product, and the regulations and labeling issues. Kiwi juices are rich in sugars, vitamins, and minerals; they are therefore processed to produce wine through conversion sugars into ethanol rapidly by using yeasts (alcoholic fermentation). Likewise, *lactobacilli* are able to ferment fruits juices such as kiwi spontaneously, regarding their reliability and consistency of performance [9]. Furthermore, fortification of milk with fruit juices (e.g. kiwi) has been reported for increasing the bioavailability of the nutritional constituents [10]. The main objective of this study was to determinate the production rates of tryptophan and glutamic acid during fermentation of milk with and without added kiwi juice by *lactobacilli* and *Saccharomyces boulardii*.

2. Material and Methods

2.1. Materials

Skimmed milk powder was purchased from Guang Ming Co. Ltd (Wenzhou, China). Kiwi (*Actinidia chinensis*) was purchased from a local retail market in China. Chloramphenicol, Ampicillin, Cycloheximide (*BioReagents*), and MF-Millipore membrane filter (mixed cellulose esters, asbestos monitoring, 0.22 µm, 25 mm, white, gridded) were purchased from Sigma-Aldrich (Shanghai, China). All chemicals used in the experiments were of analytical grade and high purity.

2.2. Microorganism and Medium

Saccharomyces boulardii (Reflor; Biocodex, Cedex, France), and all of the *lactobacilli* strains used in this study were obtained from the Synergistic Innovation Center for Food Safety and Nutrition, Jiangnan Uni. (Wuxi, China). Yeast extract peptone dextrose (YPD) supplemented with 250 µg/ml of Chloramphenicol [11] and 30 µg/ml of Ampicillin [12] were used to activate *S. boulardii*. DeMan, Rogosa, Sharpe (MRS) supplemented with 100 µg/ml of Cycloheximide [13] was used to activate *Lactobacillus* strains.

2.3. Preparation of the Media Substrates and Fermentation

Skimmed milk powder was reconstituted at a concentration of 11% (w/v), pasteurized for 15 sec at 72°C and then sterilized by using MF-Millipore membrane filter (0.22 µm/25 mm). Kiwi Juice was extracted by using Santos blender and sterilized by using MF-Millipore membrane filter (0.22 µm/25 mm). Skimmed milk was then fortified with kiwi juice at a concentration of 4% (v/v). Skimmed milk with and without added kiwi juice was distributed in 150 ml quantities into sterile, Erlenmeyer flasks (250 ml), and then inoculated with an active culture of *S. boulardii* alone or with *S. boulardii* and preselected active culture of *lactobacilli* strains together (Table 1). The inoculated substrates were allowed to ferment for 12 h at 37°C under anaerobic conditions. Samples were withdrawn at regular intervals for analysis.

Table 1. *An designed model for Saccharomyces boulardii and the preselected strains of lactobacilli inoculated into skimmed milk with and without added kiwi juice (4% v/v).*

S. boulardii
‡ Lb. casei 20975 + S. boulardii
† Lb. casei LCS + S. boulardii
† Lb. plantarum RS (35-11) + S. boulardii
‡ Lb. plantarum JXJ (6-12) + S. boulardii
† Lb. fermentum F9 + S. boulardii
‡ Lb. fermentum F16 + S. boulardii

‡ The bacterial strains are classified by their rapid rates of growth. † The bacterial strains were classified by their slow rates of growth.

2.4. Gas-Chromatography-Mass Spectrometry Analysis

Derivatization for GC-MS was performed according to a previous protocol [14] with few modifications. The dissolved sample (50 µL) was supplemented with Ribitol (15 µL), and then dried via SpeedVac Concentrator (Thermo Electron, SAVANT, SPD131DDA, CA). Methoxyamine hydrochloride - pyridine (100 µL, 10 mg/mL) was added to the dried sample, and then incubated (90 min/40°C). A 40 µL of BSTFA-TMCS (99:1) was added to the mixture, and then incubated (60 min/75°C). The extract aliquot (70 µL) was transferred for determination of the lower molecular weight compounds. A 10 µL was mixed with Dichloromethane (990 µL) for determination of the higher molecular weight compounds. Tryptophan and glutamic acid were determined via Thermo/GC-MS/Trace 1310 (TSQ 8000 Evo, Switzerland) by using a column of the Rtx-5MS capillary (30 m, 0.25 mm /0. 25 µm). The carrier gas was Helium (35.0 cm/s). The temperature was 320°C (90°C/min), and then held for 5 min.

2.5. Statistical Analysis

Expression of the triplicate data was designed as means ±

SD by using the statistical software of SPSS 19.0. The significant differences ($P < 0.05$) among the results were tested through Duncan's multiple ranges.

3. Results and Discussions

Milk alone (Control) and Kiwi-based milk as media substrates were inoculated with *S. boulardii* alone, or with *S. boulardii* and the preselected *lactobacilli* strains together. The effects of fermentation periods and microbial combinations on production of tryptophan (Figure 1A and B) and glutamic acid (Figure 2A and B) were studied. Fewer amounts of tryptophan and glutamic acid were produced if *S. boulardii* inoculated alone into milk with and without added kiwi juice. The maximum of 0.66 mg/L (tryptophan) and 8.26 mg/L (glutamic acid) were produced in the absence of *lactobacilli* inoculation in the case where the milk was fortified with kiwi juice, while the maximum cell number declined to 0.53 and 8.10 (mg/L) if the milk was free of added kiwi juice, respectively. It appears that the presence of *lactobacilli* in the formulations stimulated the production of tryptophan and glutamic acid. Therefore, the results showed that the production of tryptophan and glutamic acid increased at a rapid rate within 6 h, and to a greater extent differed significantly ($P < 0.05$) with the other cases, if *Lb. casei* 20975 had a chance to grow in the milk with and without added kiwi juice where *S. boulardii* was inoculated. In detail, when the *Lb. casei* 20975 was inoculated with *S. boulardii*, the production increased to maximum of 5.40 mg/L (tryptophan) and 12.09 mg/L (glutamic acid) within 6 h if the milk was fortified with kiwi juice. Even when the milk was free of added kiwi juice, the increased production still occurred but was reduced to 3.64 mg/L (tryptophan) and 10.30 mg/L (glutamic acid). When production of the tryptophan (1.17–1.64 mg/L) and glutamic acid (8.53–9.75 mg/L) was at a rapid rate, as when the *S. boulardii* was grown with the *Lb. plantarum* JXJ (6-12) or *Lb. fermentum* F16, the produced amounts were much more than those observed for the formulations inoculated with *S. boulardii* and other *lactobacilli* such as *Lb. plantarum* RS (35-11), *Lb. casei* LCS, or *Lb. fermentum* F9. *Lactobacilli* could exercise its proteolytic system through cleavage of the higher molecular weight nitrogenous compounds, produced tryptophan and glutamic acid. *Lactobacilli* employed the extracellular activity for liberating peptides from milk casein's. These peptides undergo breakdown resulting in the amino acids once their passage into cells through transfer system of the *lactobacilli* [15]. In spite of the resulted significant differences ($P < 0.05$), the small amounts of tryptophan and glutamic acid produced at a slow rate within 8 h in the milk fortified with kiwi juice where the *S. boulardii* was inoculated with each of *Lb. casei* LCS, *Lb. plantarum* RS (35-11) and *Lb. fermentum* F9 together were similar and varied from ≤0.95 mg/L (tryptophan) to 8.48 mg/L (glutamic acid).

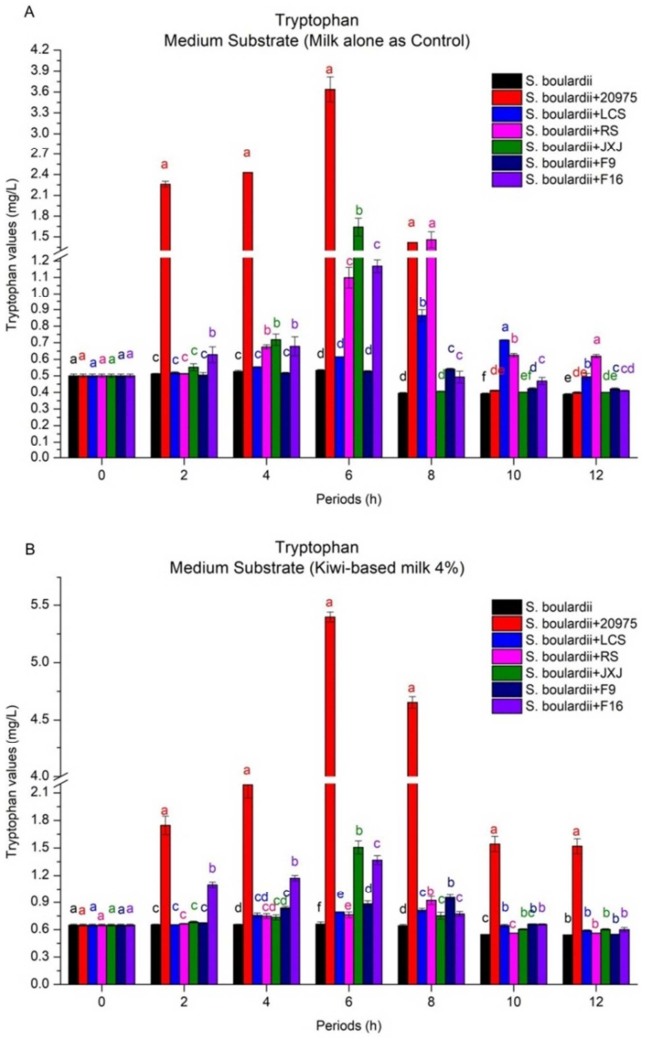

Figure 1. *Changes in tryptophan amounts (mg/L) during fermentation of milk with and without added kiwi juice by Saccharomyces boulardii, or by a combination of S. boulardii and preselected lactobacilli strains. S. boulardii is a treatment inoculated with S. boulardii alone; S. boulardii+20975 is a treatment inoculated with S. boulardii and Lb. casei 20975 together; S. boulardii+LCS is a treatment inoculated with S. boulardii and Lb. casei LCS together; S. boulardii+RS is a treatment inoculated with S. boulardii and Lb. plantarum RS 35-11 together; S. boulardii+JXJ is a treatment inoculated with S. boulardii and Lb. plantarum JXJ 6-12 together; S. boulardii+F9 is a treatment inoculated with S. boulardii and Lb. fermentum F9 together; S. boulardii+F16 is a treatment inoculated with S. boulardii and Lb. fermentum F16 together. Data are expressed as the mean standard deviation (SD) of seven treatments (triplicate samples) which were analyzed in each treatment. Different superscripts of lowercase letters ($^{a-f}$) in the same row denote the significant differences ($P < 0.05$) among treatments for the same fermentation period.*

It is apparent that by the fermentation of milk free of added kiwi juice, the maximum amounts of tryptophan and glutamic acid produced within 8 h were about the same in the cases where *S. boulardii* was inoculated with LCS or F9. Furthermore, inoculation of *S. boulardii* with RS (35-11) was an exception in the case where the milk was free of added kiwi juice, just when the production increased to 1.46 mg/L (tryptophan) and 8.88 mg/L (glutamic acid) differed nonsignificantly ($P > 0.05$) with *Lb. fermentum* F16 before reaching a maximum. Certainly, *Lb. plantarum* is efficient to

hydrolyze β-casein completely and α_{s1}-casein partially, thereby synthesizing the amino acids [16]. On the other hand, whey protein is restricted to the proteolytic activities of *Lactobacillus* in the skim milk. However, *Lb. plantarum* possesses the proteolytic activity to hydrolyze β-Lactoglobulin in part that was not reported for hydrolysis of the α-Lactalbumin. Our findings are compatible with the other study stated that the high proteolytic activity of *Lb. casei* [17]. Other study referred to the low capacity of *Lb. casei* to degrade of the casein fraction in such as β-casein. The other fractions of milk casein were not considered as one of the hydrolysis indexes for *Lb. casei* [16].

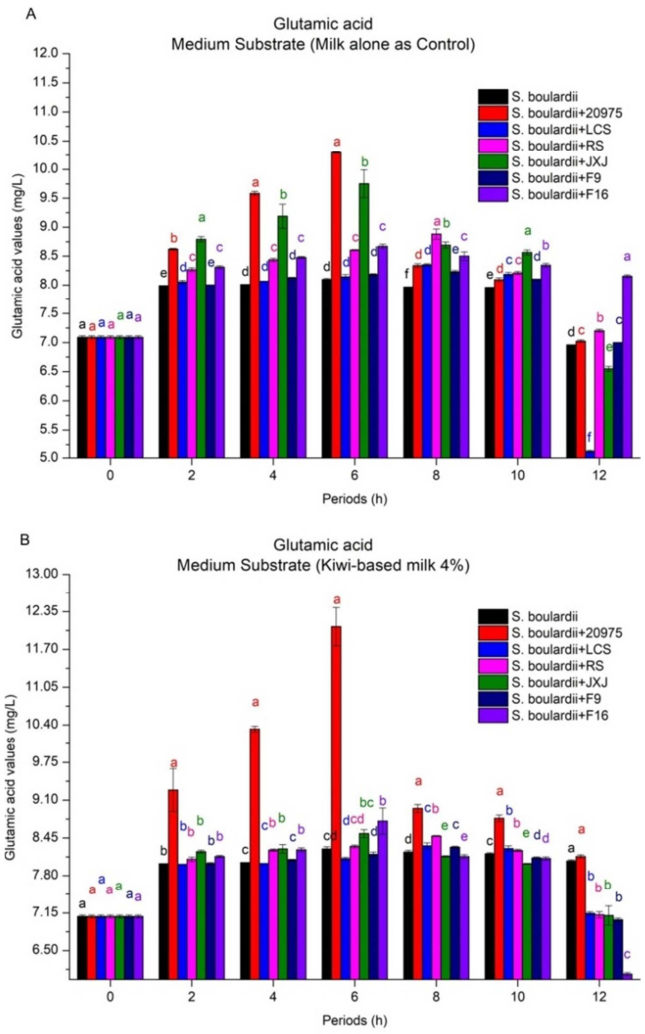

Figure 2. *Changes in glutamic acid amounts (mg/L) during fermentation of milk with and without added kiwi juice by Saccharomyces boulardii, or by a combination of S. boulardii and preselected lactobacilli strains. For symbol details, see Figure 1.*

Another assumption is that the essential amino acids (e.g., glutamic acid and tryptophan) generate from intermediaries during metabolism of the organic acids [18]. Subsequently, glutamic acid as dicarboxylic acid predominated in all of the treatments due to the higher metabolic activity of *Lactobacillus* strains. The ability of *S. cerevisiae* in internalizing different sources of the nitrogen compounds has been reported [19]. In view of that, *S. cerevisiae* could control the nitrogen uptake across the plasma membrane. In consequence, the whole internalization of nitrogen compounds could induce the synthesis of tryptophan as resulted in this study. In this interim, the tryptophan biosynthesis by *S. cerevisiae* could modulate the cellular metabolism under the adverse conditions [20]. After reaching a maximum, the tryptophan and glutamic acid contents of the formulations declined remarkably, indicating probable uptake of tryptophan and glutamic acid as assimilable nitrogen sources by the *lactobacilli* through dehydrogenation reactions [9]. The beginning of the decline of tryptophan and glutamic acid coincided with the exhaustion of essential nutrients supply from the medium. Still, the facts on catabolism of the amino acids by *lactobacilli* or yeast are much scarce.

4. Conclusions

It is clear from this study that fortification of milk with kiwi juice could induce the metabolic activity of *lactobacilli* much more than *S. boulardii*, regarding the produced tryptophan and glutamic acid amounts during fermentation. However, the competition for the nutritive supply impacted the metabolic dynamics in such as proteolysis systems. *S. boulardii* lacked somewhat characteristics of *lactobacilli*, but it's could assimilate and ferment the metabolites of *lactobacilli* regarding the increased production of the tryptophan and glutamic acid. In addition, hydrolysis of milk proteins into amino acids served as a sign of distinctive activity for the *S. boulardii*. Eventually, this study recommends that proliferation of bacteria coincided with presence of yeast established a scalable strategy in the fermented dairy industry.

Acknowledgements

This work was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Conflict of Interest

The authors declare that no conflict of interest.

References

[1] Phillips M, Kailasapathy K, Tran L. Viability of commercial probiotic cultures (L. acidophilus, Bifidobacterium sp., L. casei, L. paracasei and L. rhamnosus) in cheddar cheese. International journal of food microbiology 2006; 108: 276–280.

[2] S. Lahtinen, A. C. Ouwehand, S. Salminen and A. von Wright, Lactic acid bacteria: microbiological and functional aspects. Ed. Boca Raton, Florida, United States: CRC Press, 2011.

[3] Wu R, Wang W, Yu D, Zhang W, Li Y, Sun Z, Wu J, Meng H, Zhang H. Proteomics Analysis of Lactobacillus casei Zhang, a New Probiotic Bacterium Isolated from Traditional Home-made Koumiss in Inner Mongolia of China. Molecular & Cellular Proteomics 2009; 8: 2321–2338.

[4] SILVA CFGD, SANTOS FL, SANTANA LRRD, SILVA MVL, CONCEIÇÃO TDA. Development and characterization of a soymilk Kefir-based functional beverage. Food Science and Technology 2018; 38: 543–550.

[5] Gaon D, Garcia H, Winter L, Rodríguez N, Quintas R, Gonzalez SN, Oliver G. Effect of Lactobacillus strains and Saccharomyces boulardii on persistent diarrhea in children. Medicina (Buenos Aires) 2003; 63: 293–298.

[6] Lourens-Hattingh A, Viljoen BC. Growth and survival of a probiotic yeast in dairy products. Food Research International 2001; 34: 791–796.

[7] Van der Aa Kühle A, Skovgaard K, Jespersen L. In vitro screening of probiotic properties of Saccharomyces cerevisiae var. boulardii and food-borne Saccharomyces cerevisiae strains. International journal of food microbiology, 2005; 101: 29–39.

[8] Parvez S, Malik KA, Ah Kang S, Kim H. Probiotics and their fermented food products are beneficial for health. Journal of applied microbiology 2006; 100: 1171–1185.

[9] Di Cagno R, Coda R, De Angelis M, Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology 2013; 33: 1–10.

[10] García-Nebot MJ, Alegría A, Barberá R, Clemente G, Romero F. Addition of milk or caseinophosphopeptides to fruit beverages to improve iron bioavailability?. Food Chemistry 2010; 119: 141–148.

[11] Vanhee LME, Goemé F, Nelis HJ, Coenye T. Quality control of fifteen probiotic products containing Saccharomyces boulardii. Journal of Applied Microbiology 2010; 109: 1745–1752.

[12] Srinivas B, Rani GS, Kumar BK, Chandrasekhar B, Krishna KV, Devi TA, Bhima B. Evaluating the probiotic and therapeutic potentials of Saccharomyces cerevisiae strain (OBS2) isolated from fermented nectar of toddy palm. AMB Express 2017; 7: 2.

[13] Ali AA. Isolation and Identification of lactic acid bacteria isolated from traditional drinking yoghurt in Khartoum State, Sudan. Current Research in Bacteriology 2011; 4: 16–22.

[14] Zhao N, Zhang C, Yang Q, Guo Z, Yang B, Lu W, Li D, Tian F, Liu X, Zhang H. Selection of taste markers related to lactic acid bacteria microflora metabolism for Chinese traditional paocai: a gas chromatography–mass spectrometry-based metabolomics approach. Journal of agricultural and food chemistry 2016; 64: 2415–2422.

[15] Kok J. Genetics of the proteolytic system of lactic acid bacteria. FEMS Microbiology Letters 1990; 87: 15-42.

[16] Khalid NM, Marth EH. Proteolytic activity by strains of Lactobacillus plantarum and Lactobacillus casei. Journal of Dairy Science 1990; 73: 3068–3076.

[17] Solieri L, Rutella GS, Tagliazucchi D. Impact of non-starter lactobacilli on release of peptides with angiotensin-converting enzyme inhibitory and antioxidant activities during bovine milk fermentation. Food microbiology 2015; 51: 108–116.

[18] de Nadra MCM, Arena ME, Saguir FM. Nutritional requirements and amino acids utilization by lactic acid bacteria from wine-A short review. JOURNAL OF FOOD AGRICULTURE AND ENVIRONMENT 2003; 1: 76–79.

[19] Ljungdahl PO, Daignan-Fornier B. Regulation of amino acid, nucleotide, and phosphate metabolism in Saccharomyces cerevisiae. Genetics 2012; 190: 885–929.

[20] Zhao XQ, and Bai FW. Mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. Journal of Biotechnology 2009; 144: 23–30.