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# GROWTH ASSOCIATION RELATIONSHIP BETWEEN THE YEAST *Saccharomyces cerevisiae* AND LACTIC ACID BACTERIA IN PRODUCTION OF FERMENTED MILKS.

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## ABSTRACT

Growth association relationship between yeast as probiotioc organism and lactic acid bacteria in fermented milks for their probiotic functions enrichment was investigated. The survival and growth of yeast and lactic acid bacteria were monitored over 3-weeks storage period. Specific counts, pH and sensory properties were determined to evaluate the possible contribution of yeasts towards the products. The yeast *Saccharomyces cerevisiae* was able to survive in yoghurt and in the other products containing *Lactobacillus acidophilus* and/or *Bifidobacterium bifidum*. Yeast grew well also in milk as a pure culture while its growth was sharply decreased with increasing the storage period. The inclusion of yeast in fermented milk products stimulated growth of both lactic acid bacteria and yeast-maximum counts, exceeding 9.8 and 6.6 log/ml respectively. pH values were slightly increased upon inclusion of yeast with yoghurt starters and conversely they were slightly decreased in the products containing *L. acidophilus* and/or *B. bifidum*. Growth association between yeast and lactic acid bacteria contributed much for obtaining fermented milk products with good sensory qualities.

**Keywords:** *Saccharomyces cerevisiae*, Lactic acid bacteria, *Bifidobacterium bifidum*, Associative growth, Fermented milk.

## INTRODUCTION

Probiotics can be defined as microbial cell preparations or components of microbial cells that have a beneficial effect on health and well-being of the host (*Salminen et al. 1999)*. Van der Ae Kühle *et al. (2004)* reported that, food–borne *Sac. cerevisiae* strains may have a potential beneficial effect as probiotics, while they were able to tolerate low pH and bile salts. Some strains exhibited pronounced adhesive properties and have protective effects against bacterial infections via reduction of the intestinal proinflammatory response. Furthermore, *Sac. boulardii* (a strain of *Sac. cerevisiae*) was described in the clinical literature as biotherpeutic agent and reported to be efficacious in the prevention or recurrence of antibiotic – associated diarrhea and treatment of acute diarrhea in children and colitis in humans (Surawicz *et al.* 1989, Cetina-Sauri and Sierra Basto 1994).

Probiotic microorganisms are increasingly incorporated into food as dietary adjuncts for the purpose to benefit the human health, and they are typically lactobacilli or bifidobateria (Lourens-Hattingh and Viljoen 2001). Yeast have been reported to be involved in several different types of indigenous fermented foods and beverages (Zulu *et al.* 1997, Gadaga *et al.* 2001).It appears that Sac. cerevisiae can survive passage through the intestinal tract, which further accentuates the possible use of yeasts as probiotics. Several specific antagonistic interactions have been reported between yeast culture like Sac. cerevisiae and enteric pathogens e.g.

enteropathogenic *Escherichia coli*, *Shigella* and *Salmonella* as reviewed by Gedek (1991).

The frequent occurrences of yeast in dairy related products indicate the ability of yeast to survive and metabolise milk constituents (Good-enough & Klein 1976). Some milk products that include yeast in their starter culture like acidophilus yeast milk (long and long 1975), Kefir, Koumiss and Labna. They are produced by fermentation of milk by a mixture of lactic acid bacteria, Yeasts and other bacteria such as acetic acid bacteria Subramanian and Shankar (1983). The possible function of yeast in fermented foods may contribute to fermentation of carbohydrates, production of aroma compounds (esters, organic acids, carbonyls, etc.), stimulation of lactic acid bacteria, providing essential metabolites and growth factors, inhibition of mycotoxin producing moulds and degradation of mycotoxins, and cyanogenic glycosides (linamarase activity), and probiotic properties (Jespersen 2003). Therefore, yeasts must have increasing interest be included in our search to find out new starter cultures for fermented milk products and introducing new cultures with desired charactertics.

Recently the active use of yeasts as dietary adjuncts for human are going to be increased. Lourens–Hattingh and Viljoen (2001- 2002) concluded that the application of yeast species of Sac. Cerevisiae as probiotic microorganisms seems promising especially in milk and plain yoghurts since no gas or alcohol were formed.

They recommended investigation and contribution of yeasts to taste, texture and odours in yoghurt.

The current study focus upon the ability of *Sac. cerevisiae* to grow in yoghurt and bio-yoghurt and evaluating its contribution to the sensoric properties of fermented milk.

## MATERIALS AND METHODS

**Lactic acid Bacteria.**

Cultures of *Lactobacillus delbreuckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacteria bifidum* were obtained from Dairy Microbiology Lab., National Research Centre, Dokki, Cairo, Egypt. Lactobacilli strains were cultured for 24 h in deMan-Rogosa-Sharpe (MRS) broth (Oxoid, Hampshire, United Kingdom (UK) Str. *thermophilus* was cultured on M17 (Oxoid). *B. bifidum* was cultured for 24 h in modified (MRS) supplemented with 0.05% L-cysteine HCl (Merck, Germany) and incubated in anaerobic environment (BBL Gas Pak, Becton Dickinson, Cockeysville AM, USA).

**Yeast culture:** *Saccharomyces cerevisiae* was isolated on malt extract agar from bakers yeast product of sugar and integrated industries- El Hawamdia, Egypt.

**Milk:** Fresh raw buffaloes milk was obtained from the Animal production Research Institute, Agriculture Research center, Ministry of Agriculture, Cairo, Egypt.

**Methods:**

1- Yoghurt-Like products manufacture yoghurt – like products were manufactured according to Robinson and Tamime (1990). They were prepared from heated (95°C/10min) fresh whole buffaloes milk. Starters were then added as the following:

**Control:** ***Str. thermophilus*** and ***L. delbreuckii subsp bulgarcus*** in proportion 1:1 (v/v)

**Treatment I:** ***Str. thermophilus*** and ***L. bulgaricus*** and **Sac.** ***cerevisiae*** were mixed in proportion 1:1:1 (v/v).

**Treatment II:** ***Str. thermophilus*** and ***Sac. Cerevisiae*** were mixed in proportion (1 :1) (v/v).

**Treatment III:** ***L. delbreuckii sub sp. bulgaricus*** and ***Sac. cerevisiae*** were mixed in proportion 1:1 (v/v).

**Treatment IV:** ***Sac. cerevisiae*** was used to inoculate milk at ratio of 2% v/v, then fermentation process was carried out at 42°C. After completion of coagulation, the set yoghurt-like products cooled rapidly and stored in a refrigerator at ≃ 5-7°C.

**2- Fermented milk manufacture.**

Six batches of fermented milk were prepared from fresh whole buffaloes milk, heated to 95°C for 10 min. The first batch was inoculated with 2% (v/v) of ***L. acidophilus*** single culture. The second was inoculated with *B. bifidum*. The third was inoculated with 2% (v/v) of yeast *Sac. cerevisiea*. The forth one was inoculated with 1% (v/v) of *L. acidophilus* and 1% (v/v) of yeast, *Sac. cerevisae*, double mixed culture. The fifth portion was inoculated with 1% (v/v) *B. bifidium* and 1% (v/v) of yeast, *Sac. cerevisiae*. The final portion (6th) was inculcated with 1% (v/v) *L. acidophilus*, 1% (v/v) *B. bifidum* and 1% (v/v) of yeast, triple mixed culture.

*L. acidophilus*. The second was inoculated with 2% (v/v) of all these cultures were incubated at 42°C till coagulation and then stored at refrigerator temperature ≃ 5°C.

The experiments were replicated and duplicate samples were used.

**Analytical procedures:**

Yoghurt and fermented milk samples were taken periodically for analysis when fresh and after 3, 6, 10, and 25 days, during storage period.
pH was measured using a digital pH meter with glass electrode model Hanna HT 481.

The organoleptic properties of fresh yoghurt and fermented milk were assessed by 20 panelists of the experienced staff members of Dairy Department, National Research Centre. Samples were evaluated for flavour (50 points), body and texture (30 points) and appearance (20 points) according to Keating and Rand white (1990).

## RESULTS AND DISCUSSION

**Growth association relationship between yeast and yoghurt starter.**

Growth association relationship between the yeast *Sac. cerevisiae* and yoghurt starter was studied. Table (1) show the results obtained that it could be noticed from treatment 4 that the growth of both *Str. thermoqhilus* and *L. bulgaricus* was enhanced in the presence of yeast and up to the end of incubation period with reduction of survival rate of yeast. This result could be also noted in treatment (1) and (2). However *L. bulgaricus* exhibited a higher level of growth when compared with *Str. thermophilus*. This was concomitant with decreasing of the yeast survival rates with generally increasing time in all the treatments. Goodenough and Klein (1976) summarized the growth association relationship between yeast and lactic acid bacteria (LAB). They stated that LAB ferment about 35% of the lactose in milk through hydrolysis process to glucose and galactose. Only the glucose is changed into lactic acid while galactose moiety is released mainly by *Str. thermophilus* into the extracellular environment. The high concentration of galactose presence (about 1%) was the main reason for the growth of galactose positive and non–fermenting yeast in yoghurt. On the other hand, the pH values of the yeast incorporated cultures showed higher values than those detected in yoghurt starter cultures.

Lourens-Hattingh and Viljoen (2002) reported that when Candida yeast was grown with *St. Thermophilus* and *L. bulgaricus* it has induced synergistic peptonization fallowed by a gradual decrease in acidity.

**Table (1): Impact of growth association between yeast and yoghurt starter on their survival and pH development on yoghurt like-stored products at refrigerator temperature.**

| Storage period | Treatment 1 | | | Treatment 2 | | | Treatment 3 | | Treatment 4 | | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Log/cfu/ml | | | Log/cfu/ml | | | Log/cfu/ml | | Log/cfu/ml | | | | Log/cfu/ml | | |
| | S.th | L.b | pH | L.b | Y | pH | Y | pH | S.th | L.b | Y | pH | S.th | L.b | pH |
| 0 Time | 8.5 | 5.3 | 5.6 | 7.8 | 4.5 | 5.4 | 5.4 | 5.5 | 8.1 | 7.7 | 5.5 | 5.6 | 6.3 | 7.9 | 4.3 |
| 3 Days | 8.7 | 4.7 | 5.2 | 8.7 | 5.4 | 5.6 | 5.2 | 5.2 | 8.4 | 8 | 5.1 | 5.2 | 8.1 | 7.8 | 4.1 |
| 6 Days | 8.6 | 4.9 | 4.7 | 8.5 | 4.7 | 5.6 | 5 | 5 | 8.6 | 8.5 | 4 | 5.1 | 8.3 | 8.1 | 4.0 |
| 10 Days | 8.4 | 4 | 4.6 | 9.2 | 6.3 | 5.0 | 4.9 | 4.9 | 6.3 | 8.6 | 3.8 | 5.0 | 8.4 | 8 | 3.4 |
| 25 Days | 8 | 3.2 | 4.5 | 9 | 6 | 4.9 | 3.7 | 3.0 | 9.3 | 9 | 3 | 4.9 | 8.6 | 8 | 3.1 |

S.th: ***Streptococcus thermophelus***
L.b: ***Lactobacillus delbreuckii subsp bulgarcus***
Y: ***Saccharomyces cerevisiae***

## Growth association relationship between yeast, *B. bifidum* and *L. acidophilus*

Incorporation of yeast *Sac. cerevisiae*, *B. bifidum* and *L. acidophilus* for milk fermentation was investigated Table (2) shows the results obtained. Associative growth between *L. acidophilus* and yeast led to increase the growth of both organisms up to 10 days of incubation and then declined by the end of incubation. Similar results were obtained with the mixed culture of *B. bifidum* and yeast although *B. bifidum* growth was decreased after the sixth day of incubation. It could be noted as well that the growth association relationship between *L. acidophilus*, *B .bifidum* and yeast has induced higher growth of all the members of the mixed culture at least during the first ten days of incubation. However, *L. acidophilus* was not detected by the end of incubation. This may be attributed to depletion of some growth limiting factors perhaps consumed by the other two organisms or due to the accumulation of some inhibitory materials which made the organism not to survive.

Table (2): Impact of growth association between yeast, *L. acidophilus* and *B.bifidum* on their survival and pH development of biofermented milk stored at refrigerator.

| Storage period | 1 | | 2 | | 3 | | 4 | | | 5 | | | 6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *L.acidophilus* Log/cfu | | *B.bifidum* Log/cfu | | *Sac.cerervisiae* Log/cfu | | *L.acidophilus + Sac.cerevisiae* Log/cfu | | | *B.bifidum + Sac.cerevisiae* Log/cfu | | | *L.acidophilus+B.bifidu-m+ Sac.cerevisiae* Log/cfu | | | |
| | L.a | pH | B.b | pH | Y | pH | L.a | Y | pH | B.b | Y | pH | L.a | B.b | Y | pH |
| 0 Time | 9.2 | 5.3 | 9.2 | 4.9 | 9.2 | 5.2 | 8.9 | 3.2 | 5.0 | 7.4 | 3.2 | 4.5 | 6.2 | 8.8 | 3 | 4.5 |
| 3 Days | 9.4 | 5.2 | 8.6 | 4.5 | 6.6 | 5.1 | 9.5 | 5 | 5.0 | 8.8 | 4.9 | 4.6 | 9.4 | 9.1 | 6.6 | 4.6 |
| 6 Days | 9.1 | 5 | 8.6 | 4.4 | 4.6 | 4.9 | 9.2 | 5.5 | 4.8 | 8.9 | 4.7 | 4.5 | 9.8 | 9.4 | 6.1 | 4.6 |
| 10 Days | 8.9 | 4.9 | 9.2 | 4.3 | 6.4 | 4.4 | 9 | 8.1 | 4.4 | 7.0 | 5 | 4.5 | 8.9 | 9.4 | 5.7 | 4.6 |
| 25 Days | 7.3 | 4.5 | 8.7 | 4.0 | 5.8 | 4.2 | 6.4 | 6.1 | 4.0 | 4.1 | 3.3 | 4.1 | 0 | 7.3 | 4.4 | 4.5 |

1) *Lactobacillus acidophilus.* 4) *Lactobacillus* acidophilus + *Saccharomyces cerevisiae.*
2) *Bifidobacterium bifidum.* 5) *Bifidobacterium bifidum + Saccharomyces cerevisiae.*
3) *Saccharomyces cerevisiae* 6) *Lactobacillus acidophilus + Bifido + Saccharomyces cerevisiae*

The pH values of pure and mixed cultures showed no marked decrease and was expected as a result of the associative growth relationship between yeast and the lactic acid cultures. This result may be attributed to the possible preferable ability of the employed yeast strain to metabolise galactose derived from the break down of lactose rather than metabolizing organic acids produced by lactic acid bacteria. Furthermore the employed yeast strain exhibited decreasing of pH values in its pure culture by increasing the time of incubation which reflects its capability for acid production.

Thus the employed strain didn't contribute for acidity reduction under the described conditions. This is not agreed with that reported by Fleet and Main 1987 who reported the ability of many yeasts to metabolize lactic acid. However, Suriyarachchi and Fleet (1981) reported that although *Sac. boulardii* was able to utilize lactic acid as a carbon source which is unlikely the substrate could assimilated under the anaerobic conditions that exist in yogurts.

## Sensory evaluation:

### 1-Sensory evaluation of yoghurt like products.

The sensory data of fresh yoghurt like product are given in Table(3). It is obvious that the incorporation of yeast *Sac. cerevisiae,* with the yoghurt starter in milk had no adverse effects on the sensory properties of the resultant fermented milk. On the contrary the addition of yeast imparted a rich smooth taste with a good flavour, body texture and good appearance when compared with the traditional yoghurt (control). However the three other products gave lower scores compared with control. Thus the addition of yeast, *Sac. cerveisiae* to the yoghurt starter contributed for obtaining fermented milk product with a good sensoric quality.

**Table (3): Sensory evaluation of Fresh Yoghurt like product.**

| Starter used | Flavor (50) | Body and Texture (30) | Appearance (20) | Total (100) |
|---|---|---|---|---|
| Control | 41 | 24 | 18 | 83 |
| Treatment I | 48 | 25 | 19 | 92 |
| Treatment II | 35 | 20 | 17 | 72 |
| Treatment III | 40 | 22 | 16 | 78 |
| Treatment IV | 30 | 20 | 16 | 66 |

**Scores are in averages**

**2-Sensory evaluation of biofermented milk.**

Table (4) shows that the addition of yeast to the lactic acid cultures still contributing for obtaining good types of fermented milk, specially with *L. acidophilus* which attained maximum scores in flavour, body and texture and appearance even when compared with the acidophiius milk. The bifidus-yeast milk was also comparable to the bifidus milk. However the associated cultures of *L. acidaphilus, B. bifidum* and yeast gave a relatively lower score for the resultant fermented milk compared with the former described products. In general, all of the fermented milk products containing involved yeast exhibited a good body texture, good appearance and acceptable flavour. It was also noteworthy the absence of synopsis in these products till the end of storage period. Further more no alcoholic taste was detected by the panelists and no gas formation was observed. Similar results were reported by Lourens-Hattimgh and Viljoen (2001) who confirmed that the application of yeast species as probiotic microorganisms seems promising especially in milk and plain yoghurts since no gas or alcohol were formed.

**Table (4): Sensory evaluation of Fermented milk.**

| Starter used | Flavor (50) | Body and Texture (30) | Appearance (20) | Total (100) |
|---|---|---|---|---|
| L[a] | 45 | 24 | 17 | 86 |
| B[b] | 41 | 24 | 17 | 82 |
| Y[c] | 34 | 24 | 19 | 75 |
| L +Y[d] | 49 | 25 | 18 | 92 |
| B +Y[e] | 39 | 25 | 17 | 81 |
| L+B +Y[f] | 38 | 25 | 18 | 81 |

**a) L = *L. acidophilus.***
**b) B = *B. bifidum.***
**c) Y = yeast (*Sac. cerevisiae*).**
**d) L + y = *L. acidophilus* + yeast (*Sac. cerevisiae*)**
**e) B + y = *B. bifidum* + yeast (*Sac.cerevisiae*).**
**f) L +B + Y = (*L. acidophilus* +*B. bifidum* + *Sac. cerevisiae* )**
**Scores are in averages**

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## النمو المشترك لخميرة الساكرومايسس سيرفيسيا وبكتريا حامض اللاكتيك فى انتاج الآلبان المتخمرة

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تم دراسة العلاقة بين نمو الخميرة كمدعم حيوى لبكتريا حامض اللاكتيك .و تم تتبع نمو وبقاء كل من الخميرة وبكترياحامض اللاكتيك لمدة ثلاثة اسابيع كفترة تخزين مع تقدير الاعداد النوعية ودرجة الآس الهيدروجينى والخواص الحسية للتعرف على مدى اسهام الخميرة فى تحسين المنتج. وقد وجد أن الخميرة قادرة على النمو والبقاء فى الزبادى والمنتجات اللبنية المتخمرة الاخرى المحتوية على بكتريا الاسيدوفولس العصوية و البيفيدوبكتريا او مع كليهما كما اظهرت الخميرة كمزرعة نقية نموا جيدا فى اللبن بينما تناقص اعدادها بحده مع زيادة فترة التخزين.وقد وجد ان احتواء المنتجات اللبنية المتخمرة على الخميرة يشجع كل من بكتري حامض اللاكتيك والخميرة معا وقد قدرت اعدادها بمقدار٩،٨ & ٦،٦ لوغاريتمى/مل على الترتيب وقد زادت قيم الأس الهيدروجينى مع وجود الخميرة وبادىء الزبادى وبالعكس فقد تناقصت اعداد الخميرة فى المنتجات المحتوية على عصويات الاسيدوفلس والبيفيدوباكتريا او كليهما.
كما وجد ان النمو المشترك للخميرة وبكتريا حامض اللاكتيك قد اسهم كثيرا فى الحصول على آلبان متخمرة ذات خواص حسية جيدة.