

## Effect of protective culture on the growth of *Candida maltosa* YP1 in yoghurt

DENISA LIPTÁKOVÁ - LUBOMÍR VALÍK - BARBORA BAJÚSOVÁ

### Summary

Effect of commercial protective cultures consisting of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* subsp. *shermanii* on growth of resistant yeast contaminants *C. maltosa* YP1 was examined in fresh yoghurt products. The partial inhibition of growth of *C. maltosa* and its temperature dependence were observed. Adding of protective culture has no relevant effect on the lag time, however the temperature effect was significant. Growth rate of *C. maltosa* was significantly influenced by both factors protective culture and temperature of incubation as was described by the following Ratkowsky model  $\sqrt{Gr_p} = 0.0112T + 0.0373$  ( $R^2 = 0.9569$ ) for the trials with protective culture and  $\sqrt{Gr_c} = 0.0126T + 0.0489$  ( $R^2 = 0.9696$ ) for the control samples, respectively. Furthermore, the equations resulting from secondary models were used for predicting the time yeast contaminants need to reach the count of  $1.10^6$  CFU.ml<sup>-1</sup> in yoghurts at different storage temperatures. Predictions of this time were on the order of 8 d and 6 d longer in yoghurt containing protective culture at 6 and 8 °C, respectively. However our results confirmed the effects of protective culture on growth of yoghurt yeast contaminant, the application of protective culture in fermented dairy products cannot be considered a substitution for good hygiene or good manufacturing practice.

### Keywords

*Candida maltosa*; protective cultures; yoghurt; growth modelling

Yoghurt and yoghurt-based products belong to the favourite consumer dairy products because of their delightful taste, health-promoting effects and last but not least because of safety. Fermentation of milk with yoghurt bacteria *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* results in the reduction of lactose content, production of lactic acid, some organoleptic substances (e.g. acetaldehyde, diacetyl), small peptides, amino acids and fatty acids [1-6].

The acidity of the yoghurt products is equivalent to about 0.9% lactic acid, which corresponds to pH values from 4.2 to 4.6. Except for the yoghurt low pH value, fast growth of yoghurt bacteria in dairy environment and their high numbers are the main natural barriers for potential growth of contaminants. However, some of the yeasts and yeast-like organisms are able to grow at these unfavourable conditions; some of oxidative yeasts can even assimilate lactic acid. In consequence low pH is increased and the fermented product usually spoiled [7]. Growth of yeasts in dairy products may lead to inhibition of starter cultures, formation of

gas and off-flavours, and product discoloration [8]. The most prevalent and frequently isolated yeast strains represent the genera *Kluyveromyces*, *Debaryomyces*, *Yarrowia* and *Candida* [8-10]. LAUBSCHER and VILJOEN [11] reported resistance of dominant dairy associated yeasts to commercial sanitizers and cleaning agents. For example, the species *Debaryomyces hansenii*, *Candida versatilis*, *Torulaspora delbrueckii* and others showed strong resistance, even after 60 min of exposure of commercial sanitizers.

Numerous studies [12-17] link the increasing presence of yeasts and moulds in fermented dairy products to insufficient hygiene precautions before and during the production, failures in sanitation of equipment, air-contamination, insufficient heat treatment or inadequate microbiological quality of the supplements used. Therefore, yeast and moulds are considered efficient indicators of the level of good manufacturing and hygiene practices during production fermented dairy products [8].

In order to eliminate undesirable effects of microbial contamination on dairy products, many re-

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search studies promoted the idea of bioprotection by using various cultured microorganisms capable of producing specific inhibitory metabolites [18]. Preservative effect of lactic acid bacteria aimed e.g. at yeasts and moulds is primarily connected with the production of weak organic acids, especially lactic, acetic and propionic acids respectively. In addition, these bacteria also produce other inhibitory compounds such as hydrogen peroxide, diacetyl, acetoin, benzoic acid, formic acid and bacteriocins which enhance the effect of low pH against spoilage and action of pathogenic microorganisms, thus keeping them under control [19-21].

This work focuses on the effect of commercial protective culture consisting of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* subsp. *shermanii* on growth of oxidative yeast *Candida maltosa* YP1 inoculated into yoghurt. Another aim of the study was to predict time for *C. maltosa* YP1 to reach  $1.10^6$  CFU.ml<sup>-1</sup> in protected and control yoghurt samples.

## MATERIAL AND METHODS

### Microorganism

*Candida maltosa* YP1 was isolated as a contaminant from the surface of fruit yoghurt samples taken from several batches. Most probably they were contaminated via microfilter used for filtration of the air keeping gauge pressure in the fermentation tank. The morphological and biochemical characteristics of yeast strain were reported in our previous studies [7, 22].

### Substrate inoculation

The strain of *C. maltosa* YP1 was kept on Plate count agar (PCA, Imuna, Šarišské Michalany, Slovakia) at  $5 \pm 1$  °C. The suspension of the strain used for inoculation was prepared from 48h culture of *C. maltosa* YP1 grown at defined surface of nutrient agar in tubes by standard rinsing with sterile pepton water/saline water (0.85% NaCl and 0.1% peptone). The inoculum was applied into fresh yoghurt with or without commercial protective culture so that the initial cell density of *C. maltosa* YP1 reached initial numbers  $\leq 10^3$  CFU.ml<sup>-1</sup>. The trials were repeated twice in two or three parallel test runs at the temperatures of 6 °C, 8 °C, 12 °C, 18 °C and  $21 \pm 0.5$  °C respectively.

Fresh yoghurt bases were produced with yoghurt starter culture Yomix™ (Danisco, Brabrand, Denmark) consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. Yoghurt with protective culture and control samples were taken from the sub-cool-

ing tank in dairy, in which the fresh yoghurt was cooled to 22 °C after fermentation. The samples were transferred to the laboratory immediately.

Number of *C. maltosa* YP1 in inoculated yoghurt samples was determined similarly as in the yeasts by dilution method according to STN ISO 7954 [23].

### Fitting of growth curves, calculating and validation of growth parameters

Growth parameters were calculated using modelling technique of BARANYI et al. [24]. The dependence of growth parameters on temperature was modelled using Ratkowsky or simple linear mathematical equations.

To compare mathematical models to each other as well as with experimental results, the accuracy factor and "percent of discrepancy" were calculated according to BARANYI et al. [25]:

$$A_f = \exp \left( \sqrt{\frac{\sum_{k=1}^m (\ln f(x^k) - \ln \mu^k)^2}{n}} \right)$$

$$\% D_f = (A_f - 1) \cdot 100$$

where  $\mu$  = specific growth rate obtained from growth curve,  $f(x^k)$  = specific growth rate calculated from the equations describing experimental values,  $n$  = number of measurements,  $\% D_f$  = percent of discrepancy.

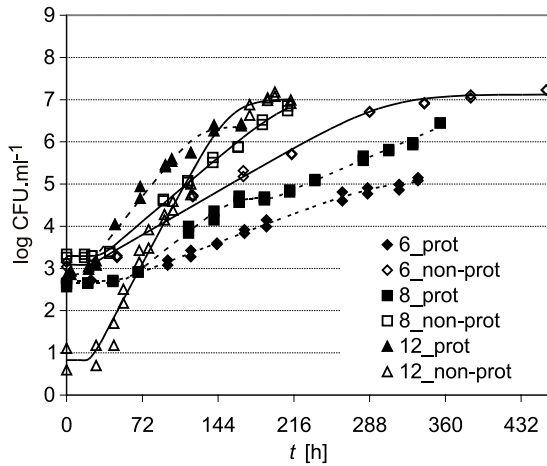
## RESULTS AND DISCUSSION

Growth dynamics of *Candida maltosa* YP1 in yoghurt with protective culture and control yoghurts

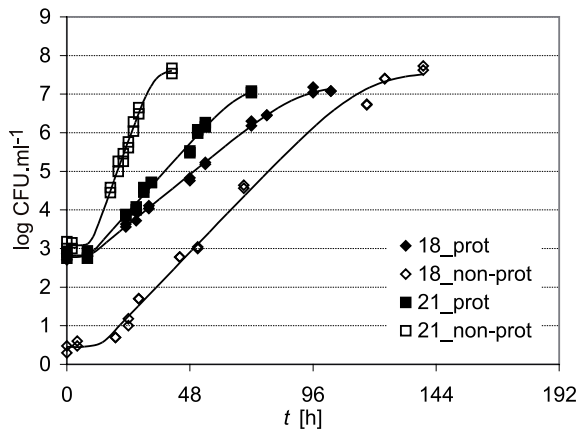
Growth dynamics of *C. maltosa* YP1 during the challenge tests with or without protective culture was dependent on storage temperature (Fig. 1 and 2).

In control yoghurts without protective culture, maximal lag time of *C. maltosa* 26.7 h and minimal growth rate ( $Gr_c = 0.014$  h<sup>-1</sup>,  $GT = 21.5$  h) were found at  $6 \pm 0.5$  °C. At 21 °C, the tested yeast strain reached maximum density with the lag time about 9 h and growth rate increased to 0.185 h<sup>-1</sup>.

Growth data plotted in Fig. 1 and 2 confirmed the expected effect of commercial protective culture on growth curves of *Candida maltosa*. Lag time was prolonged and growth rates decreased especially at low temperature region from 12 °C to 6 °C. For example, lag time of *C. maltosa* found



**Fig. 1.** Growth curves of *Candida maltosa* YP1 in yoghurts with/without protective culture at the temperature of 6, 8, and 12 ± 0.5 °C.



**Fig. 2.** Growth curves of *Candida maltosa* YP1 in yoghurts with/without protective culture at 18 °C and 21 ± 0.5 °C.

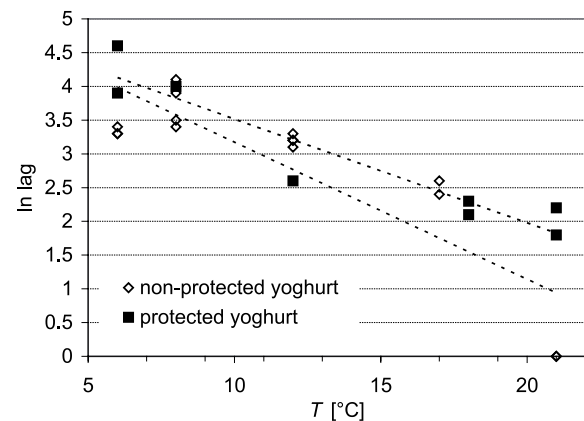
in protected yoghurt at 6 was almost twice as long (48.8 h) than in control tests without the protective culture. Its growth rate at 6 °C of 0.0096 h<sup>-1</sup> that was equivalent to  $GT = 31.5$  h was about 31.4% slower than in control yoghurt ( $Gr_c = 0.014$ h<sup>-1</sup>). With increasing storage temperature the growth rates of yeast strain naturally increased. However, maximal growth rate  $Gr_{Pc} = 0.0746$  h<sup>-1</sup> determined at 21 °C was about 2.5 times slower than in control yoghurts kept at the same temperature ( $Gr_c = 0.185$  h<sup>-1</sup>).

The effect of protective cultures added to the activity of yoghurt bacteria can be similarly explained by antimicrobial effect of weak organic acids that in combination with lactic acid is generally associated with diffusion of undissociated organic acid molecule through the plasma membrane

into the neutral cytoplasm. Once inside the microbial cell, the process of acid dissociation results in intracellular acidification and accumulation of the acid anion. On the other hand, mechanisms of yeast resistance to weak acids include decreased access of the acid to the cell via improved ability to extrude H<sup>+</sup> protons, conversion of the preservative into an innocuous compound and inducible extrusion of the anion [26-28]. Moreover, competitiveness of the cultures of lactic acid bacteria is also determined by their adaptation to a substrate as well as by intrinsic and extrinsic environmental factors. Antagonism referred to inhibition of undesirable microflora is mostly concerned with production of primary and secondary antimicrobial metabolites by lactic acid bacteria [29].

### Secondary mathematical modelling and validation of growth parameters

The effect of storage temperature on lag time and growth rates of *C. maltosa* YP1 was described applying secondary mathematical models (Fig. 3 and Fig. 4). The results showed that adding of protective culture had no relevant effect on lag time, although the temperature effect was significant. Lag time is generally influenced by many factors associated with the history of growth of substrates that in our case were various batches of yoghurt, and also with history of *C. maltosa* inoculum and added protective culture. In spite of this fact, lag time of tested yeast strain in yoghurts with protection culture ( $\ln\lambda_{Pc}$ ) and control yoghurt ( $\ln\lambda_c$ ) could be described by the linear equations  $\ln\lambda_{Pc} = -0.1537T + 5.0508$  ( $R^2_{Pc} = 0.892$ ) and  $\ln\lambda_c = -0.2031T + 5.2031$  ( $R^2_c = 0.7457$ ), respectively. The growth rate of *C. maltosa* was significantly influenced by both protective culture and temperature of incubation and described by the following



**Fig. 3.** Effect of temperature and protective culture on the lag time of *Candida maltosa* YP1.

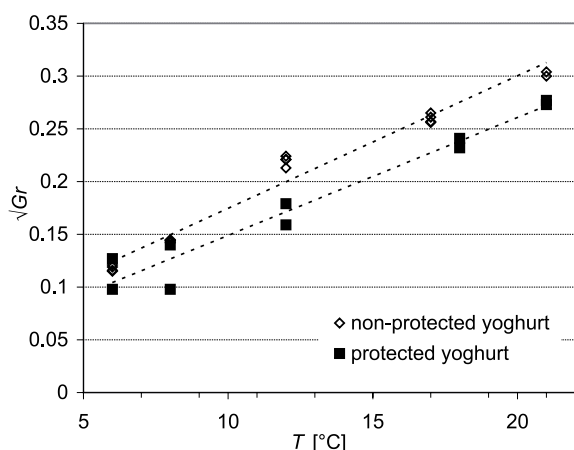


Fig. 4. Effect of temperature and protective culture on growth rate of *Candida maltosa* YP1 in yoghurt.

Ratkowsky linear equations as dependence on storage temperature:

$$\sqrt{Gr}_{pc} = 0.0112T + 0.0373 \quad (R^2_{pc} = 0.9569)$$

$$\sqrt{Gr}_c = 0.0126T + 0.0489 \quad (R^2_c = 0.9696)$$

The observed growth rates of *C. maltosa* YP1 in protected yoghurts calculated from the experimental growth curves of *C. maltosa* YP1 using D-model [28] and the growth rates calculated using Ratkowsky model were validated according to BARANYI *et al.* [25]. The discrepancies between the observed growth rates and those calculated from the square root model were 25.4%, with the accuracy factor of 1.25. Similar discrepancies were mentioned by Pin and Baranyi [30] and observed also in our earlier study [31].

The prediction of time for *C. maltosa* YP1 to reach relevant density in yoghurts may represent valuable information from the point of quality control. Results obtained from the secondary mathematical modelling were used for calculation

of the prediction of time to reach  $1.10^6$  CFU.ml<sup>-1</sup> of *C. maltosa* in yoghurts at different storage temperature (Tab. 1). This time was about 8 to 4 days longer in yoghurt containing protective culture at 6 °C and 8°C than in control yoghurt samples respectively.

## CONCLUSION

*C. maltosa* YP1 as one of the yeast contaminants of dairy products showed the ability to compete with lactic acid bacteria starters, even with some protective cultures in yoghurt. This kind of mutual microbial relations will need quantification based on predictive microbiology approach not only within food research but also in food practice where both safety or quality point of view play the most important role.

The growth dynamics of *C. maltosa* was different in yoghurts containing commercial protective cultures than it was in the control product. However, protective culture consisting of *L. rhamnosus* and *Propionibacterium freudenreichii* subsp. *shermanii* caused partial inhibition of the growth of highly resistant yeast strain studied in this work, this tools should not substitute good hygiene or manufacturing practice.

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Tab. 1. Prediction of time required to reach  $1.10^6$  CFU.ml<sup>-1</sup> of *C. maltosa* YP1 in yoghurts with and without protective culture and initial density of  $N_0 = 1$  CFU.ml<sup>-1</sup>.

Temperature [°C]	Time prediction [d] to reach $1.10^6$ CFU.ml <sup>-1</sup> in yoghurts	
	with protective culture	without protective culture (control yoghurts)
6	25	17
8	17	13
12	9	8
18	5	4
21	4	2



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