Characterisation of *Lactobacillus rhamnosus* VT1 and Its Effect on the Growth of *Candida maltosa* YP1

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Abstract

Liptáková D., Valík Ľ., Lauková A., Strompfová V. (2007): **Characterisation of** *Lactobacillus rhamnosus* **VT1 and its effect on the growth of** *Candida maltosa* **YP1.** Czech J. Food Sci., **25**: 272–282.

The combined effect of initial amount of 18 h *L. rhamnosus* VT1 inoculum and incubation temperature on the growth of *Candida maltosa* YP1, an oxidative food spoilage yeast strain, was primarily modelled and studied by standard response surface methodology. This study resulted in the following linear regression equations characterising lag time and growth rate of *C. maltosa* YP1 in milk in competition with the potentially protective lactobacillus strain. Lag-phase of *C. maltosa* was strongly influenced by the amount of lactobacillus inoculum (V_0) and incubation temperature ($1/T$). The synergic effect of both these factors was also evident as results from the equation lag = $-33.50 + 186.38 \times V_0 \times 1/T + 512.27 \times 1/T - 5.511 \times V_0$ ($R^2_{(\lambda)} = 0.849$). The growth rate was sufficiently described by the linear relation: $Gr_{Cm} = -0.00046 + 0.0033 \times T - 0.0016 \times V_0$ ($R^2_{(Gr)} = 0.847$). On the basis of these equations, the mutual microbial interactions and the potential application of the lactobacillus strains to food protection are discussed.

Keywords: *Candida maltosa* YP1; *Lactobacillus rhamnosus* VT1; mathematical modelling

Microbial spoilage of milk and other pH neutral dairy products is generally associated with the growth of bacteria. However, yeasts possess the ability to grow under conditions unfavourable to many bacteria, and they also play a role as spoilage organisms, e.g. in fermented milks, yoghurts, cheeses, and beverages (Viljoen & Greyling 1995; Jakobsen & Narvhus 1996; Consentino *et al.* 2001). The most prevalent yeast strains frequently isolated from dairy products are representatives of the genera *Kluyveromyces*, *Debaryomyces*, *Yarrowia*, and *Candida* (Welthagen & Viljoen 1998, 1999; Viljoen 2001). Laubscher and Viljoen (1999) reported on the resistance of the dominant dairy-associated yeasts to commercial sanitisers and cleaning compounds. Yeasts such as *Debaryomyces hansenii*, *Candida versatilis*, *Torulaspora delbrueckii*, and other showed a strong surviving resistance, even after 60 min of exposure.

Numerous studies (Roostita & Fleet 1996; Gadaga *et al.* 2000, 2001; Abdelgadir *et al.* 2001; Corbo *et al.* 2001) link the increasing presence of yeasts and moulds in fermented dairy products to insufficient hygiene during production, sanitation of the equipment, air-contamination, insufficient heat treatment, or the inadequate microbiologi-

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cal quality of the supplements used. Therefore, yeasts and moulds can be considered as efficient indicators of the level of good manufacturing or hygienic practices during the production of these fermented products (Viljoen 2001).

The interest in biological preservation methods as a significant tool in the ensuring of microbiological safety of foods has increased over the past decade. Many research studies have supported the idea of using antagonistic microorganisms and their metabolites to slow down or inhibit the growth dynamics of undesirable microorganisms, bacteria, yeasts, and toxinogenic fungi in food (Schillinger *et al*. 1996). The preservative effect of lactic acid bacteria against a wide spectrum of undesirable microorganisms is primarily due to the production of weak organic acids, especially lactic and acetic. Other secondary lactic acid bacteria metabolites such as hydrogen peroxide, diacetyl, acetoin, benzoic, formic, and other acids and bacteriocins act in the control of the growth and multiplication of spoilage and pathogenic microorganisms synergistically with the decrease of pH (Helander *et al*. 1997; Caplice & Fitzgerald 1999).

Candida maltosa is oxidative, hydrocarbon-utilising, imperfect yeast widespread in the environment (Kasüske *et al*. 1992; Slávikova & Vadkertiová 1997). This yeast species is well-known due to its inducible cycloheximide resistance, killer activity against other yeast species, pseudohyphal growth in response to environmental conditions, biofilm formation, alkanes and short chain fatty acids assimilation (Mutoh *et al*. 1995; Nakazawa *et al*. 1998; Schmitz *et al*. 2000; Buzzini & Martini 2001; Jirků *et al*. 2001). Lauková and Valík (2003) pointed out that *C. maltosa* contaminated yoghurt products through the fermentation tank filters used for air sterilisation. Improper servicing of the filter, longer usage than recommended by the producer, enabled the yeast to colonise the filter and survive its treatment with an unspecified mixture of steam and hot water. The heat resistance of *Candida maltosa* YP1 tested in this work in comparison with *Saccharomyces* sp. was studied within the study by Lauková *et al*. (2003a).

Despite the sound potential of predictive microbiology as a tool for the prediction of microbial behaviour in foods, few studies have been devoted to the interaction between yeasts and lactic acid bacteria during improper storage of dairy products (Viljoen 2001; Viljoen *et al*. 2003). More studies are also needed on the quantitative evaluation of the possibilities of suppressing the growth of yeasts by some lactic acid bacteria.

This work was focused on the characterisation of the strain *Lactobacillus rhamnosus* VT1 and the effect of its various initial concentrations on the growth of oxidative yeast *Candida maltosa* YP1 in co-culture.

MATERIAL AND METHODS

Microorganisms. *Candida maltosa* YP1 was isolated as a contaminant from the fruit yoghurts and subsequently from the tested microbiological filter used for the filtration of the air in the fermentation tank. The strain was identified fenotypically by E. Slávikova from the Culture Collection of Yeasts (Slovak Academy of Sciences, Bratislava, Slovak Republic).

Lactobacillus rhamnosus VT1 was isolated from tartar sauce and identified at the Department of Dairy and Fat Technology, Institute of Chemical Technology in Prague, Czech Republic, and kindly provided by M. Plocková. Its antifungal activity was described by Plocková *et al*. (2001). To gain more information on the potential probiotic character of the strain, the following tests were performed: its sensitivity to antibiotics, lactic acid production, survival of VT1 strain under low pH, tolerance of VT1 strain to oxgall-bile, and its adhesion ability to human mucus.

Antibiotic testing and lactic acid production. The testing was provided by the standard diffusing agar technique on Mueller-Hinton agar (Merck, Darmstadt, Germany) with the use of the following antibiotic discs supplied by Becton and Dickinson (Cockeysville, USA): kanamycin – KAN, vancomycin – VAN, rifampicin – RIF, chloramphenicol – CHC, tetracycline-TCT (30 μg), streptomycin – STM (50 μg), erythromycin (15 μg), ampicillin – AMP, gentamycin-GEN (10 μg), neomycin – NEO (5 μg). These antibiotics are commonly used against gram-positive bacteria. The inhibitory zones were calculated according to manufacturers' instructions. *Lactobacillus rhamnosus* GG strain (ATCC 53103, Arthur Ouwehand, Danisco, Finland) was used as the positive control. The production of lactic acid was determined according to Pryce (1969).

Survival of** L. rhamnosus **VT1 strain under low pH and its tolerance to bile salts. Cells sus-

pension of the 18 h culture of VT1 strain in de Mann-Rogosa-Sharpe broth (MRS, Oxoid, Basingstoke, Hampshire, UK) was checked in an acidic environment up to pH 3. The amount of surviving cells on MRS agar was expressed in colony forming units (CFU) per ml.

Tolerance to bile salts was checked according to Gilliland and Walker using oxgall-bile (Becton and Dickonson, Mayle, France). A 5% concentration of oxgall-bile was used in the MRS broth inoculated with the strain tested. The viable cell count was enumerated at time 0 and after 24 h of the growth at 37°C (Gilliland & Walker 1990).

Adhesion ability of VT1 strain to human mucus. Adhesion ability to human colonic mucus was studied and performed by Satu Vesterlund according to Ouwehand *et al.* (1999, 2002). The use of human intestinal material had been approved by the joint ethical committee.

Inoculation and cultivation conditions. The strain of *C. maltosa* YP1 was kept on Plate count agar (PCA, Imuna, Šarišské Michaľany, Slovak Republic) at 5 ± 1°C. The standard suspension of the strain was prepared from 48 h culture of *C. maltosa* YP1 grown on the defined surface of agar in tubes by standard rinsing with sterile pepton water/saline water and used in the individual experiments for inoculation of ultra-pasteurised milk samples with an initial cell density of *C. maltosa* YP1 less than 10^3 CFU/ml. *L. rhamnosus* VT1 was cultivated at 37°C for 18 h in MRS broth (Oxoid, Basingstoke, Hampshire, UK). This 18 h culture was used as a co-culture with milk samples inoculated with the yeast strain. Its concentration ranged from 1.0 % to 15.0% (v/v). The experiments were repeated twice in duplicates or triplicates, respectively.

Number of C. maltosa YP1 in milk. The actual counts of *C. maltosa* YP1 in parallel ultra-pasteurised milk samples (Rajo, Bratislava, Slovak Republic) inoculated with an increasing concentration of *L. rhamnosus* VT1 were determined on the medium with chloramphenicol according to Slovak Technical Standard STN ISO 7954 (1997) in order to gain sound growth curves.

Determination of titratable acidity. Titratable acidity was determined using NaOH solution (c_{NaOH} = 0.25 mol/l) and phenolphthalein as the indicator. The results were expressed as percentage of lactic acid (w/v). Acidity was determined at regular intervals equally as microbiological examinations so that lactic acid production was also involved in the microbial growth evaluation.

Fitting the growth curves and calculating the growth parameters. The growth parameters of the strain *C. maltosa* YP1 in inoculated ultra-pasteurised milks incubated at the temperatures of 8, 10, 17, and 21 ± 0.5°C were calculated as a function of time in relation to the different lactobacilli concentrations, using the modelling technique of Baranyi *et al.* (1993). The dependence of the growth parameters on temperature and initial *L. rhamnosus* VT1 density was studied by the standard response surface methodology of the Statistica 7.1 software package.

Validation of the growth parameters. To validate the mathematical equations describing yeast strain responses to various lactobacillus inocula, the accuracy and discrepancy factors were calculated as defined by Baranyi *et al.* (1999):

$$A_f = \exp\left(\sqrt{\frac{\sum_{k=1}^{m}(\mathrm{Ln}f(Gr^k) - \mathrm{Ln}Gr^k)^2}{n}}\right) \quad (1)$$

$$\%D_f = (A_f - 1) \times 100 \quad (2)$$

where:

Gr – growth rate obtained from the growth curve

$f(Gr^k)$ – growth rate calculated from the equations describing experimental values

n – number of measurements

A_f – accuracy factor

$\%D_f$ – per cent discrepancy

Standard error of prediction (SEP) was calculated by Zurerra-Cosano *et al.* (2006):

$$\%\mathrm{SEP} = \frac{100}{Gr_{\text{mean obs}}} \sqrt{\frac{\Sigma\,(Gr_{\text{obs}} - Gr_{\text{pred}})^2}{n}} \quad (3)$$

RESULTS AND DISCUSSION

Characterisation of the strains

Lactobacillus rhamnosus VT1 was most sensitive to the vancomycin with inhibitory zones of +26 mm, rifampicin (+27 mm), chloramphenicol (+32 mm), tetracycline (+35 mm), and ampicillin (+37 mm), respectively. Depleted antibiotic sensitivity of *L. rhamnosus* VT1 was determined against erythromycin (inhibitory zones +15 mm) and streptomycin (+10 mm). The VT1 strain tested was kanamycin, gentamycin, and neomycin resistant. It was able to survive in an acidic environment up to pH 3 (after 180 min. exposure to pH 3 survive

94% of VT1 cells surviving) and 5% concentration of oxgall; its cell number after 24 h exposure decreased from 9.1 × 10^8 CFU/ml to 3.5 × 10^6 CFU per ml. The adhesion ability of *L. rhamnosus* VT1 to mucus was lower (0.49%) in comparison with the adhesion ability of the commercial probiotic strain *L. rhamnosus* GG (10.7%).

Viability of *L. rhamnosus* VT1 at 6°C did not change in ultra-pasteurised milk over 32 days. The growth was observed already at 8°C with the rate of 0.011 per hour and the corresponding time to double T_d = 27.4 h (Table 1); however, the lag time period was 202 h (8.4 days). The growth rate increased eight times to the value of 0.089 per hour and no lag time was observed when lactobacillus was incubated in milk at 24°C.

Growth dynamics of *Candida maltosa* YP1 in milk

The growth curves of *C. maltosa* YP1 as a single culture related to the incubation temperature of three inoculated samples of ultra-pasteurised milk is demonstrated in Figure 1. Generally, as it was expected, the lag time was reduced and the growth rate increased with increased incubation temperature. The maximal average lag time (86.4 h) and minimal growth rate of 0.0115 $\log_{10}$ CFU per hour (T_d = 26.2 h) were found at the lowest temperature of 6 ± 0.5°C used in the trials. Maximal growth parameters were observed at 25°C, which meant that the average lag time was 6.3 h, and the growth rate increased 20 times to 0.23 per hour.

The effect of incubation temperature on lag time and the growth rates of *C. maltosa* YP1 was analysed secondarily by transforming the lag values to their natural logarithm, which enabled its linearisation to the equation as follows: ln lag = $-0.1263T + 4.8525$ (R^2 = 0.9499, Figure 2). The growth rates calculated from the parallel growth curves using the D-model (Baranyi *et al.* 1993) were analysed according to the Ratkowsky square root model (Ratkowsky *et al.* 1982) with a highly significant result: $\sqrt{Gr} = 0.0186T + 0.0139$ (R^2 = 0.9914, Figure 3). As a result of validation, an accuracy factor of 1.16 was estimated, which according to Baranyi *et al.* (1999) resulted in 15.5% of the discrepancy between the model and the growth rates observed. Similar discrepancies were mentioned by Pin and Baranyi (1999) and observed also in our previous studies (Lauková *et al.* 2003b; Lauková & Valík 2003, 2004).

Table 1. Average values of the growth parameters of *Lactobacillus rhamnosus* VT1 in ultra-pasteurised milk at temperatures 8, 10, 15, 17, 21, and 24°C

T (°C)	Gr ($\log_{10}$ CFU/h)	λ (h)
8	0.011	202.0
10	0.022	186.0
15	0.029	13.7
17	0.05	14.7
21	0.085	3.9
24	0.089	0

C. maltosa YP1 in co-culture with *L. rhamnosus* VT1

In order to get the yeast growth under control, further work was focused on the growth of *Can-*

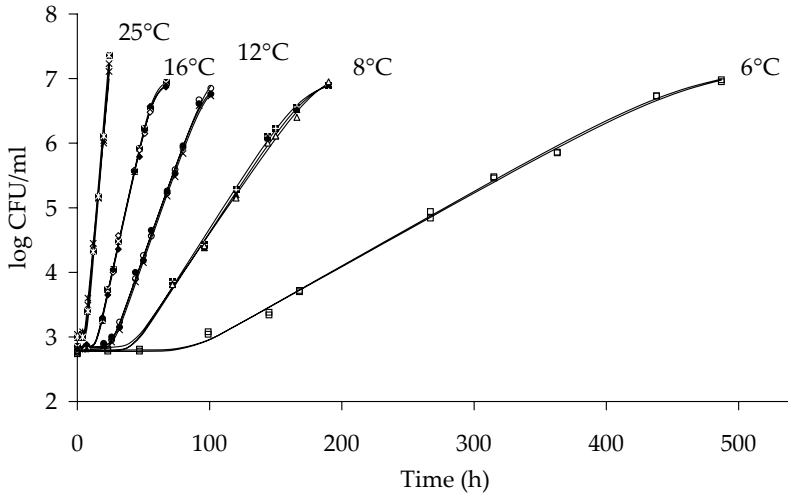


Figure 1. Growth of *Candida maltosa* YP1 in milk at 6, 8, 12, 16, and 25°C

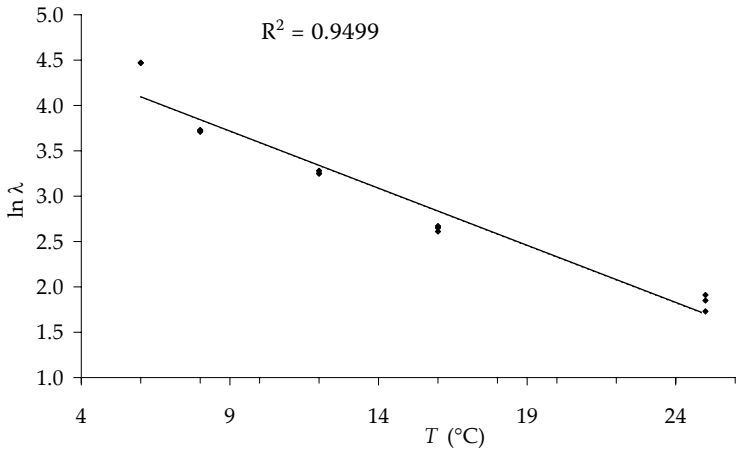


Figure 2. Lag-time (natural logarithm) of *C. maltosa* YP1 in milk versus temperature

dida maltosa as influenced by the increase of the initial *L. rhamnosus* VT1 concentration ranging from 1.0 to 15.0% (v/v). The kinetic parameters as the growth rate (*Gr*) and lag-time calculated from the growth data fitted by Baranyi and Roberts (1994) mathematical model are summarised in Table 2. To determine which of the variables has the significant influence on the growth of *C. maltosa*, multifactorial linear regression was carried out using Essential Regression Software Package by Steppan *et al.* (1998). The results of the comparison are presented in Table 3. Based on the statistical and validation parameters, taking the simplicity into account, two factorial linear equations can be preferred in order to plot the influence of *L. rhamnosus* and temperature on the behaviour of *C. maltosa* during their mutual growth in milk. For lag-phase duration and the growth rate, the following equations:

$$\text{lag} = -33.50 + 186.38 \times T^{-1} \times V_0 + 512.27 \times T^{-1} - 5.511 \times V_0 \quad (R^2 = 0.849) \quad (4)$$

$$Gr = 0.000073 + 0.0032 \times T - 0.00137 \times V_0 \quad (R^2 = 0.887) \quad (5)$$

represent the data in Figures 4 and 5.

The equations in Table 3 show that, apart from the temperature, the initial volumes of inoculum and initial lactic acid concentration were the most relevant factors for the growth description of the yeast in the three factor and six parameter Eq. 1. However, the other simple equations also include the initial lactic acid concentration (LA_{in}, e.g. Eq. 2) or the effect of lactic acid production (LA_{rate}, Eq. 2), but the natural logarithm transformation of the growth rate used in Eq. 1 generally homogenised the variance of residuals. This transformation showed the best statistical parameters as %SEP = 5.24, B_f = 1.00, and A_f = 1.04. However, the accuracy of each of the response surface models was influenced by the fact that *C. maltosa* was able to assimilate lactic acid and, on the other hand, the lactobacillus strain could produce other compounds with inhibitory effects as reported by Plocková *et al.*

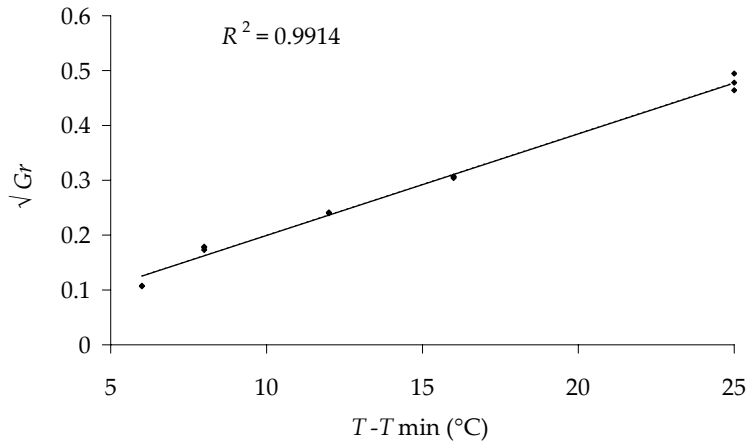


Figure 3. Square root growth rate of *C. maltosa* YP1 in milk versus temperature

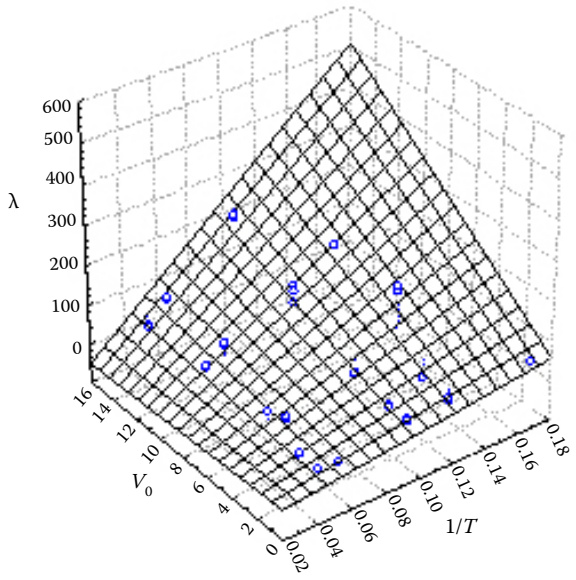
Table 2. Values of observed and predicted growth rate (*Gr*) and lag-time (λ) by linear regression model of *Lactobacillus rhamnosus* VT1 in ultra-pasteurised milk

T (°C)	V_0 (% v/v)	*Gr* ($\log_{10}$ CFU/h)		λ (h)		T (°C)	V_0 (% v/v)	*Gr* ($\log_{10}$ CFU/h)		λ (h)	
		OBS	PRED	OBS	PRED			OBS	PRED	OBS	PRED
21	15	0.045	0.047	23.9	41.4	10	15	0.014	0.012	222.3	214.6
21	15	0.039	0.047	18.9	41.4	10	15	0.013	0.012	213.5	214.6
21	15	0.042	0.047	20.6	41.4	10	15	0.013	0.012	218.1	214.6
21	10	0.051	0.054	35.6	24.5	10	10	0.010	0.018	151.8	149.0
21	10	0.055	0.054	40.2	24.5	10	10	0.009	0.018	109.7	149.0
21	10	0.053	0.054	38.1	24.5	10	10	0.010	0.018	136.7	149.0
21	5	0.058	0.060	42.3	7.7	10	5	0.021	0.025	49.1	83.4
21	5	0.058	0.060	44.9	7.7	10	5	0.019	0.025	39.9	83.4
21	5	0.058	0.060	43.7	7.7	10	5	0.020	0.025	44.7	83.4
21	2.5	0.053	0.064	1.2	–0.7	10	2.5	0.027	0.029	27.1	50.6
21	2.5	0.054	0.064	5.5	–0.7	10	2.5	0.027	0.029	23.4	50.6
21	2.5	0.053	0.064	3.3	–0.7	10	2.5	0.027	0.029	25.2	50.6
21	1	0.065	0.066	0.1	–5.7	10	1	0.027	0.031	25.2	30.9
21	1	0.065	0.066	0.1	–5.7	10	1	0.028	0.031	24.2	30.9
21	1	0.065	0.066	0.1	–5.7	10	1	0.028	0.031	24.7	30.9
17	15	0.041	0.034	73.1	78.4	8	1	0.030	0.024	30.4	48.3
17	15	0.043	0.034	76.4	78.4	8	1	0.033	0.024	31.1	48.3
17	10	0.047	0.041	75.3	51.2	8	1	0.032	0.024	30.9	48.3
17	10	0.046	0.041	72.9	51.2	8	3	0.029	0.022	40.2	83.9
17	10	0.046	0.041	74.2	51.2	8	3	0.028	0.022	36.1	83.9
17	5	0.055	0.048	11.2	23.9	8	5	0.015	0.019	206.3	119.5
17	5	0.052	0.048	5.5	23.9	8	5	0.016	0.019	221.4	119.5
17	5	0.053	0.048	8.1	23.9	8	10	0.014	0.012	213.9	208.4
17	1	0.065	0.053	0.4	2.1	8	10	0.014	0.012	209.6	208.4
17	1	0.063	0.053	0.1	2.1	8	10	0.014	0.012	211.7	208.4
17	1	0.072	0.053	0.1	2.1	6	0	0.013	0.019	76.7	51.9
						6	0	0.013	0.019	76.4	51.9
						6	0	0.013	0.019	76.6	51.9

(2001). Our results showed that in specific cases for practical purposes, the influence of bacterium with potential inhibitory effects might be described also with such simple and "rough" factors as are the initial volume of lactobacillus inoculum or lactic acid concentration.

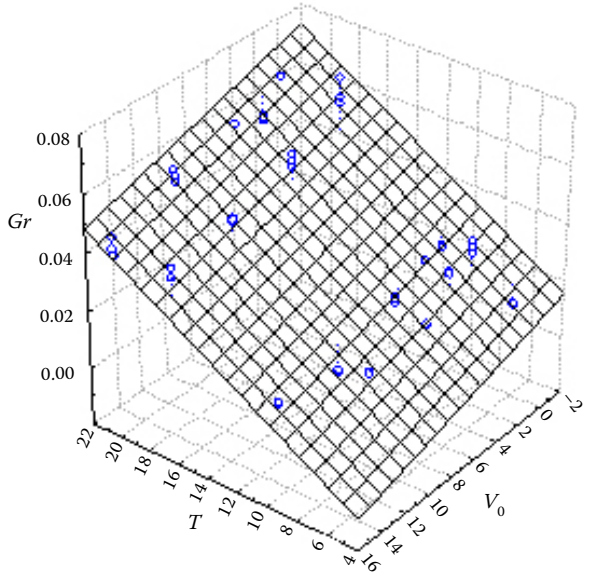
Validation of the response surface models

The validation of the response surface models (RSM) was performed in accordance with Baranyi *et al.* (1999). For internal validation, Zurera-Cosano *et al.* (2006) used the % SEP parameter that, within their response surface model of *Leuconostoc mesenteroides* (data measured by optical density) in aerobic and anaerobic conditions, ranged from 6.58 to 27.63. Naturally, the higher values of SEP were related with the prediction of lag time. Baranyi *et al.* (1999), proceeding on their previous works, reported values of standard errors in the range of 5–15%. The SEP of the presented RSM for the growth rate of *Candida*



$$\lambda = -33.5 + 186.38 \times V_0 \times T^{-1} + 512.27 \times T^{-1} - 5.511 \times V_0$$
$$(R^2 = 0.849)$$

Figure 4. Lag-time of *C. maltosa* YP1 in milk as dependence of initial *L. rhamnosus* VT1 density and storage temperature



$$Gr = 0.000073 + 0.0032 \times T - 0.00137 \times V_0$$
$$(R^2 = 0.887)$$

Figure 5. Growth rate of *C. maltosa* YP1 in milk as dependence of initial *L. rhamnosus* VT1 density and storage temperature

maltosa in co-existence with *L. rhamnosus* of various initial concentrations was comparable to those published by Zurerra-Cosano *et al.* (2006). RSM for lag time of *C. maltosa* provided worse results in terms of % SEP.

The growth rate RSM provided B_f and A_f values close to unity, which indicates a good fit between the predictions. An exceptional case in validation was the lag time model, which could be explained by a variability of the physiological states of both

Table 3. Results of comparison and validation for response surface model of *Candida maltosa* as influenced with the inoculum and growth of *Lactobacillus rhamnosus* in milk

	Response surface models	R^2	RMSE	%SEP	B_f	A_f	SS
1	$\text{Ln } Gr = -5.219 + 0.149 \times T - 0.197 \times V_0 + 6.42 \times \times LA_{in} + 0.011 \times T \times V_0 - 0.475 \times T \times LA_{in}$	0.910	0.183	5.24	1.00	1.04	18.25
2	$\text{Ln } Gr = -1.063 - 8.944 \times V_0 \times T^{-2} - 0.333 \times \ln LA_{rate}$	0.866	0.216	6.29	1.00	1.05	2.385
3	$Gr = -0.0097 + 0.0043 \times T - 0.0041 \times T \times LA_{in}$	0.842	0.007	20.52	1.04	1.23	0.003
4	$\text{lag} = -33.50 + 186.38 \times T^{-1} \times V_0 + 512.27 \times T^{-1} - - 5.511 \times V_0$	0.849	27.226	43.40	1.38	2.00	233 070
5	$Gr = 0.000073 + 0.0032 \times T - 0.00137 \times V_0$	0.887	0.006	17.33	1.03	1.20	0.002
6	$Gr = -0.0302 + 0.0086 \times T - 0.0025 \times V_0 - 0.0002 \times \times T^2 + 6.5\text{E-}0.5 \times V_0^2$	0.917	0.005	14.87	1.01	1.19	0.002
7	$Gr = -0.0372 + 0.0097 \times T - 0.003 \times V_0 - 0.0002 \times \times T^2 + 0.071 \times LA_{in}^2 + 6.7\text{E-}5 \times V_0^2$	0.925	0.005	14.17	1.00	1.18	0.017

T – temperature, V_0 – volume of *L. rhamnosus* inoculum, LA_{in} – initial lactic acid concentration (% w/v), LA_{rate} – rate of *LA* production, RMSE – root mean square error, %SEP – standard error of model prediction, B_f – bias factor, A_f – accuracy factor, SS – sum of square

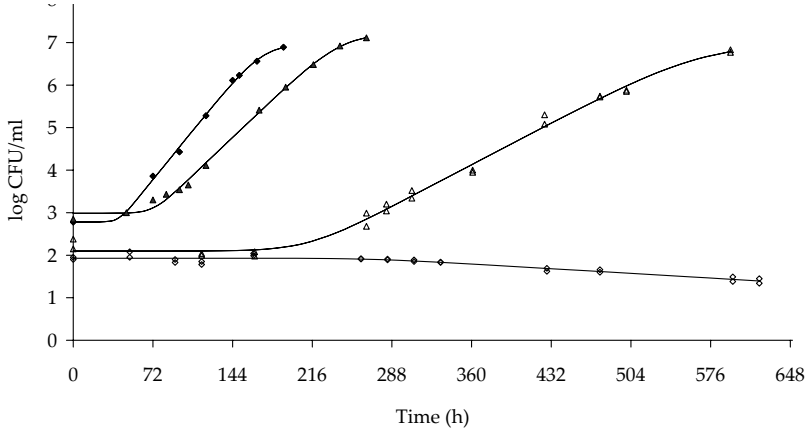


Figure 6. Comparison of growth dynamics of *C. maltosa* YP1 in ultra-pasteurised milk with or without *L. rhamnosus* VT1 (10%) and yeast extract broth with glucose, 0.3% lactic acid and 0.3% pyroglutamic acid

◆ *C. maltosa*
▲ *C. maltosa* + 0.3% *LA*
△ *C. maltosa* + 10% *L. rhamnosus*
◇ *C. maltosa* + 0.3% PCA

bacterial and yeast populations within the experiments.

Mutual behaviour of LAB and spoilage organisms

The co-existence of lactic acid bacteria and spoilage microorganisms is determined by their adaptation to the substrate and by a number of intrinsic and extrinsic factors including redox potential, water activity, pH, and temperature. Antagonism refers to the inhibition of undesirable microflora caused by competition for nutrients and by the production of anti-microbial metabolites (Holzapfel *et al.* 1995). The experimental results in our study refer to the fact that the antagonistic relationship between *L. rhamnosus* VT1 and *C. maltosa* YP1 was not only the consequence of the primary product of metabolism-lactic acid and pH-value, but it was also influenced by other secondary substances. The anti-microbial effect of weak organic acids is generally connected with the diffusion of the undissociated forms of organic acid molecules through the plasma membrane into the neutral cytoplasm where dissociation occurs, resulting in intracellular acidification and the accumulation of anions. The mechanisms of the resistance of spoilage yeast to weak acids include a decreased access of the acid to the cell, an improved ability to extrude H^+ protons, the conversion of the preservative into an innocuous compound, and inducible extrusion of the anions (Adams & Nicolaides 1997; Loureiro 2000). According to Brul and Coote (1999) the resistance of spoilage yeast to weak organic acids is dependent on the H^+-ATPase activity and the existence of a multidrug resistance pump that actively extrudes preservative anions from the cell.

Plocková *et al.* (2001) investigated the inhibitory activity of non-proteinaceous and non-saccharidic substances produced by *L. rhamnosus* VT1, which were anti-microbially effective against the fungi *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., and *Rhizopus*

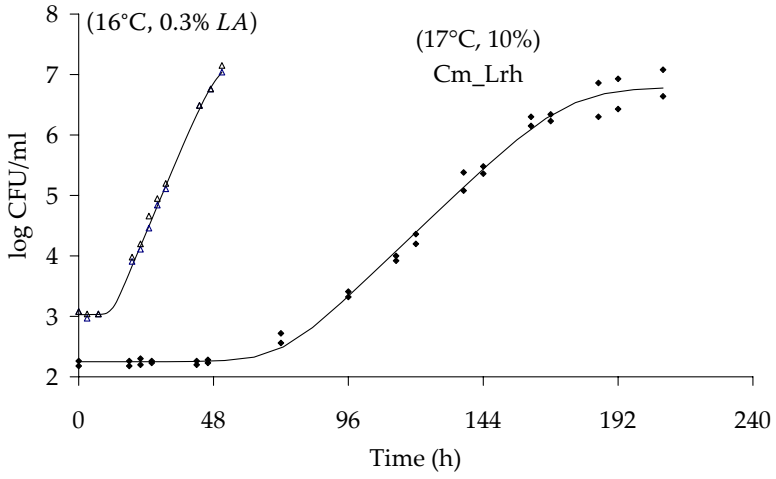


Figure 7. Comparison of the effect of *L. rhamnosus* VT1 (10%) and 0.3% lactic acid on growth of *C. maltosa* YP1 in ultra-pasteurised milk and in yeast extract broth with glucose, respectively

spp., for example *P. expansum*, *P. verrucosum*, *F. proliferatum*, *F. graminearum*, *A. candidus* C25, *A. repens* NRRL 13, *Alternaria alternata* NRRL 5255, *R. stolonifer* NRRL 1519, and *Cladosporium cladosporioides* NRRL 6421.

Another substance with inhibitory activity against *C. maltosa* YP1, which we found using capillary isotachophoretic analysis (capillary electrophoretic analyser EA 202M-VILLA, Spišská Nová Ves, Slovak Republic) in the concentration of 603 mg/kg in MRS broth (Merck, Darmstadt, Germany) after incubation at 37°C for 24 h, and of 768 mg/kg after incubation at 37°C for 48 h, was 2-pyrrolidone-5-carboxylic acid, known as pyroglutamic acid. 2-pyrolidon-5-carboxylic acid is also produced by *L. casei* subsp. *casei*, *L. acidophilus*, *L. rhamnosus* GG, and *L. rhamnosus* LC-705 (Huttunen *et al.* 1995; Yang *et al.* 1997; Työppönen *et al.* 2003).

In our follow-up study, the concentration of 0.1% (w/v) of pyroglutamic acid (Sigma-Aldrich, Steinheim, Switzerland) in yeast extract broth with glucose at pH = 3.5 decreased the growth rate of *C. maltosa* YP1 by about 37% in comparison with the control sample at the same pH value. The concentration of 0.3% pyroglutamic acid in broth (pH = 3.0) caused much more expressed inhibition of *C. maltosa* YP1 in comparison with lactic acid added at the same concentration (pH = 3.8). The lag time of *C. maltosa* YP1 observed in a glucose solution with the yeast extract and 0.3% pyroglutamic acid was 4 times longer (272 h) than in the broth with 0.3% lactic acid (65 h). In the case of lactic acid addition, a slow growth of yeast (Gr = 0.0246 $\log_{10}$ CFU/h) was found but on the other hand, pyroglutamic acid caused the decline of *C. maltosa* YP1 cells with a death rate of –0.0015 $\log_{10}$ CFU/h at the same concentration (Figure 6). The strain of *L. rhamnosus* VT1 might be used as a part of the protective culture against yeast contaminants. The example demonstrated in Figure 7 could support this idea. The inhibitory effect of 10% inoculation of *L. rhamnosus* VT1 in UHT milk at 17°C was higher in comparison with the influence of lactic acid alone, e.g. 0.27% at 16°C. Lag time and time to double (T_d) of *C. maltosa* YP1 in UHT milks with initial 10% v/v of 18 h culture of *L. rhamnosus* VT1 were, respectively, 6.5 or 2.5 times longer than in the case of lactic acid. The growth rates of the yeast in milks inoculated with 10% lactobacillus decreased up to a value of 0.044 $\log_{10}$ CFU/h in comparison with 0.11 $\log_{10}$ CFU/h found in 0.27% lactic acid. Moreover, *L. rhamnosus* VT1 was able to survive the effect of pH = 3 for 180 min in 84%. This is comparable with the known commercial strains *L. acidophilus* LA-1 or *L. rhamnosus* GG found by Prasad *et al.* (1998). Their survival was 94% and 83% at pH = 3 during 180 min, respectively. Similarly, the survival of *L. rhamnosus* VT1 after 24 h exposure to 5% (w/v) of bile salts was 73% while the commercial strains mentioned showed 96% survival but 3 h exposure to 1% (w/v) of Oxgall.

CONCLUSION

Our results confirmed that *C. maltosa* YP1 was able to compete with lactic acid bacteria in fermented dairy products. Applying our prediction of the time for a wild yeast strain *C. maltosa* YP1 to reach a relevant density in fermented products, e.g. in yoghurts, may represent valuable information for quality control related to the growth of *C. maltosa* YP1 in such products. The inhibitory effect of *L. rhamnosus* VT1 regarding the lag time or the growth rate of generally resistant *C. maltosa* YP1 was described by response surface equations. The results presented in this study could be considered as our contribution to the discussion of the possible growth of oxidative yeast contaminants such as *Candida maltosa* YP1 in acid food environments.

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References

Abdelgadir W.S., Hamad S.H., Moller P.L., Jakobsen M. (2001): Characterisation of the dominant microbiota of Sudanese fermented milk Rob. International Dairy Journal, **11**: 63–70.

Adams M.R., Nicolaides L. (1997): Review of the sensitivity of different foodborne pathogens to fermentation. Food Control, **8**: 227–239.

Baranyi J., Roberts T.A., McClure P. (1993): A non-autonomous differential equation to model bacterial growth. International Journal of Food Microbiology, **10**: 43–59.

Baranyi J., Roberts T.A. (1994): A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, **23**: 277–294.

Baranyi J., Pin C., Ross T. (1999): Validating and comparing predictive models. International Journal of Food Microbiology, **48**: 159–166.

Brul S., Coote P. (1999): Preservative agents in foods. Mode of action and microbial resistance mechanisms. International Journal of Food Microbiology, **50**: 1–17.

Buzzini P., Martini A. (2001): Large-scale screening of selected *Candida maltosa*, *Debaryomyces hansenii* and *Pichia anomala* killer toxin activity against pathogenic yeasts. Medical Mycology, **39**: 479–482.

Caplice E., Fitzgerald G.F. (1999): Food fermentations: role of microorganisms in food production and preservation. International Journal of Food Microbiology, **50**: 131–149.

Consentino S., Fadda M.E., Deplano M., Mulargia A.F., Palmas F. (2001): Yeasts associated with Sardinian ewe's dairy products. International Journal of Food Microbiology, **69**: 53–58.

Corbo M.R., Lanciotti R., Albenzio M., Sinigaglia M. (2001): Occurence and characterization of yeasts isolated from milks and dairy products of Apulia region. International Journal of Food Microbiology, **69**: 147–152.

Gadaga T.H., Mutukumira A.N., Narvhus J.A. (2000): Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. International Dairy Journal, **10**: 459–466.

Gadaga T.H., Schwan R.F., Mendoca A.T., Da Silva J.J., Rodrigues J.A. (2001): The growth and interaction of yeasts and lactic acid bacteria isolated from Zimbabwean naturally fermented milk in UHT milk. International Journal of Food Microbiology, **68**: 2–32.

Gilliland S.E., Walker D.K. (1990): Factors to consider when selecting a culture of *Lactobacillus acidophillus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. Journal of Dairy Science, **73**: 905–911.

Helander I.M., von Wright A., Mattila-Sandholm T.M. (1997): Potential of lactic acid bacteria and novel antimicrobials against gram-negative bacteria. Trends in Food Science & Technology, **8**: 146–150.

Holzapfel W.H., Geisen R., Schillinger U. (1995): Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. International Journal of Food Microbiology, **24**: 343–362.

Huttunen E., Noro K., Yang Z. (1995): Purification and identification of antimicrobial substances produced by two *Lactobacillus casei* strains. International Dairy Journal, **5**: 503–513.

Jakobsen M., Narvhus J. (1996): Yeasts and their possible beneficial and negative effects on the quality of dairy products. International Dairy Journal, **6**: 755–768.

Jirků V., Masák J., Čejková A. (2001): Significance of physical attachment of fungi for bio-treatment of water. Microbiology Research, **156**: 383–386.

Kasüske A., Wedler H., Schulze S., Becher D. (1992): Efficient electropulse transformation of intact *Candida maltosa* cells by different homologous vector plasmids. Yeasts, **8**: 691–697.

Laubscher P.J., Viljoen B.C. (1999): The resistance of dairy yeasts against commercially available cleaning compounds and sanitizers. Food Technology and Biotechnology, **37**: 281–286.

Lauková D., Valík Ľ. (2003): Quantitative analysis of the influence of the substrate and temperature on the growth of *Candida maltosa* YP1. Bulletin of Food Research, **42**: 39–254.

Lauková D., Valík Ľ. (2004): Mathematical modelling of temperature on growth dynamics of *Candida maltosa* YP1 in yoghurt. Bulletin of Food Research, **43**: 89–99.

Lauková D., Valík Ľ., Görner F. (2003a): Heat resistance of vegetative cells of *Candida maltosa* YP1. Agriculture, **49**: 375–380.

Lauková D., Valík Ľ., Görner F. (2003b): Effect of lactic acid on the growth dynamics of *Candida maltosa* YP1. Czech Journal of Food Sciences, **21**: 43–49.

Loureiro V. (2000): Spoilage yeasts in foods and beverages: characterisation and ecology for improved diagnosis and control. Food Research International, **33**: 247–256.

Mutoh E., Ohta A., Takagi M. (1995): Studies on cycloheximide-sensitive and cycloheximide-resistant ribosomes in the yeast *Candida maltosa*. Gene, **224**: 9–15.

Nakazawa T., Horiuchi H., Ohta A., Takagi M. (1998): Isolation and characterization of EPD1, an essential gene for pseudohyphal growth of a dimorphic yeast, *Candida maltosa*. Journal of Bacteriology, **180**: 2079–2086.

Ouwehand A.C., Kirjavainen P.V., Gronlund M.M., Isolauri E., Salminen S.J. (1999): Adhesion of probiotic microorganisms to intestinal mucus. International Dairy Journal, **9**: 623–630.

Ouwehand A.C., Salminen S.J., Tolkko S., Roberts P., Ovasha J. (2002): Resected human colonic tissue: new model for characterizing adhesion on lactic acid bacteria. Clinical and Diagnostic Laboratory Immunology, **9**: 184–186.

Pin C., Baranyi J. (1999): Predictive models as means to quantify the interactions of spoilage organisms. International Journal of Food Microbiology, **41**: 59–72.

Plocková M., Stiles J., Chumchalová J., Halfarová R. (2001): Control of mould growth by *Lactobacillus rhamnosus* VT1 and *Lactobacillus reuteri* CCM 3625 on milk agar plates. Czech Journal of Food Sciences, **19**: 46–50.

Prasad J., Gill H., Smart J., Gopal P.K. (1998): Selection and characterisation of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. International Dairy Journal, **8**: 993–1002.

Pryce J.D. (1969): A modification of the Barker-Summerson method for the determination of lactic acid. Analyst, **94**: 1151–1152.

Ratkowsky D.A., Olley J., McMeekin T.A., Ball A. (1982): Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology, **149**: 1–5.

Roostita R., Fleet G.H. (1996): Growth of yeasts in milk and associated changes to milk composition. International Journal of Food Microbiology, **31**: 205–219.

Schillinger U., Geisen R., Holzapfel W.H. (1996): Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. Trends in Food Science & Technology, **7**: 158–164.

Schmitz C., Goebel I., Wagner S., Vomberg A., Klinner U. (2000): Competition between n-alkane-assimilating yeasts and bacteria during colonization of sandy soil microcosms. Applied Microbiology and Biotechnology, **54**: 126–132.

Sláviková E., Vadkertiová R. (1997): Seasonal occurence of yeasts and yeast-like organisms in the river Danube. Antonie van Leeuwenhoek, **72**: 77–80.

StatSoft Inc. (2004): Statistica Cz (software for data analysis). Availabe at www.StatSoft.cz

Steppan D.D., Werner J., Yeater R.P. (1998): Texbook, Germany. Aavailable at http://www.jowerner.homepage.t-online.de/download.htm

STN ISO 7954 (1997): Microbiology – General principles for enumeration of yeasts and molds – Colony count technique at 25°C. Bratislava, Slovak Republic.

Työppönen S., Petäjä E., Mattila-Sandhom T. (2003): Bioprotectives and probiotics for dry sausages. International Journal of Food Microbiology, **83**: 233–244.

Viljoen B.C. (2001): The interaction between yeasts and bacteria in dairy environments. International Journal of Food Microbiology, **69**: 37–44.

Viljoen B.C., Greyling T. (1995): Yeasts associated with Cheddar and Gouda making. International Journal of Food Microbiology, **28**: 79–88.

Viljoen B.C., Lourens-Hattingh A., Ikalafeng B., Peter G. (2003): Temperature abuse initiating yeast growth in yoghurt. Food Research International, **36**: 193–197.

Welthagen J.J., Viljoen B.C. (1998): Yeast profile in Gouda cheese during processing and ripening. International Journal of Food Microbiology, **41**: 185–194.

Welthagen J.J., Viljoen B.C. (1999): The isolation and identification of yeasts obtained during the manufacture and ripening of Cheddar cheese. Food Microbiology, **16**: 63–73.

Yang Z., Suomalainen Z., Mäyra-Mäkinen A., Huttunen E. (1997): Antimicrobial activity of 2-pyrrolidone-5-carboxylic acid produced by lactic acid. Journal of Food Protection, **60**: 786–790.

Zurerra-Cosano G., Garcia-Gimeno R.M., Rodríguez-Pérez R., Hervás-Martínez C. (2006): Performance of response surface model for prediction of *Leuconostoc mesenteroides* growth parameters under different experimental conditions. Food Control, **17**: 429–438.

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