Effect of Lactic Acid on the Growth Dynamics of *Candida maltosa* YP1

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Abstract

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The growth dynamics of the oxidative imperfect yeast strain *Candida maltosa* YP1 isolated from the surface of fruit yoghurt was studied in relation to the lactic acid concentration ranging from 0 to 1.6% (w/v). The maximal specific growth rate of 0.36 h^{-1} and minimal lag-phase duration of 2.9 h were found in the glucose solution without lactic acid at 25°C. The decrease of the natural logarithm of both the specific growth rate (ln μ) and the lag-phase prolongation (ln λ) in the dependence on the increase of lactic acid concentration (0–1.59%) was significantly linear (ln μ = –1.1458 – 0.6056 *c*; $R^2_{(\mu)}$ = 0.9526; ln λ = 1.0141 + 1.9766 *c*; $R^2_{(\lambda)}$ = 0.9577). Based on these equations, the prediction of the time necessary for *C. maltosa* YP1 to reach 1 × 10^6 CFU/ml in the dependance on lactic acid concentration and, the initial density of the yeast culture was calculated. For example, *C. maltosa* YP1 was able to reach the level of 1 × 10^6 CFU/ml in a model glucose solution at the initial concentration N_0 = 1 CFU/ml, 0.9% lactic acid and 25°C within 2 d. The growth predictions presented indicate a considerable resistance of *C. maltosa* YP1 to lactic acid in the concentration of up to 1.3% (w/v).

Keywords: *Candida maltosa*; growth parameters; lactic acid

Yeasts possess well-developed systems able to maintain intracellular pH that plays an essential role in the yeast physiology and growth (Henriques *et al.* 1997). That is why they tolerate low pHs ranging from 4.0 to 3.0 and belong naturally to the main spoilage microflora of yoghurt. Some of them are able to grow at cooling temperatures, to ferment lactose or to assimilate lactic acid. For these properties as well as their proteolytic and lipolytic activity, numerous yeast species were mentioned in connection with dairy products by Richter *et al.* (1992), Betts *et al.* (1999) and Jakobsen and Narvhus (1996).

Varnan and Sutherland (1994) stated that *Saccharomyces* spp. and *Kluyveromyces marxianus* have often been involved in the spoilage of yoghurt. On the other side, oxidative yeasts *Candida* spp., *Debaryomyces* spp., *Metschnikowia* spp., *Pichia* spp., *Rhodotorulla* spp., *Torulaspora* spp., *Trichosporon* spp., and *Yarrowia* spp. may also cause the deterioration of yoghurts. These yeasts can grow on the surface and form there smooth colonies or films. Their ability to oxidatively degrade lactic acid leads to a slow production of carbon dioxide and water. Consequently, the acidity of yoghurt on its surface is decreased and the growth of other saprophytic microorganisms including bacteria may appear. The spoilage of yoghurt by yeasts results in defects manifested by changes in the yoghurt texture or consistency, by yeasty, fruity or bitter flavour, and by unpleasant off-flavour (Rašic & Kurmann 1978; Robinson & Tamime 1990; Viljoen & Greyling 1995).

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

moulds can be considered as efficient indicators of the standards of the manufacturing or hygiene practices during the production of these fermented products.

The aim of this work was to characterise the growth of the oxidative wild yeast strain *Candida maltosa* YP1 isolated from the surface of yoghurt. As the isolation of this yeast species in connection with the spoilage of fermented dairy products has not been mentioned in the literature available to this time, the experiments were focused mostly on the description of the growth dynamics in relation to the lactic acid concentration ranging from 0 to 1.6% (w/v) at 2°C.

MATERIAL AND METHODS

Microorganism. Strain *C. maltosa* YP1 was isolated as a contaminat from the surface of fruit yoghurt. Its identification was confirmed by Dr. E. Sláviková from the Collection of Yeasts Cultures (Slovak Academy of Science Bratislava).

Inoculation. Strain of *C. maltosa* YP1 was kept on Plate count agar (PCA, Imuna, Šarišské Michaľany, Slovakia) at 5 ± 1°C. Suspensions of the strain were prepared from 48 h culture of *C. maltosa* YP1 grown on a defined surface of agar in tubes by standard rinsing with sterile pepton water/saline water. The suspensions were used in the individual growth experiments in glucose solutions (Mikrochem, Pezinok, Slovakia) containing yeast extract (Fluka, Buchs, Switzerland) and lactic acid (Pliva Lachema, Brno, Czech Republic). The actual acid concentrations were determined by titration with a NaOH solution (c_{NaOH} = 0.246 mol/l) using phenolphthalein as indicator. The active acidity was also measured using pH-meter (Radelkis OP-211/1, Budapest, Hungary). Inoculations were carried out so as to reach initial numbers of ≤ 10^3 CFU/ml. The experiments were repeated twice in two or three parallels, respectively.

Number of wild strain** C. maltosa* **YP1** ***in glucose solution with yeast extract and lactic acid. Number of strain *C. maltosa* in parallel model solutions with increasing lactic acid concentration was determined according to Slovak Technical Standard STN ISO 7954 as the yeasts.

Growth parameters and mathematical modelling. Growth parameters of strain *C. maltosa* YP1 in model mediums with different lactic acid concentrations were calculated from the experimental growth curves by D-model (Baranyi *et al.* 1993). The specific growth rates of *C. maltosa* YP1 obtained were related to the lactic acid concentration. The prediction for *C. maltosa* YP1 to reach the level of 1 × 10^6 CFU/ml indicating the food spoilage were validated by Baranyi *et al.* (1999).

RESULTS AND DISCUSSION

Characterisation of wild strain *C. maltosa* YP1

The strain of *C. maltosa* YP1 formed smooth, soft and cream colonies that were zonal drab stripped on malt extract agar after incubation at 28°C for 3 d. The colony center was usually gently elevated and the border was undulated. The vegetative cells were round to gently cylindrical, with the size of 3.3 × 6.6 μm. The shape of 24 h cells grown in apple juice is shown in Figure 1.

C. maltosa is asporogenic yeast able to exhibit pseudohyphal growth in response to the environmental conditions (Nakazawa *et al.* 1998). The

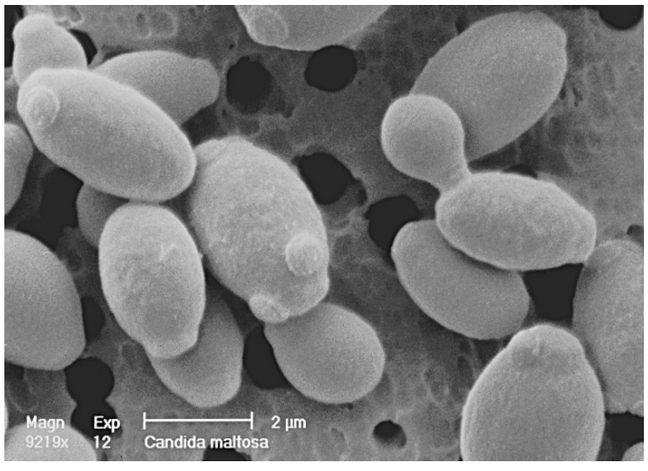

Figure 1. Yeast *Candida maltosa* YP1 (dr. M. Kaláb, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada: http//anka.livstek.lth.se:2080/C_maltosa.htm)

production of any pigments and polysaccharides based on starch was not observed. A wild strain of *C. maltosa* under study was tolerant to 50% of glucose but, on the other hand, the absence of growth factors in the environments limited its multiplication. The strain was able to grow fast at 37 and 42°C. Biochemical properties of this strain are summarised in Table 1.

Effect of lactic acid on the growth of wild strain *C. maltosa* YP1

The growth curves of the wild strain *C. maltosa* YP1 were analysed in parallel glucose solutions containing the yeast extract in relation to the lactic acid concentrations ranging from 0.28 to 1.59% (w/v). Data summarised in Figure 2 confirmed the expected effect of lactic acid on the growth curves where the lag-phase was prolonged with increasing acid concentration. The growth curves show that the lactic acid concentration as low as 0.28% (w/v) caused a temporary slight decrease of the cell number during lag-phase. This fact was more evident at higher lactic acid concentrations.

Table 1. Biochemical properties of wild strain *C. maltosa* YP1

Saccharide/substance	Fermentation	Assimilation
Maltose	–	+
Sucrose	+ (weakly)	+
Lactose	–	–
Glucose	+	+
Galactose	–	+
Raffinose	–	–
Trehalose	+	+
D-xylose		+
L-arabinose		–
KNO_3		–
Celobiose		+
Inulin		–
Starch solution		–
Ethanol		+ (weakly)
Glycerol		+ (weakly)

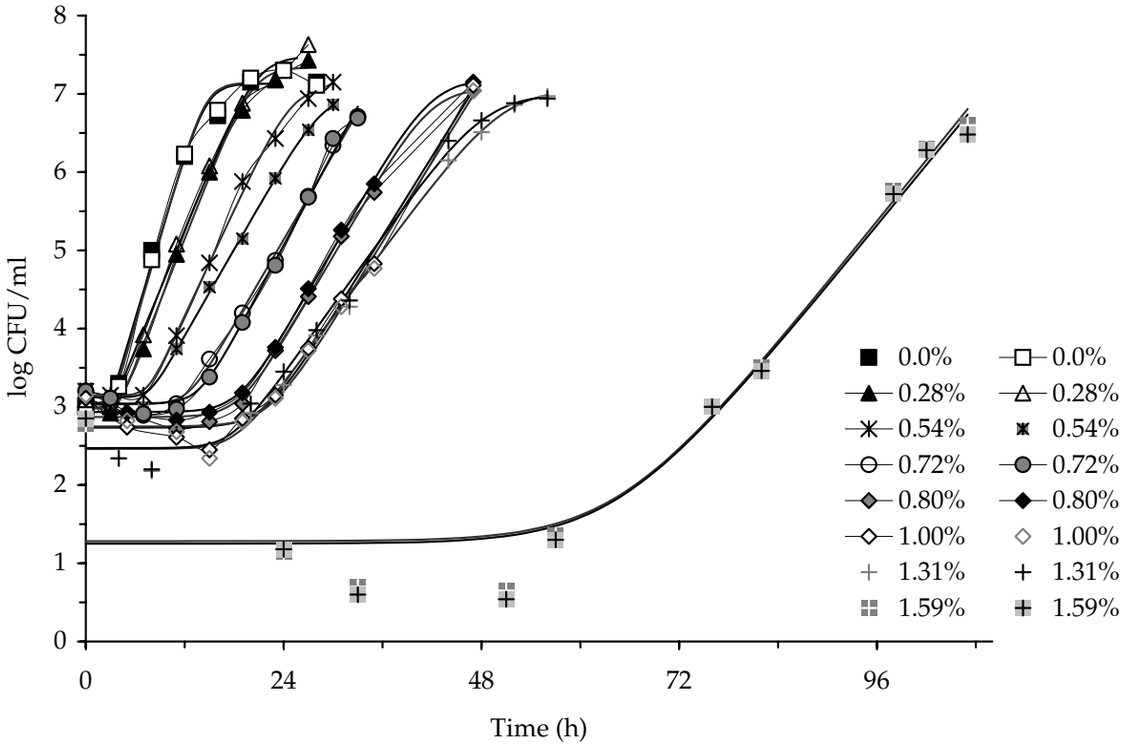

Figure 2. Growth curves of the wild strain *C. maltosa* YP1 in glucose solution containing yeast extract corresponding to lactic acid concentrations at 25°C

However, the number of growing cells at the end of the lag-phase always reached the initial level of inoculation and after that period the wild strain of *C. maltosa* YP1 was able to grow exponentially even at the highest lactic acid concentration of 1.59% (w/v). The specific growth rate of wild strain *C. maltosa* YP1 was influenced a small extent by lactic acid, especially within the interval of 0.54 to 1.01%. The increase of lactic acid to the concentration of 1.59% had a dramatic effect for the adjustment of *C. maltosa* YP1 to high acidity of the medium (Figure 2). For example, the lag-phase of *C. maltosa* YP1 was 21-times longer at this concentration as compared to the medium without lactic acid addition. On the other hand, the growth rate of *C. maltosa* YP1 as found at the highest concentration of 1.59% reached just 36% of its maximal value found at no lactic acid addition. It seems that the highest concentration of lactic acid in glucose medium can be close to the acid resistance limit of *C. maltosa* YP1. The corresponding growth parameters are summarised in Table 2.

Table 2. Growth parameters of wild strain *C. maltosa* YP1 in glucose solution containing yeast extract as a function of lactic acid concentration at 25 ± 0.5°C

Lactic acid concentration (%)	Specific growth rate (h^{-1})	Lag-phase duration (h)	Generation time (h)
0.00	0.35	2.7	0.86
0.00	0.36	3.0	0.83
0.28	0.27	4.1	1.12
0.28	0.26	3.3	1.17
0.54	0.23	7.5	1.33
0.54	0.18	7.7	1.63
0.72	0.18	12.4	1.66
0.72	0.20	13.8	1.53
0.79	0.18	18.6	1.66
0.79	0.18	18.5	1.66
1.01	0.17	21.6	1.77
1.01	0.17	22.2	1.77
1.31	0.14	18.1	2.15
1.31	0.15	17.6	2.0
1.59	0.13	64.8	2.32
1.59	0.13	64.5	2.33

These findings comply with the well-developed systems known in yeast cell biology for maintaining intracellular pH. Intracellular pH and the H^+ pump are thought to play an important role in the yeast growth. They are regulated by plasma membrane ATPase that forms the transmembrane H^+ gradient driving force in nutrient transportation (Imai & Ohno 1995). This system plays an essential role in the yeast physiology, in the lower permeability of the plasma membrane to the acid, the active extrusion of the preservative, and the mediated transport and conversion of preservatives (Henriques *et al.* 1997).

Further, the lag-phase and the specific growth rate were analysed in relation to the lactic acid concentrations within the second part of the mathematical modelling. The results are presented in Figures 3 and 4 including the corresponding equations. As can be seen, both the natural logarithm of the lag-phase and the specific growth rate of the wild strain *C. maltosa* YP1 were significantly linearly dependent on the lactic acid concentration. The lag-phase prolongation of the wild strain *C. maltosa* YP1 as a function of the increasing lactic acid concentration (c) was characterised by the following equation: $\ln \lambda = 1.0141 + 1.9766\ c$ ($R^2_\lambda = 0.9577$). Similarly, the specific growth rates of the wild strain *C. maltosa* YP1 decreased with the increasing lactic acid concentration according to the equation $\ln \mu = -1.1458 - 0.6056\ c$ ($R^2_\mu = 0.9526$).

For practical reasons, the time for the wild strain *C. maltosa* YP1 to reach 1×10^6 CFU/ml was calculated using these linear equations since the strain under study was involved in the spoilage of dairy

Table 3. Prediction of time (d) for wild strain *Candida maltosa* YP1 to reach 1×10^6 CFU/ml in glucose solution containing yeast extract adjusted with lactic acid at 25 ± 0.5°C

Lactic acid concentration (%)	Time to reach 1×10^6 CFU/ml as a function of initial density N_0 (d) =		
	1 CFU/ml	100 CFU/ml	1000 CFU/ml
0.0	0.9	0.6	0.5
0.3	1.2	0.8	0.7
0.6	1.5	1.1	0.9
0.9	2.0	1.6	1.4
1.2	2.9	2.3	2.0
1.5	4.2	3.5	3.2
1.8	6.4	5.6	5.2

products. The respective predictions are summarised in Table 3 as results of varying initial densities of *C. maltosa* YP1. Our results confirmed that the strain used in the study was able to reach the density of 1×10^6 CFU/ml very fast in the presence of lactic acid. The initial concentrations of the cells had no practical effect on the time need to reach the spoilage yeast density of 1×10^6 CFU/ml.

Considering the growth of *C. maltosa* YP1 in yoghurt products, we should take into account the fact that there are some other factors such as the growth and metabolites of yoghurt bacteria and a low storage temperature (< 8°C) apart from low pH-values acting against the contaminants in yoghurt. Experiments focused on these factors are presently subjects of our study.

Validation of growth predictions

Generally, validation is defined as a comparison of the predicted responses (specific growth rate, lag-phase or generation time) with the actual findings (Ross 1996). Baranyi *et al.* (1999) stated that the main goal of validation is to determine the accuracy and discrepancy of mathematical models compared with one another as well as with the experimental results. For our purposes, we calculated the accuracy factor and the "per cent discrepancy". These factors were defined by Baranyi *et al.* (1999) as follows:

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

$$D_f = (A_f - 1) \times 100$$

where: μ – specific growth rate obtained from the growth curve

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

n – number of measurements

$\%D_f$ – per cent discrepancy

The calculation of the accuracy and discrepancy factors was based on the comparison of the growth rates determined experimentally and the values calculated from the equation presented in Figure 4. Similarly, validation of the growth predictions of the wild strain *C. maltosa* YP1 to reach the density of 1×10^6 CFU/ml was based on the comparison of the predictions resulting from the experimental growth parameters and those resulting from the parameters calculated from the equation as presented in Figures 2 and 3. The results of validation are summarised in Table 4 and show that in both cases, the discrepancies between the predicted and the observed values were close to 13%. In our opinion, these values of the validation parameters are acceptable for the microbial growth. Similar values were found by Te Giffel and Zwietering (1999) and Valík *et al.* (2002).

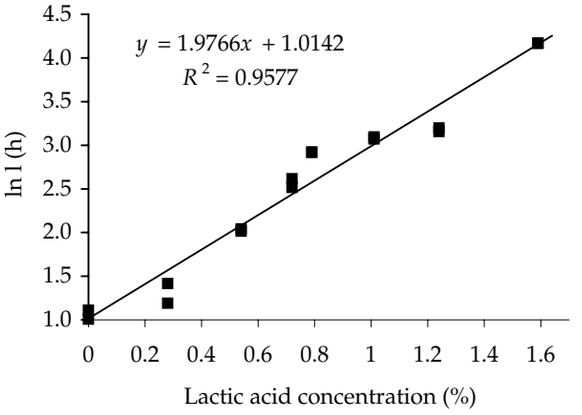

Figure 3. Lag-phase of the wild strain *C. maltosa* YP1 (ln λ) as a function of lactic acid concentration in glucose solution containing yeast extract at the temperature of 25 ± 0.5°C

The application of our prediction of the time needed for the wild yeast strain *C. maltosa* YP1 to reach a relevant density in fermented products, e.g. in yoghurts, may provide valuable information for the quality control since the growth of *C. maltosa* in such products can be affected by lactic acid bacteria or other nutritive and environmental

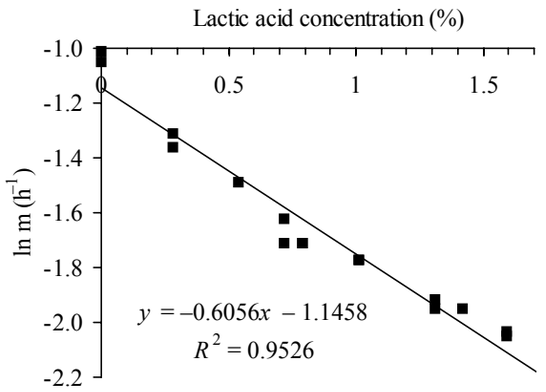

Figure 4. Specific growth rate of the wild strain *C. maltosa* YP1 (ln μ) as a function of lactic acid concentration in glucose solution containing yeast extract at the temperature of 25 ± 0.5°C

Table 4. Parameters validating the model for the specific growth rate of the wild strain *C. maltosa* YP1 in the dependence on lactic acid concentration in glucose solution with yeast extract, and the time prediction to reach the density of 1×10^6 CFU/ml

Factor	Validation index for	
	equation	prediction of time to reach
	$\ln \mu = -1.1458 - 0.6056c$	1×10^6 CFU/ml for *C. maltosa*
A_f	1.13	1.12
% D_f	13.1	12.3

factors which should also be taken into account in practice.

The results presented in this study may be considered as our contribution to the discussion of possible growth of the oxidative wild yeast strain *Candida maltosa* YP1 in acid food environments. We presume that our work will continue with a study of the quantitative effects of other factors on the growth of this yeast.

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Súhrn

Lauková D., Valík Ľ., Görner F. (2003): **Vplyv kyseliny mliečnej na dynamiku rastu *Candida maltosa* YP1**. Czech J. Food Sci., **21**: 43–49.

Popísali sme dynamiku rastu oxidatívnej kvasinky *Candida maltosa* YP1 izolovanej z povrchu ovocného jogurtu vo vzťahu ku koncentráciám kyseliny mliečnej v intervale 0 až 1,6 %. Maximálna špecifická rastová rýchlosť 0,36 h^{-1} a minimálne trvanie lag-fázy 2,9 h boli zistené v modelovom roztoku s glukózou a kvasničným autolyzátom bez prídavku kyseliny mliečnej pri teplote 25 °C. Znižovanie prirodzeného logaritmu špecifickej rastovej rýchlosti (ln μ) a predlžovanie prirodzeného logaritmu lag-fázy (ln λ) *C. maltosa* v závislosti od stúpajúcej koncentrácie kyseliny mliečnej boli významne lineárne (ln μ = –1,1458 – 0,6056 × *c*; $R^2_{(\mu)}$ = 0,9526; ln λ = 1,0141 + 1,9766 × *c*; $R^2_{(\lambda)}$ = 0,9577). Na základe týchto závislostí sa v práci prezentujú predpovede, kedy kvasinka *C. maltosa* YP1 v závislosti od koncentrácie kyseliny mliečnej a vlastného počiatočného počtu dosiahne počty 1 × 10^6 KTJ/ml. Túto koncentráciu dosiahne *Candida maltosa* YP1 napríklad pri počiatočnom počte N_0 = 1 KTJ/ml, 0,9% kyseliny mliečnej a 25 °C po 2 dňoch. Uvádzané predikcie rastu indikujú značnú toleranciu *C. maltosa* YP1 voči kyseline mliečnej až po koncentráciu 1,3 % (w/v).

Kľúčové slová: *Candida maltosa*; rastové parametre; kyselina mliečna

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