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Saccharomyces cerevisiae PDE genes influence medium acidification and cell viability

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The yeast *Saccharomyces cerevisiae* contains two genes, *PDE1* and *PDE2*, which encode a low-affinity and a high-affinity cAMP phosphodiesterase, respectively, and are regulators of the amount of the secondary messenger cAMP. Deletion of *PDE2* and *PDE1* changes the ability of yeast cells to survive stress conditions such as heat shock and nitrogen starvation. In this study, we analysed the influence of low-affinity and high-affinity cAMP phosphodiesterases on medium acidification (caused by a peculiarity in yeast metabolism) during cell growth and on yeast cell viability during a gradual medium acidification and in acid stress conditions. A statistically significant increase in *ΔPde1* cell viability during a gradual acidification of the medium and also in acid stress conditions allows us to suggest that the *PDE1* gene is a negative regulator of cell viability in acidic conditions. Our study also shows that inactivation of the *PDE2* gene decreases cell viability after acid shock induction. It can be suggested that *Pde2p* is a positive regulator of cell viability in acid stress conditions. The two yeast phosphodiesterases play different roles in the regulation of cell viability in acidic conditions.

Key words: *Saccharomyces cerevisiae, PDE1, PDE2 phosphodiesterases,* medium acidification, acid stress, cell viability

INTRODUCTION

The Ras / cAMP pathway is a major determinant of stress resistance of the yeast *Saccharomyces cerevisiae*. The efficiency of this pathway is determined by the amount of cAMP. The cAMP quantity can be regulated at the level of its synthesis (Ras / adenylatcyclase module) and degradation (cAMP phosphodiesterases) [1].

The yeast *Saccharomyces cerevisiae* contains two genes, *PDE1* and *PDE2*, which encode a low-affinity and a high-affinity cAMP phosphodiesterase, respectively. The high-affinity cAMP phosphodiesterase *Pde2* belongs to the well studied class of phosphodiesterases, their representatives having been found in many species, including mammals [2]. *Pde2p* is an Mg^{2+}-requiring, zinc-binding enzyme with a *K*m for cAMP of 170 nM [2–4] which controls the basal cAMP level in the cell and protects it from interference of extracellular cAMP [5]. *Pde2* phosphodiesterase has also a key role in the control of cAMP levels in the stationary growth phase [1]. The intracellular level of cAMP dramatically increases upon addition of exogenous cAMP to *pde2* mutants, suggesting that *Pde2p* is responsible for breaking down exogenous cAMP [5]. Significant changes in the transcriptome have recently been described for the *S. cerevisiae pde2Δ* mutant [6]. These changes, which represent a constitutive activation of the cAMP pathway, manifest themselves in a range of cell-wall-related phenotypes, supporting the role for *PDE2* (and / or cAMP) in the maintenance of cell wall integrity in *S. cerevisiae*, as previously suggested [7, 8].

Pde1 displays a low affinity for cAMP with the K_m value varying between 20 and 250 μM [9]. Londesboroughand and Lukari (1980) have suggested that *Pde1* may be significant in the degradation of the high cAMP concentration that occurs in yeast cells after addition of glucose [10].

Deletion of *PDE1*, but not *PDE2*, results in a higher cAMP accumulation upon addition of glucose or upon intracellular acidification [11]. Under most conditions, *pde1Δ* and *pde2Δ* mutants have a wild-type phenotype; however, they are more sensitive to a heat shock and nutrient starvation. Previously it has been reported that a deletion of *PDE2* and *PDE1* changes the ability of yeast cells to survive stress conditions such as heat shock and nitrogen starvation [3, 4].

Pde1 and *Pde2* activity in yeast might be regulated by PKA-mediated phosphorylation [11]. As opposed to the *Ras2*^{val19} strain, the *Ras2*^{val19} *pde1Δ pde2Δ* strain displays a very high cAMP level. In the *Ras2*^{val19} strain, phosphodiesterases

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are able to prevent hyperaccumulation of cAMP. However, in a strain with a reduced PKA activity, phosphodiesterases are apparently unable to prevent cAMP hyperaccumulation [12]. The aim of our research was to determine the role of *PDE1* and *PDE2* in acid stress conditions and also during a gradual acidification of the medium.

MATERIALS AND METHODS

Yeast strains, plasmids, media and growth conditions

Yeast strains used in this study, containing mutations in phosphodiesterases genes (Table 1), are isogenic to SP1 (a kind gift of Prof. D. Engelberg) [13] and were kindly provided by Prof. J. M. Thevelein (Katholieke Universiteit, Leuven) [11].

For the transformation of the strains, two low-copy number plasmids were used. The transformation was carried out by electroporation according to the Gietz et al. transformation protocol [14].

The composition of the two growth media was as follows. The rich medium (YPD) contained 2% of glucose, 2% of peptone, 1% yeast extract. The synthetic medium (SD) contained 0.67% of yeast nitrogen base (w / o amino acids, with ammonium sulfate), 2% of glucose supplemented according to appropriate auxotrophic requirements. For the buffering of the media, 2-morpholinoethanesulfonic acid (MES) was used. The starting pH was 6.2 of the YPD medium and 5.4 of the SD medium. The cells were grown for 78 h at 30 °C on an orbitee shaker at 130 rpm. For acid stress induction, the solution pH 2.1 was used; 1M sorbitol solution pH 5.4 was used as a control.

Viability assay

The viability assay was carried out as previously described [15]. Briefly: yeast strains were grown in four liquid media (YPD, YPD-MES, SD, SD-MES). For the microscopy and flow-cytometry analysis, samples were taken at 22nd, 46th, 72nd hour of growth, stained with propidium iodide (PI) as described in [16], and immediately analysed by fluorescent microscopy at 560 nm wavelength (Olympus Provis AX70TRF microscope) or by flow cytometry. For the colony formation ability assay, yeast strains were grown till the late exponential phase (72nd hour of growth). 100 μl of known amount of the cells was plated on YPD plates, and after incubation for 3 days at 30 °C the colonies were counted. Acid stress induction was performed by placing yeast cells into a 2.1 pH 1M sorbitol solution for 4 and 6 hours (as the control, we used the same conditions except that the solution pH was 5.4). After incubation, evaluation of the cell viability analysis was performed by microscopy, flow cytometry and colony formation ability (as described above).

RESULTS

In this study, we analysed the influence of low-affinity and a high-affinity cAMP phosphodiesterases on medium acidification (caused by peculiarities in yeast metabolism) during cell growth, as well as on yeast cell viability during a gradual medium acidification and in acid stress conditions.

Deletion of the *PDE1* (strain JT135) induces an increased medium acidification and cell growth in SD medium as compared to SP1 ($p < 0.05$) (Fig. 1). Deletion of the *PDE2* (strain

Table. ***Saccharomyces cerevisiae* strains**

Strain	Genotype	Reference
SP1	MATa his3 leu2 ura3 trp1 ade8 can^R	[13]
JT134	MATa his3 leu2 ura3 trp1 ade8 can^R pde2::HIS3	[11]
JT135	MATa his3 leu2 ura3 trp1 ade8 can^R pde1::LEU2	[11]
JT136	MATa his3 leu2 ura3 trp1 ade8 can^R pde1::URA3 pde2::HIS3	[11]
Tr4	MATa his3 leu2 ura3 trp1 ade8 can^R pde2::HIS3 (YCpPDE2(URA3))	This study
Tr5	MATa his3 leu2 ura3 trp1 ade8 can^R pde1::HIS3 (YCpPDE1(LEU2))	This study

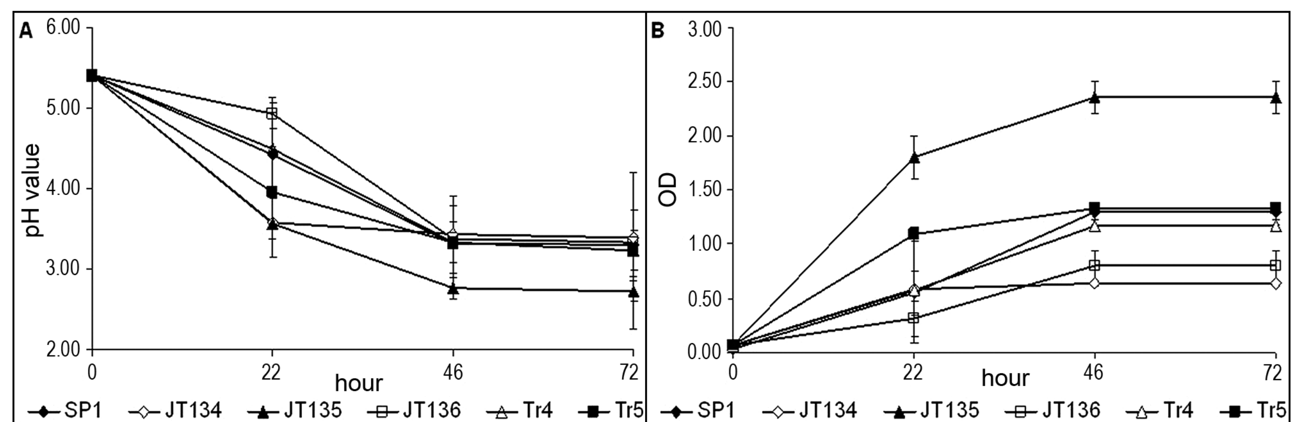

Fig. 1. SD medium acidification and yeast growth. Error bars indicate standard deviation. Experiments were independently repeated three times

JT134) gene shows no effect on the medium acidification and cell growth (Fig. 1). Deletion of both phosphodiesterase genes (JT136 strain) does not affect medium acidification, either.

During the yeast cell growth in YPD, no significant medium pH changes can be seen. All the analysed strains had slightly acidified media (from 6.2 to 4.96), and no statistically significant changes between the strains were observed (data not shown).

Analysis of the growth of transformants *Tr4* ant *Tr5* showed that transformation with the low-copy number plasmid and high stability of the plasmid (84.5 ± 0.568) completely restored cell metabolisms to the wild-type level; medium acidification and cell growth rate did not differ from those of SP1 ($p > 0.05$).

Previously, we have shown that a long-lasting, gradual acidification of the medium is related to cell death [15], and the regulation of yeast cell viability depends on the Ras / PKA signal transduction pathway activity. Hyperactivation of the pathway (*Ras2*^{Val19}) causes an increased cAMP accumulation and leads to cell death in the acidic environment [12]. Inactivation of the phosphodiesterase genes also leads to an enhanced cAMP accumulation, and we expected to observe a decrease also in cell viability. Therefore, yeast cell viability was evaluated by microscopy, flow cytometry and ability to produce colonies in the exponential, early stationary and late stationary phases.

The viability of the exponential phase cells remained over 95% in all media (YPD, YPD-MES, SD, SD-MES) and applying different methods. No differences between the analysed strains and different media were seen in the early stationary phase, either; cell viability remained over 80% in this case.

Analysis of the late stationary phase features showed no significant changes in cell viability when grown in YPD and YPD-MES media. Strain viability in all cases remained over 88%.

After 72 hours of growth in SD medium, only 26.13% ± ± 2.231% of SP1 cells were still viable, and pH at this measuring point was 3.30 ± 0.022 (Fig. 2). The disruption of the *PDE2* (JT134) gene showed similar results: cell viability was 24.04 ± 1.798% and pH 3.39 ± 0.026. Analogous results were also obtained during transformant *Tr4* growth: cell viability was 25.57 ± 0.438% and pH 3.23 ± 0.248. A slightly increased ($p > 0.05$) cell viability was seen after 72 hours of the transformant *Tr5* growth in SD medium (cell viability 33.57 ± 0.735%, pH 3.23 ± 0.972). A statistically significant increase of cell viability as compared to SP1 ($p < 0.05$) was seen in the strain with the disrupted *PDE1* (*JT135*) gene; cell viability at the 72nd hour of growth was 69.51 ± 0.550%, the pH value at this measuring point being 2.26 ± 0.128. In comparison to SP1, the cell viability of *JT136* (both phosphodiesterase genes, *PDE1* and *PDE2*, were disrupted) was also significantly higher ($p < 0.05$) – 55.68 ± 0.827%, pH being 3.33 ± 0.408. Increase in *JT135* and *JT136* strain cell viability could be observed even in SD-MES media, where the pH value was 5.4 (Fig. 2).

All these results were confirmed also by microscopy and flow cytometry analysis (data not shown). The slight, statistically insignificant differences between the methods may be due to sensitivity of the method. During microscopy analysis, only fully red stained cells are registered as "dead", therefore, in flow cytometry even light red stained cells are counted as "positive". Therefore, the most precise method of evaluating cell viability is evaluation of the colony formation abilities.

Results of our analysis show a possible negative effect of *PDE1* on yeast cell survival in acid stress conditions during a natural medium acidification. To confirm this assumption,

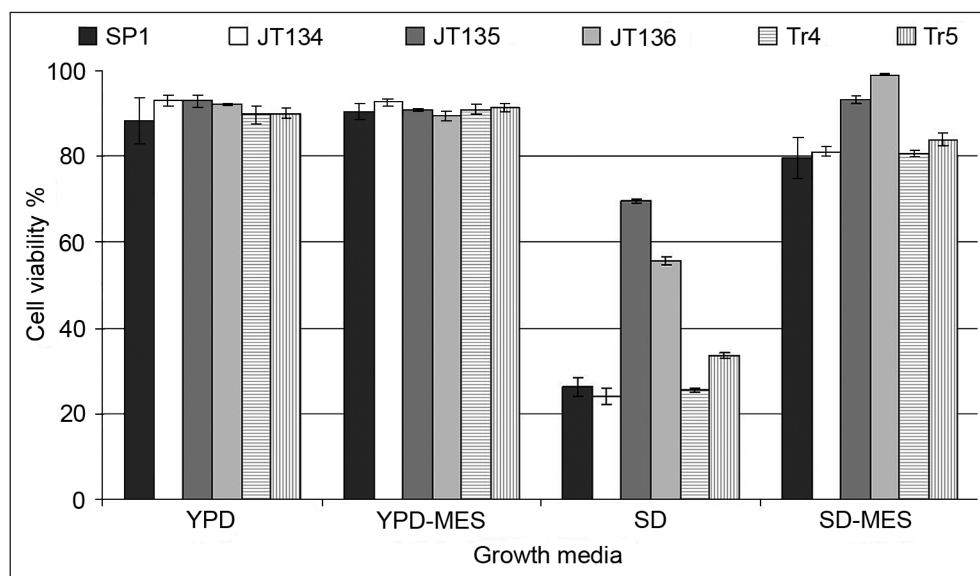

Fig. 2. Colony formation abilities after 72 h of yeast cell growth in 4 different media. Yeast cells were grown in liquid media for 72 h, later samples were taken, and a known amount of the cells was plated on SD plates. Colonies were counted after 48 h. Error bars indicate standard deviation. Experiments were independently repeated 3 times

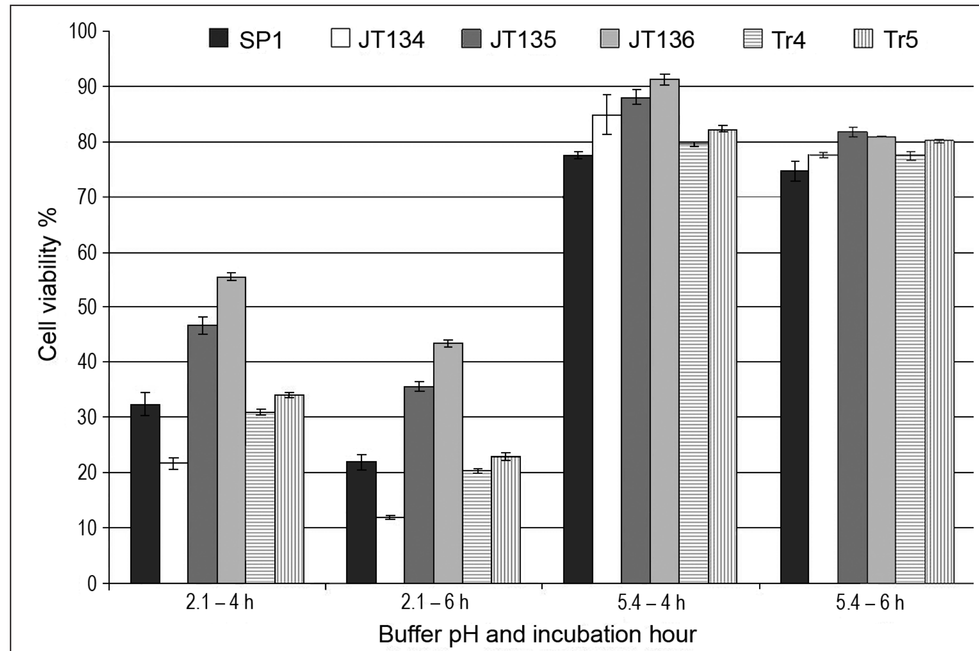

Fig. 3. Acid stress induction. Yeast cells were placed into two solutions (pH 5.4 and pH 2.1) for 4 h and 6 h. After acid stress induction, for the evaluation of cell viability, the colony formation ability test was performed. Error bars indicate standard deviation. Experiments were independently repeated 3 time

we tested the ability of yeast strains to survive in sudden acid stress conditions. For this purpose, pH 2.1 and 5.4 (control) solutions were used.

No differences between the used strains occurred after incubation in the control pH 5.4 solution. The viability of SP1 decreased after incubation in the pH 2.1 solution. The cell viability of *JT135* and *JT136* was statistically significantly higher as compared to SP1 ($p < 0.05$). Disruption of the *PDE2* gene reduced cell viability significantly ($p < 0.05$ in comparison to SP1). Only 11.95 ± 0.290% of cells were still viable after incubation for 6 hours in the pH 2.1 solution. Therefore, at this measuring point, 35.62 ± 0.898% of *JT135* and 43.42 ± 0.647% of *JT136* were still viable. Cell viability in the *Tr4* and *Tr5* transformants did not differ from that in SP1.

All these results were also confirmed by flow cytometry and microscopy analysis (data not shown). Regardless the differences between the methods, the tendency of cell behaviour in acid stress conditions remained the same.

DISCUSSION

During the growth in a glucose medium, deletion of the *PDE1* gene causes changes in yeast cell metabolisms, induces growth medium acidification and increased growth rate, whereas deletion of the *PDE2* gene and also a double deletion of *pde1Δ pde2Δ* has no effect on these features of the cell (Fig. 1). It is known that deletion of *Pde1*, but not of *Pde2*, results in a much higher glucose- and acidification-induced cAMP accumulation and overexpression of *Pde1* and abolishes glucose- and intracellular acidification-induced cAMP accumulation [11]. In response to signals of glucose or intracellular and extracellular acidification, an increased synthesis of cAMP is observed [12, 11]. In a *pde1Δ* mutant lacking the low-affinity cAMP phosphodiesterase, this cAMP signal was much higher (approximately threefold) and also longer-lasting. In the *pde2Δ* mutant which lacks the high-affinity cAMP phosphodiesterase, the cAMP signal was insignificantly changed or partially reduced. In the *pde1Δ pde2Δ* strain, the cAMP signal was virtually absent. The reduction or disappearance of the cAMP signal in the *pde2Δ* and *pde1Δ pde2Δ* strains seems at first sight contradictory, but it can be explained by an enhanced feedback inhibition of PKA in cAMP synthesis [11]. This allows us to predict a possible relationship between feedback inhibition in cAMP regulation and the induction of medium acidification. Enhanced feedback inhibition in the *pde2Δ* and *pde1Δ pde2Δ* strains decreased strain growth and medium acidification (cell metabolism rate) in the JT134 and JT136 strains (Fig. 1 A, B).

A cell viability assay revealed a specific role of *PDE1* in the regulation of cell viability in acidic conditions (Figs. 2, 3). Previously it has been shown that the stress-induced cAMP increase is enhanced in *pde1Δ* stains and that overexpression of the *Pde1p* abolishes acidification- and glucose-induced cAMP accumulation, and indicated a possible specific role of the *Pde1* in controlling the antagonist-induced cAMP signaling [11]. A statistically significant increase in *PDE1* cell viability during the gradual acidification of the medium and also in acid stress conditions implies that the *PDE1* gene is a negative regulator of cell viability in acidic conditions.

Deletion of *PDE2* reduced the ability of yeast cells to survive stress conditions such as heat shock and nitrogen starvation [4, 17], and the overexpression of *Pde2* enhanced the basal heat resistance of the cells [11] and prevented ROS accumulation [18]. Mutants with the *PDE2* deletion are highly sensitive to a heat shock treatment [19]. In this relation, our study shows that inactivation of the *PDE2* gene also decreases cell viability after acid shock induction (Fig. 3). Figure 3 shows a statistically significant decrease of JT134 cell viability as compared to SP1 after incubation in the pH 2.1 solution. However, JT134 cell viability did not differ from that of SP1 during a gradual acidification of the media (Fig. 2). Acidification of the medium to 3.39 ± 0.026 (during the JT134 growth) and depletion of the nutrient source generated stress conditions for the yeast cells and an increase in cAMP level, so there is a possibility that absence of *PDE2* can be compensated by the activity of *PDE1*. It is known that when the increased level of cAMP reaches the K_m of the *Pde1* enzyme, *Pde1p* regulates the stress tolerance of yeast cells [11, 1]. It can be also suggested that *Pde2p* is a positive regulator of cell viability in acidic stress conditions.

There are reports that a double deletion of the *PDE1* and *PDE2* genes increases sensitivity to the heat shock and nutrient starvation [17]. However, other authors report that single mutations of phosphodiesterase genes are more pronounced [11]. Our data on the JT136 (*pde1 pde2*) strain viability are somewhat contradictory. On the one hand, there are no differences between JT136 and SP1 during the growth in SD medium (Fig. 2). On the other hand, in acidic stress conditions, the cell viability of JT136 is statistically significantly increased ($p < 0.05$) as compared to SP1. These results show a possible effect of the other components of the Ras / PKA signal transduction pathway on the viability of yeast cells when both phosphodiesterase genes are inactivated.

Stress sensitivity, in general, correlates with the cellular levels of cAMP, and the basal cAMP level, especially in the stationary phase, is mainly affected by cAMP phosphodiesterases [1]. Ma et al. (1999) showed a specific role of *Pde1*, as opposed to *Pde2*, in controlling glucose- and also acidification-induced stimulation of cAMP accumulation [11]. The cell viability of the *PDE2* mutant strain does not differ from that of the wild-type strain during the natural gradual acidification of the media (Fig. 2); however, it is significantly decreased in acid stress conditions (Fig. 3), whereas deletion of the *PDE1* gene causes a significant increase in cell viability, which is indicated also in the *pde1Δ pde2Δ* strain (Figs. 2, 3). These results show that two yeast phosphodiesterases play different roles in the regulation of cell viability in acidic conditions.

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Eglė Lastauskienė, Donaldas Čitavičius

SACCHAROMYCES CEREVISIAE MIELIŲ GENAI VEIKIA TERPĖS RŪGŠTĖJIMĄ IR LĄSTELIŲ GYVYBINGUMĄ

Santrauka

Saccharomyces cerevisiae mielės turi du genus – *PDE1* ir *PDE2*, kurie atitinkamai koduoja aukšto ir žemo gimininguмо substratui fosfodiesterazes ir reguliuoja antrinio informacijos nešėjo cAMP kiekį. *PDE1* ir *PDE2* delecija keičia mielių ląstelių gebėjimą išgyventi stresinėmis sąlygomis, tokiomis kaip karščio šokas ar azoto badas. Šio tyrimo metu mes analizavome aukšto ir žemo giminingumo fosfodiesterazių poveikį terpės rūgštėjimui (kurį sukėlė pakitimai mielių metabolizme) augant ląstelėms ir ląstelių gyvybingumui laipsninio rūgštėjimo bei rūgštinio šoko sąlygomis. Statistiškai patikimas ląstelių gyvybingumo padidėjimas *ΔPde1* kamieno atveju laipsninio rūgštėjimo bei rūgštinio šoko metu leidžia daryti prielaidą, kad *PDE1* yra neigiamas ląstelių gyvybingumo reguliatorius rūgštinėje aplinkoje. Mūsų tyrimas rodo, kad *PDE2* geno inaktyvacija sumažina ląstelių gyvybingumą po rūgštinio šoko indukcijos. Galima teigti, kad *Pde2p* yra teigiamas ląstelių gyvybingumo reguliatorius rūgštinio šoko sąlygomis. Dvi mielių fosfodiesterazės vaidina skirtingus vaidmenis ląstelių gyvybingumo reguliacijoje rūgštinėmis sąlygomis.