Short-chain fatty acids as metabolic inhibitors and a carbon source for yeasts

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Summary. The aim was to study C_1—C_5 fatty acids as inhibitors and inhibitory substrates for yeasts and the sequence of their utilization in the mixtures of acids with glucose. *Candida tropicalis* cannot metabolize formic acid as the only carbon source. In the presence of another carbon source formic acid is metabolized to CO_2. C_2—C_5 fatty acids can be used as the sole carbon source for the studied microorganisms in cultivation media in the range about pH 6,0. Acetic acid is utilized simultaneously with glucose. Diauxic growth in the mixtures of saccharides and propionic or butyric acid was observed.

Short-chain fatty acids form a substantial part of the total organic carbon of various secondary sources. Therefore, they should be considered as potentially important substrates for biomass production [1, 2]. On the other hand it is generally known that the short-chain fatty acids act as inhibitors of microbial metabolism [3, 4]. Yeasts and yeast-like microorganisms metabolize short-chain fatty acids in the cultivation media at subinhibitory concentrations depending on physical conditions such as temperature, pH value, presence of other natural substrates such as assimilable and fermentable sugars, presence or absence of oxygen and also depending on the metabolic properties of microorganisms [5,6].

Material and methods

Yeasts and yeast-like microorganisms from the Czechoslovak Collection of Yeasts in Bratislavy were used:

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Leucosporidium scotii CCY 64-1-1

Rhodotorula gracilis CCY 20-8-1

Candida tropicalis CCY 29-7-33 isolated from beech wood prehydrolysate sulution

Rhodotorula glutinis CCY-20-2-1.

Ustilago sp. [7] and *Rhodotorula glutinis* [7].

Microorganisms were cultivated in synthetic medium [8] buffered by phosphate to pH 6,0 with glucose and/or short-chain fatty acids as a sole carbon source.

Aerobic cultivation was carried out in a laboratory fermentor or in cultivation flasks on a rotary shaker at 28 °C.

The growth of the microorganisms was determined by measuring changes in optical density at 620 nm (SPECORD UV VIS), converted to dry matter concentrations.

The inhibitory effect of acids was estimated from the growth curve shapes.

The consumption of glucose was measured by the o-toluidine method (Biola test).

The concentration of short-chain fatty acids was measured by capillary isotachophoresis.

^{14}C-formic acid, 1-^{14}C-acetic acid and 1-^{14}c-propionic acid were used for radiometric assays of the rate of uptake and for metabolic studies. The chemicals were purchased from the Institute for Research, Production and Utilization of Radioisotopes, Prague.

$^{14}CO_2$ released during cultivation was trapped in sodium hydroxide and measured after dilution in a liquid scintilator solution on liquid scintilation counter LKB 1217 RACKBETA. ^{14}C incorporation into yeast cells was measured on membrane filters Synpor 2, pore 2.5 μm (Synthesia, Czechoslovakia) on Geiger-Müller tube Tesla NQZ 612. Respiration of yeasts was estimated using polarographic Clark oxygen electrode.

Results and discussion

Candida tropicalis CCY 29-7-33 can grow well in a synthetic cultivation medium with 5 g.l^{-1} glucose at pH 6.0 under aeration in the presence of formic acid, acetic acid, propionic acid, butyric acid and valeric acid in the concentration of 0.02 mol.l^{-1}. The growth is accompanied by the prolongation of lag-phase when acid concentration in the medium is increased. No growth is observed when the concentration of acids exceeds 0.4 mol.l^{-1}, with the exception of acetic acid which inhibits the growth above 1.0 mol.l^{-1} at given pH.

C_2-C_5 short-chain fatty acids can be utilized as the only carbon source by the studied microorganisms in the cultivation medium in the range about pH 6.0 (Fig. 1). *Candida tropicalis* utilized acetic acid, propionic acid, butyric and isobutyric acids in a concentration up to 0.4 mol.l^{-1} and valeric acid to a concentration 0.02 mol.l^{-1}. A significant lag-phase can be seen on the growth curves, when inoculum grew on glucose medium. It can be explained by adaptation of microorganisms in the new substrate (synthesis of new enzymes for fatty acid metabolism). Since the fatty acids are toxic substrates and there is not any other easy utilizing source of carbon in the medium, the lag-phase on the growth curves is very long.

Formic acid as a single carbon source in the synthetic medium at pH 6.0 is not utilized by any of the studied microorganisms. In the same conditions and in the presence of glucose, formic acid at the subinhibitory concentrations is degraded up to CO_2. In cell free extracts of *C. tropicalis* isolated from cells cultivated in the medium containing $H^{14}COOH$ and glucose activity of NAD-dependent formate dehydrogenase was detected. Formic acid is metabolized at the same time with glucose consumption. After the exhaustion of glucose the growth is stopped and lysis of the yeast cells occurs.

Acetate as a typical intermediate of cell metabolism is well metabolized by yeast cells under aerobic conditions, as the sole carbon and energy sources

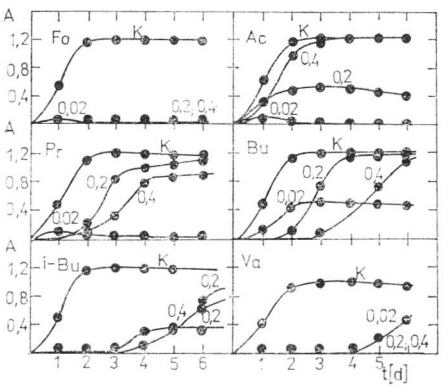

Fig. 1. Growth curves of *C. tropicalis* in the synthetic medium at 28 °C, pH 6.0 under aeration with short-chain fatty acids as the only carbon source. Fo — formic acid, Ac — acetic acid, Pr- propionic acid, Bu — butyric acid, i-Bu — isobutyric acid, Va — valeric acid. Numbers near curves indicate concentration of substrate in mol.l^{-1}. K — control, source of carbon 10 g.l^{-1} glucose.

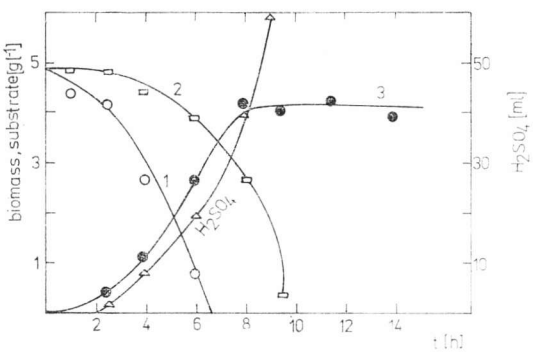

Fig. 2. Cultivation of *C. tropicalis* in the synthetic medium with glucose and acetic acid. 1 — glucose concentration, 2 — acetic acid concentration, 3 — biomass. Laboratory fermentor, batch cultivation, aeration, pH 6.0 at 28 °C. pH-stat (0.5 mol.l^{-1} H_2SO_4).

Acetate is partially oxidized to CO_2 for obtaining energy, partially incorporated into all cell fractions of the cell. Acetic acid in the presence of glucose is utilized at one stroke without diauxia (Fig. 2). In the first stage of cultivation acetate is not utilized as it was indicated by capillary isotachophoresis of the acetate estimation in the cultivation medium, even the concentration increases moderately (Fig. 3) while experiments with labelled acetate showed its decline. Acetic

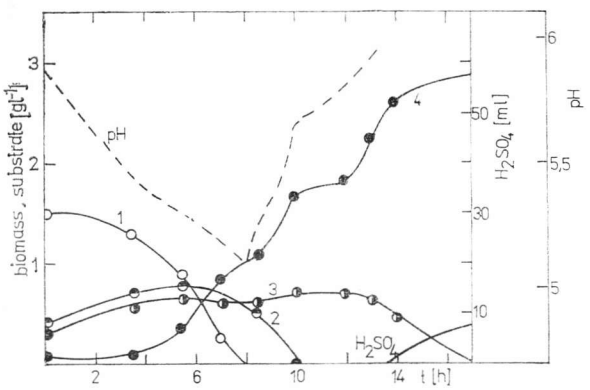

Fig. 3. Cultivation of *C. tropicalis* in the synthetic medium with glucose, acetic acid and propionic acid. 1 — glucose concentration, 2 — acetic acid concentration, 3 — propionic acid concentration, 4 — biomass. Laboratory fermentor, batch cultivation, aeration, pH = 6.0 at 28 °C pH-stat (1 mol.l^{-1} H_1SO_4).

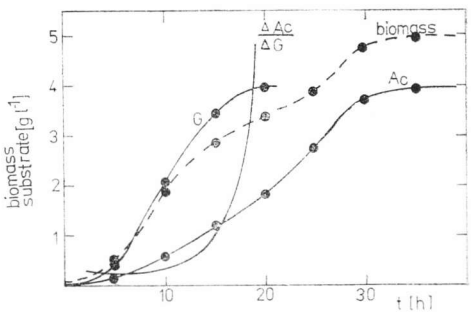

Fig. 4. Acetate consumption per unit of glucose consumption — Ac/G; consumption of acetate — Ac; consumption of glucose — G. Cultivation of *C. tropicalis*, see Fig. 3.

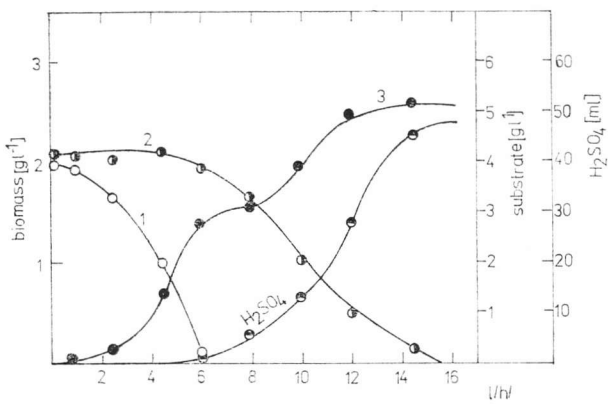

Fig. 5. Cultivation of *C. tropicalis* in the synthetic medium with glucose and propionic acid. 1 — glucose concentration, 2 — propionic acid concentration, 3 — biomass. Laboratory fermentor, batch cultivation, aeration, pH 6.0 at 28 °C. pH-stat (0.5 mol.l^{-1} H_2SO_4).

acid is utilized from the beginning of the cultivation in one stroke with glucose (Fig. 4). It is supposed that increase of acetic acid in the cultivation medium is caused by the formation of acetate „de novo". The rate of the formation is higher than its utilization.

Propionic acid in the presence of glucose is utilized only after the consumption of glucose. There is observed a typical diauxia with the second lag-phase on the growth curve (Fig. 5). The experiments with labelled 1-^{14}C-propionic acid indicate that carboxylic carbon forms CO_2. Only a part of ^{14}C (3.5%) is incorporated into the cell mass.

Butyric acid in the presence of glucose is utilized in the same way as propionic acid, that is with diauxia.

The sequence of utilization of two carbon substrates by *C. tropicalis* is shown in Tab. 1.

Acetic acid in concentrations up to 0.02 mol.l^{-1} in the synthetic cultivation medium at pH 6.0 can serve as the only carbon source for all studied yeasts (Fig. 6). *Candida tropicalis, Leucosporidium scotii, Rhodotorula glutinis* [7] to the concentration of acetic acid 0.15 mol.l^{-1}.

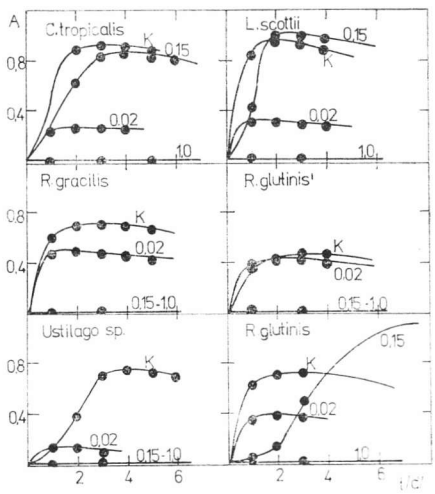

Fig. 6. Growth curves of the studied yeasts in the synthetic medium with acetic acid as the only carbon source at pH 6.0, 28 °C under aeration. Numbers near curves indicate concentration of acetic acid in mol.l^{-1}. K — control, growth on glucose (5 g.l^{-1}) without acetic acid. *R. glutinis'* — CCY 20-2-1.

C. tropicalis, R. glutinis [7] and *Ustilago* sp. [7] can use propionic and butyric acid as the only carbon source up to concentrations 0.02 mol.l^{-1}, as well.

The presence of inhibitors changes the respiratory activity of microorganisms. We have studied the conditions of the weakest inhibitory effect of short-chain fatty acids for yeasts. These studies indicated that pH values of cultivation medium influence the inhibitory effect of the respiration of yeasts. The inhibitory effect of these acids is shown in Tab. 2.

Our results are comparable with the results of another authors mentioned.

Tab. 1. The sequence of utilization of the second substrate in the presence of glucose in synthetic medium at pH 6.0 under aeration at 28 °C, *C. tropicalis*. The concentration of acids and saccharides — 5 g.l^{-1}.

Substrate	Sequence of utilization
glucose + formate	simultaneously
glucose + acetate	simultaneously
glucose + lactate	simultaneously
glucose + propionate	diauxia
glucose + butyrate	diauxia
glucose + isobutyrate	diauxia
glucose + valerate	diauxia
glucose + xylose	simultaneously
glucose + arabinose	without utilization
glucose + sorbose	diauxia
glucose + ethanol	diauxia
ethanol + acetate	simultaneously

Tab. 2. The inhibitory effect of short-chain fatty acids (concentration 10 g.l^{-1}) on respiration *C. tropicalis* after 1 hour cultivation on phosphate buffer with 20 g.l^{-1} glucose under aeration at 28 °C.

Acid	Inhibition %			
	pH			
	4.0	5.0	6.0	7.0
formic	100	0	0	0
acetic	100	20	0	0
propionic	100	60	0	0
butyric	100	50	15	25
isobutyric	100	50	35	60
valeric	100	30	20	20

The inhibitory concentration of short-chain fatty acids is dependent on cultivation conditions and yeasts studied.

References

1. ŠESTÁKOVÁ, M.: Folia Microbiol *21*, 1976, p. 384
2. MELL, H. — BRONDER, M. — KROGER, A.: Arch. Microbiol. *131*, 1982, p. 225.
3. HUŇKOVÁ, Z. — FENCL, J.: Biotechnol. Bioeng. *19*, 1977, p. 1622
4. MOON, N. J.: Appl. Bacteriol. *55*, 1983, p. 453
5. HASEGAVA, Y. — HIGACHI, K. — OBATA, H. — TOKUYAVA, T.: Canad. J. Microbiol. *28*, 1982, p. 942.
6. MANIAN, S. S. — O'GATA, F.: Arch- Microbiol. *131*, 1982, p. 51

7. KOCKOVÁ-KRATOCHVÍLOVÁ, A. — CONTRERAS, O. R.: Yeast-like microorganisms isolated from plant material on Cuba. In: Česká mykológia (in press).
8. PIRT, S. J.: J. Gen. Microbiol. *47*, 1967, p. 181

Низшие жирные кислоты в качестве ингибиторов обмена веществ и источников углерода для дрожжей

Резюме

Целью работы было изучение жирных кислот C_1—C_5 в качестве ингибиторов и ингибирующих субстратов для дрожжей, и последовательности их утилизации в смесях кислот с глюкозой. *Candida tropicalis* не способна к метаболизму муравьиной кислоты как единственного источника углерода. В присутствии другого источника углерода муравьиная кислота метаболизована на CO_3.

Жирные кислоты C_2—C_5 могут быть использованы в качестве единственных источников углерода для исследованных микроорганизмов в культурных средах с pH около 6,0. Уксусная кислота утилизирована вместе с глюкозой. Диауксический рост наблюдался в смеси сахаридов и пропионовой или масляной кислоты.

Nižšie mastné kyseliny ako inhibítory metabolizmu a zdroje uhlíka pre kvasinky

Súhrn

Cieľom práce bolo študovať C_1—C_5 mastné kyseliny ako inhibítory a inhibičné substráty pre kvasinky a sekvenciu ich utilizácie v zmesiach kyselín s glukózou. *Candida tropicalis* nie je schopná metabolizovať kyselinu mravčiu ako jediný zdroj uhlíka. V prítomnosti iného zdroja uhlíka je kyselina mravčia metabolizovaná na CO_2.

C_2—C_5 mastné kyseliny sa môžu využiť ako jediné zdroje uhlíka pre študované mikroorganizmy v kultivačných médiách, pH okolo 6,0. Kyselina octová je utilizovaná súčasne s glukózou. Diauxický rast sa pozoroval v zmesi sacharidov a kyseliny propiónovej alebo maslovej.