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ORIGINAL PAPER

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Controlled transient changes reveal differences in metabolite production in two *Candida* yeasts

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Abstract Physiological responses during growth on xylose and the xylose-degrading pathway of *Candida tropicalis* and *Candida guilliermondii* yeasts were investigated. The responses to a linearly decreasing oxygen transfer rate and a simultaneously increasing dilution rate were compared. *C. guilliermondii* produced acetate but no ethanol, and *C. tropicalis* ethanol but no acetate under oxygen limitation. Both strains produced glycerol. The D-xylose reductase of *C. guilliermondii* is exclusively NADPH-dependent, and acetate production regenerated NADPH. The xylose reductase of *C. tropicalis* has a dual dependency for both NADH and NADPH. It regenerated NAD by producing ethanol. Both strains regenerated NAD by producing glycerol. The effect of intracellular NADH accumulation to xylose uptake and metabolite production was studied by using formate as a cosubstrate. Formate feeding in *C. tropicalis* triggered the accumulation of glycerol, ethanol and xylitol. Consequently, the specific xylose consumption increased 28% during formate feeding, from 477 to 609 C-mmol/C-mol cell dry-weight (CDW)/h. In *C. guilliermondii* cultures, formate feeding resulted only in glycerol accumulation. The specific xylose consumption increased 6%, from 301 to 319 C-mmol/C-mol CDW/h, until glycerol started to accumulate.

Introduction

The first three enzymes in the xylose metabolizing pathway in yeasts are D-xylose reductase (XR; E.C. 1.1.1.21), xylitol dehydrogenase (XDH; E.C. 1.1.1.9) and xylulokinase (XK; E.C. 2.7.1.17) (Smiley and Bolen 1982; Lachke and Jeffries 1986; Slininger et al. 1987; Prior et al. 1989; Hahn-Hägerdahl et al. 1994). XR reduces xylose into xylitol, and XDH oxidizes xylitol into xylulose. In the last step before the pentose phosphate pathway, XK phosphorylates xylulose into xylulose-5-phosphate. XR can use either cofactor, NADH or NADPH, but XDH is almost exclusively NAD-dependent (Winkelhausen and Kuzmanova 1998). XK requires ATP. Under oxygen-limited conditions xylitol formation from xylose is enhanced due to the accumulation of intracellular NADH and subsequent inhibition of NAD-dependent XDH (Oh et al 1998; Hahn-Hägerdahl et al. 1994). In *C. guilliermondii*, the xylitol flux increases as a function of the linearly deepening oxygen limitation and accumulation of NADH (Granström et al. 2001). There have been several studies on the effect of oxygen on xylitol production (Hahn-Hägerdahl et al. 1994; Vandeska et al. 1995; Oh et al. 1997; Girio et al. 1994). The role of a possible xylose permease in *Candida* yeasts and its effects on xylose uptake in different conditions is currently not well understood. XK is often identified as the bottleneck enzyme in ethanol production from xylose by recombinant yeasts (Deng et al. 1990). When considering xylitol production from xylose, the most significant bottlenecks are the uptake of xylose and cofactor regeneration for XR activity.

Depending on the cofactor dependency of XR, the catabolism of xylose by yeast cells differs. Cofactor recirculation is plausible when XR depends on both NADH and NADPH. The NAD generated by XR is reduced to NADH by XDH, whereas NADP is reduced to NADPH by glucose-6-phosphate dehydrogenase (Kötter and Ciriacy 1993). According to Verduyn et al. (1985), NADH-dependent XR is a prerequisite for anaerobic alcohol fermentation of xylose by *Pichia stipitis*. Lachke and Jeffries (1986) studied mutants of *Pachysolen tannophilus* Y-2460 and concluded that a high NADH/NADPH-dependency ratio of XR is favorable to ethanol production, whereas high XK activity favors growth. Eliasson et al. (2000) constructed a xylose-utilizing recombinant *Saccharomyces cerevisiae* strain, TMB 3001. Genes from *P. stipitis* encoding an NADH/NADPH-dependent XR and an NAD-dependent XDH were integrated into

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the *S. cerevisiae* genome thereby facilitating anaerobic xylose fermentation into ethanol in the presence of glucose.

In this study we have compared two *Candida* yeasts that are able to produce high levels of xylitol from xylose, but which have a different XR cofactor dependency. The XR of *Candida tropicalis* has a dual dependency on NADH and NADPH (Horitsu et al. 1992; Granström, unpublished result), whereas the XR of *Candida guilliermondii* is exclusively NADPH-dependent (Granström et al. 2001). Does this difference in cofactor dependency have an effect on xylose uptake and xylitol production under oxygen-limited conditions? And what are the physiological consequences of intracellular accumulation of NADH under fully aerobic conditions? To answer these questions two different approaches were used. First, the combined effect of oxygen limitation and substrate flux on the redox balance was studied. The oxygen transfer rate was decreased linearly while the substrate flux was simultaneously increased. Kask et al. (1999) and Müller et al. (1985) used a similar approach but varied only one parameter at a time. These two parameters, oxygen transfer rate and substrate flux, are very informative when studying the effects of redox balance on the physiology of these yeasts. Second, we studied the effect of formate on the redox balance under fully aerobic conditions. Utilization of formate proceeds via an NAD-dependent formate dehydrogenase (FDH; E.C. 1.2.1.2) resulting in carbon dioxide and NADH. The steady-state cell yield of *Candida utilis* on glucose increased with increasing amount of formate in the medium until growth became C-limited (Bruinenberg et al. 1985). It is known that formate is not consumed as a sole carbon source by *C. tropicalis*, and its FDH is an inducible enzyme (Šmogrovicová et al. 1987). We assumed that formate feeding and subsequent accumulation of intracellular NADH would lead to enhanced xylose utilization and xylitol formation in *C. tropicalis* cultures, due to the dual cofactor dependency of its XR, but not in cultures of *C. guilliermondi*. As assumed, both of these approaches revealed the difference in metabolite production pattern between the two yeasts.

Materials and methods

Organism, maintenance and inoculum preparation

Candida tropicalis VTT-C-78086 (ATCC 1369) and *Candida guilliermondii* VTT-C-71006 were obtained from VTT Biotechnology (Espoo, Finland). Frozen stock cultures containing 20% (w/v) glycerol were stored in 2 ml ampoules at –70 °C. Inoculum for fermentation was prepared in 250-ml shake flasks grown overnight on YPD-medium at 30 °C and 200 rpm. YPD-medium contained 10 g yeast extract (Difco)/l, 20 g bactopeptone (Difco)/l and 20 g glucose (Fluka)/l.

Growth medium and formate solution

Mineral medium was prepared according to Verduyn et al. (1992). The medium contained per liter: $(NH_4)_2SO_4$ 5.0 g, KH_2PO_4 3.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, EDTA 15 mg, $ZnSO_4 \cdot 7H_2O$ 4.5 mg, $CoCl \cdot 6H_2O$ 0.3 mg, $MnCl_2 \cdot 4H_2O$ 1 mg, $CuSO_4 \cdot 5H_2O$ 0.3 mg, $CaCl_2 \cdot 2H_2O$ 4.5 mg, $FeSO_4 \cdot 7H_2O$ 3 mg, $NaMoO_4 \cdot 2H_2O$ 0.4 mg, H_3BO_4 1 mg, KI 0.1 mg and silicon-based antifoam agent 0.05 mg (BDH). Mineral medium was autoclaved for 20 min at 120 °C. After autoclaving a filter-sterilized vitamin solution was added to a final concentration per liter of 0.05 mg biotin, 1 mg calcium panthotenate, 1 mg nicotinic acid, 25 mg *myo*-inositol, 1 mg pyridoxal hydrochloride, and 0.2 mg *para*-aminobenzoic acid. Xylose was sterilized at 110 °C for 20 min and added separately to the growth medium. In the transient cultivations, all the medium components were doubled due to the high concentration of xylose in the medium (50 g/l) corresponding to 1.67 C-mol/l. Culture purity was monitored on a regular basis by phase-contrast microscopy. Formate was autoclaved and then added to a final concentration of 115 g/l (2.5 C-mol/l) using a peristaltic pump. The dilution rate range for formate feeding of *C. tropicalis* and *C. guilliermondii* was 0.00–0.015 h^{-1} and 0.00–0.044 h^{-1}, respectively.

Chemostat and transient experiments

All cultivations were started using the chemostat conditions. The cultivations were carried out in a 2-l fermenter (Braun MD) in mineral medium at 30 °C with a stirrer speed of 2,000 rpm. The culture pH was set at 5.0 and the dilution rate was adjusted accordingly. The 1-l working volume was kept constant by removing the effluent with a peristaltic pump (Watson-Marlow 505U) that was connected to a PID-controlled load cell. The actual working volume was determined at the end of each experiment. The culture pH was kept constant by addition of 2 M KOH. The airflow rate was set to maintain the dissolved oxygen concentration above 30% in all fully aerobic cultivations and it was controlled using a mass-flow controller (Bronkhurst HiTec, Ruurlo, Holland). The dissolved oxygen concentration was measured with an O_2-electrode (Ingold). Transient oxygen-limited conditions were created by a profile, in which agitation speed decreased from 2,000 rpm to 100 rpm within 10 h. Feeding profiles for the cosubstrate formate and the carbon substrate xylose were carried out with peristaltic pumps (Watson-Marlow 101U), which were controlled by the central unit of the fermenter. Five working-volume changes were allowed to take place to ensure steady-state conditions before commencing the transient-state experiments.

Exhaust gas analysis

The fermentation exhaust gas was cooled to 4 °C in a condenser to prevent the evaporation of volatile compounds before entering the mass spectrometer (VG-Prima 600). Carbon dioxide, oxygen, argon and nitrogen were analyzed from exhaust gas. In calculating the oxygen consumption and carbon dioxide production rates, a temperature of 30 °C was assumed and air pressure was taken from the daily weather forecast. During the transient-state experiment, exhaust gas values were monitored continuously.

Cell dry-weight measurements

Culture samples (10 ml) were vacuum-filtered through preweighed nitro-cellulose filters (0.45 µm, Schleicher and Schuell), washed with Milli-Q water, and then dried in a microwave oven for 20 min (Ignis, Japan). During transient-state experiments, dry-weights were measured after 0, 3, 6 and 10 h in order to minimize the culture volume used for sampling. Dry weights between the sampling points were interpolated. In all specific-rate calculations, a 45% carbon content of the biomass was assumed.

Substrate and metabolite analysis

Sample volumes of 1.2 ml collected from the chemostat cultures were centrifuged at 10,000 rpm for 5 min (Heraeus Sepatech, Bio-

fuge A, Germany) and the supernatant was analyzed during the experiment. Xylose, formate, xylitol, glycerol, acetate and ethanol concentrations were analyzed by HPLC. The set-up consisted of an HPX-87H Aminex ion-exclusion column (Bio-Rad) and two detectors in series, a Waters 410 refractive index and a Waters 486 UV-detector. The column was maintained at 60 °C and eluted with 5 mM H_2SO_4 at a flow rate of 0.6 ml/min.

Ammonia assay

Ammonium was measured according to the method of Bergmeyer (1985). The assays were carried out with a Shimadzu UV-2100 spectrophotometer.

Results

The effects of transient changes on *C. tropicalis*

Effects of a linearly increasing dilution rate and a decreasing agitation rate on specific oxygen and carbon dioxide transfer rates and dissolved oxygen value are shown in Fig. 1A, B. The physiological responses are presented in Fig. 2. The dilution rate was increased from 0.089 h^{-1} to 0.389 h^{-1} and the agitation speed was decreased from 2,000 rpm down to 100 rpm in 10 h. The specific oxygen consumption rate (qO_2) and specific carbon dioxide production rate (qCO_2) started to decrease after 4 h, when the dilution rate had reached 0.21 h^{-1}. The onset of xylitol and xylose accumulation occurred after 6 h. Both glycerol and ethanol accumulation took place after 6 h. The highest specific xylitol production, 196 C-mmol/C-mol cell dry-weight (CDW)/h, was measured at the end of the experiment with the dilution rate of 0.39 h^{-1}. The highest specific production of ethanol and glycerol was after 9 h at the dilution rate of 0.36 h^{-1} (3.93 and 4.53 C-mmol/C-mol CDW/h, respectively). No acetate was produced. The accumulation of xylose together with xylitol production occurred when qO_2 started to decrease. This was accompanied by a decrease in biomass yield on xylose (Yxs) after 6 h and continuing until the end of the experiment. The Yxs decreased from 0.469 to 0.368 C-mol CDW/C-mol xylose. This indicated a change from carbon-limited to oxygen-limited conditions. The ammonium (NH_3) content varied between 7.9 and 112 mmol/l, indicating that cultivation was not ammonium-limited. The highest accumulation of xylose was 1504 C-mmol/C-mol CDW/h and occurred after 10 h.

The effect of transient changes on *C. guilliermondii*

The same experiment was repeated with *C. guilliermondii* (Fig. 3), in which XR is exclusively NADPH dependent. The highest specific production rate of glycerol, acetate and xylitol occurred at the end of the experiment at a dilution rate of 0.39 h^{-1} (8.40, 5.46 and 92.3 C-mmol/C-mol CDW/h, respectively). The onset of acetate accumulation occurred after 6 h followed by

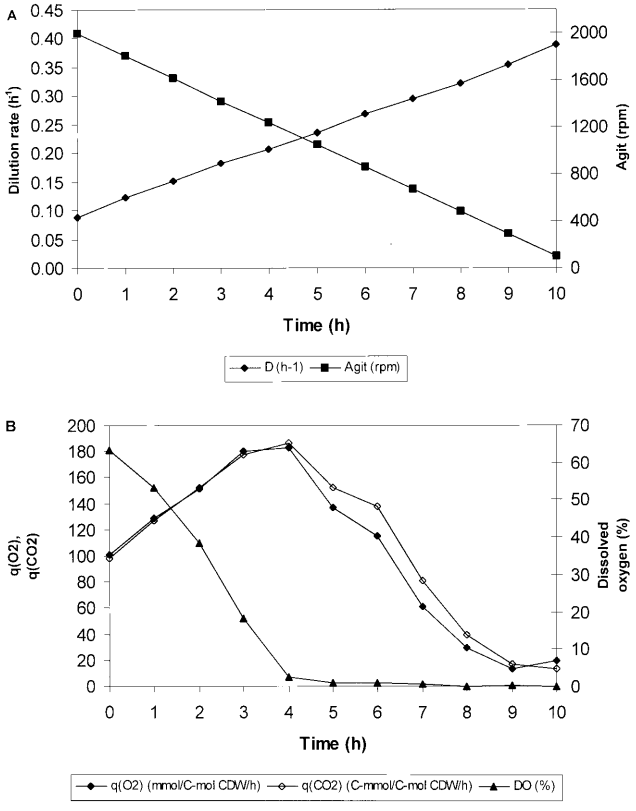

Fig. 1A,B The effect of a linearly increasing dilution rate and a decreasing agitation rate (**A**) on the specific oxygen transfer rate (qO_2), specific carbon dioxide production rate (qCO_2), and dissolved oxygen (DO) value (**B**) in *Candida tropicalis*. The profiles were introduced into steady-state culture

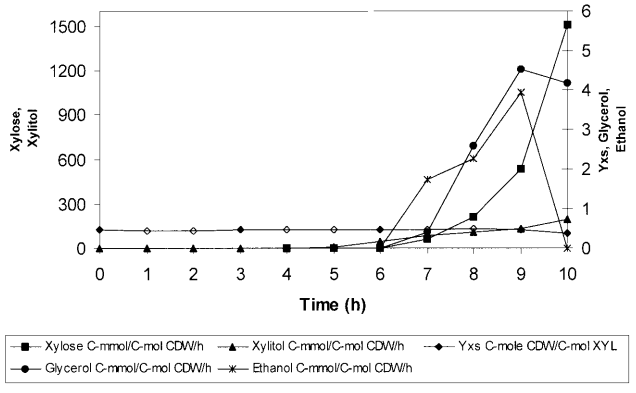

Fig. 2 The response of *C. tropicalis* to controlled transient conditions. The dilution rate increased from 0.089 h^{-1} to 0.389 h^{-1} and the agitation rate decreased from 2,000 rpm to 100 rpm within 10 h. Both profiles were introduced into steady-state culture. The yeast was grown on a mineral medium with xylose as carbon substrate at 30 °C at pH 5. *Open diamonds* on the curve showing biomass yield on xylose (*Yxs*) indicate the extrapolated value for biomass between the sampling points

glycerol after 7 h. No ethanol was produced. Xylose began to accumulate after 4 h together with xylitol, indicating a change from C-limited to O_2-limited conditions. The decrease in Yxs after 3 h and continued until the end of the experiment. The Yxs decreased from 0.490 to

Table 1 The effect of accumulation of intracellular NADH in *Candida tropicalis* was studied by introducing a formate gradient. Steady state was established before commencing the formate gradient. The dilution rate of xylose (1.67 C-mol/l) was set to 0.26 h^{-1} and the agitation speed was 2,000 rpm. The formate (2.5 C-mol/l) gradient changed from 0.0 to 0.015 h^{-1} within 10 h. *Yxs* Biomass yield on xylose, *XYL* xylose, *FOR* formate, *CDW* cell dry-weight

Time (h)	Yxs (C-mol CDW/C-mol XYL–FOR)	Consumption Xylose (C-mmol/C-mol CDW/h)	Consumption Formate (C-mmol/C-mol CDW/h)	Production Xylitol (C-mmol/C-mol CDW/h)	Production Glycerol (C-mmol/C-mol CDW/h)	Production Ethanol (C-mmol/C-mol CDW/h)
0	0.537	477	0.0	1.1		
1	0.533	479	3.8	1.2		
2	0.525	485	8.4	1.2		
3	0.515	493	13.3	1.2		
4	0.502	504	18.6	1.7	0.0	
5	0.488	517	23.2	7.3	0.9	0.0
6	0.473	533	28.4	10.4	2.0	6.6
7	0.463	545	32.1	10.5	2.0	7.0
8	0.449	562	35.1	11.1	6.3	7.4
9	0.434	583	39.1	11.5	9.2	6.7
10	0.416	609	43.4	11.3	12.2	4.9

Table 2 The effect of accumulation of intracellular NADH in *C. guilliermondii* was studied by introducing a formate gradient. The steady state was established before commencing the formate gradient. The dilution rate of xylose substrate (1.67 C-mol/l) was set to 0.16 h^{-1}. Agitation speed was 2,000 rpm. The formate (2.5 C-mol/l) gradient changed from 0.0 to 0.044 h^{-1} within 12 h. For abbreviations, see Table 1

Time (h)	Yxs (C-mol CDW/C-mol XYL–FOR)	Consumption Xylose (C-mmol/C-mol CDW/h)	Consumption Formate (C-mmol/C-mol CDW/h)	Production Glycerol (C-mmol/C-mol CDW/h)
0	0.521	301	0.0	
1	0.497	312	11.4	
2	0.482	318	23.3	0.0
3	0.475	319	34.9	0.1
4	0.474	316	46.4	0.2
5	0.476	312	56.5	0.3
6	0.480	307	67.1	0.4
7	0.482	302	77.1	0.5
8	0.483	299	87.2	0.6
9	0.479	299	97.7	0.7
10	0.470	303	109.8	0.9
11	0.453	312	124.1	1.3
12	0.427	329	142.3	1.7

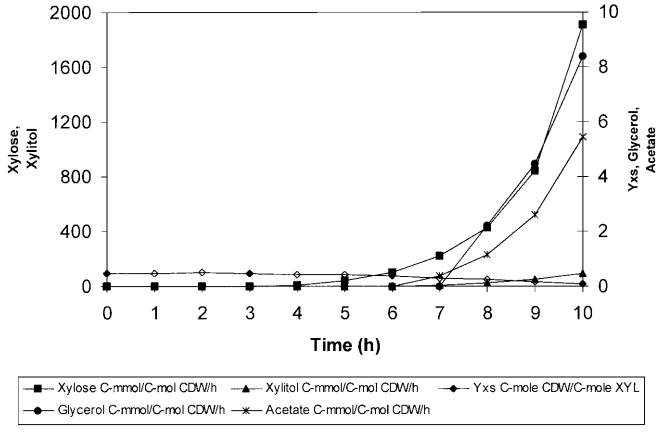

Fig. 3 The response of *Candida guilliermondii* to controlled transient conditions. The dilution rate increased from 0.100 h–1 to 0.385 h–1and the agitation rate decreased from 2,000 rpm to 100 rpm within 10 h. Both profiles were introduced into steady-state culture. The yeast was grown on a mineral medium with xylose as carbon substrate at 30 °C at pH 5. *Open diamonds* on the curve showing biomass yield on xylose (*Yxs*) indicate the extrapolated value for biomass between the sampling points

0.073 C-mol CDW/C-mol xylose. The NH_3content of the samples varied between 38.4 and 146.4 mmol/l. The highest accumulation of xylose was 1,931 C-mmol/C-mol CDW/h and occurred at 10 h.

The effect of formate feeding on *C. tropicalis*

Transient responses were studied further by using formate as a cosubstrate under fully aerobic conditions. With *C. tropicalis*, the dilution rate was set to D=0.26 h^{-1}, corresponding to the value at which xylose began to accumulate (Fig. 2). A dilution rate close to the maximum value (D_{max}) was chosen to bring about visibly the effects of formate on xylose consumption and metabolite production. The transient responses of *C. tropicalis* to formate feeding are presented in Table 1. The biomass yield on xylose and formate carbon decreased steadily from 0.537 to 0.416 C-mol CDW/C-mol xylose. Some xylitol was detected already in the beginning of formate feeding, but it began to accumulate to-

gether with glycerol after 4 h followed by ethanol after 5 h. Formate started to accumulate after 5 h, when specific formate consumption was 23 C-mmol/C-mol CDW/h (data not shown). At the end of the experiment, specific formate consumption was 43 C-mmol/C-mol CDW/h. Specific xylose consumption increased from 477 to 609 C-mmol/C-mol CDW/h (28%). Residual xylose was found after 5 h (data not shown).

The effect of formate feeding on *C. guilliermondii*

The transient responses of *C. guilliermondii* to formate feeding are presented in Table 2. The dilution rate was set to D=0.16 h^{-1}, corresponding to the value at which xylose began to accumulate (Fig. 3). The biomass yield on xylose and formate carbon decreased from 0.521 to 0.427 C-mol CDW/C-mol xylose. Glycerol began to accumulate after 3 h. No xylitol accumulated during the experiment. Formate began to accumulate after 4 h, when specific formate consumption was 46 C-mmol/C-mol CDW/h (data not shown). At the end of the experiment, specific formate consumption was 142 C-mmol/C-mol CDW/h. Specific xylose consumption increased from 301 to 319 C-mmol/C-mol CDW/h (6%), but started to decrease when glycerol began to accumulate. The experiment was continued for 12 h at which time residual xylose was found.

Discussion

The designed profile decreased the specific oxygen transfer rate linearly after 4 h and onwards (Fig. 1A, B). The conditions prevailing under the transient changes could be reproduced in a chemostat by terminating both profiles at a desired time point and by allowing cultures to achieve a steady state. Two separate oxygen-limited steady states carried out using these profiles gave mean values of 774±26 C-mmol/C-mol CDW/h, 246±12 C-mmol/C-mol CDW/h and 202±4.8 mmol/C-mol CDW/h for specific xylose consumption, qCO_2 and qO_2, respectively (Granström et al. unpublished data).

C. tropicalis and *C. guilliermondii* accumulated xylitol, glycerol and xylose but differed in ethanol and acetate accumulation. *C. tropicalis* accumulated ethanol but not acetate, whereas *C. guilliermondii* accumulated acetate, but not ethanol. Acetate production by *C. guilliermondii* indicates regeneration of NADPH through NADP-dependent acetaldehyde dehydrogenase (E.C. 1.2.1.3). Although most of the NADPH is generated through the pentose phosphate pathway (Bruinenberg et al. 1985), acetate accumulation suggests that NADPH becomes a limiting factor for xylose conversion to xylitol under these conditions. In *C. tropicalis*, NADPH limitation is partly compensated by NADH assimilation. This leads to higher xylose consumption and xylitol accumulation than observed in *C. guilliermondii*, since NADH is used both for converting xylose to xylitol and for inhibiting xylitol dehydrogenase. Ethanol and glycerol production allows regeneration of NAD. There are number of reactions that can contribute to the intracellular redox balance, such as malic enzyme, malate dehydrogenase, glucose-6-phosphate dehydrogenase, etc. However, we interpret this difference in ethanol and acetate production as being due to the differences in cofactor dependency of XR. Our results suggest that redox balance plays a significant role in xylose uptake and consumption. Furthermore, cofactor regeneration can become a primary bottleneck in xylitol production from xylose by these *Candida* yeasts. The mechanism of xylose transport is unknown under these conditions; however, many *Candida* sp. take up xylose by facilitated diffusion (Hahn-Hägerdahl et al. 1994). If this is the case, then intracellular redox balance would primarily regulate substrate uptake in these yeasts.

Formate feeding exposed both yeasts to surplus intracellular NADH, which was indicated by glycerol accumulation under fully aerobic conditions. Glycerol acts as a redox sink for excess NADH (Oura 1977). *C. tropicalis* used the surplus NADH as a cofactor together with NADPH, as evidenced by enhanced specific xylose uptake. Eventually, the synergistic effect of additional NADH and enhanced uptake of xylose will result in either total inhibition of XDH or accumulation of intracellular intermediates causing inhibition of growth. Due to the exclusive NADPH dependency of the *C. guillier-mondii* XR, the effect of formate was different. Initially it slightly facilitated specific xylose consumption, but it had no effect on either xylitol or ethanol production. Eventually, formate dissipated the proton-motive force of the cell membrane, resulting in an uncoupling effect (Dijkhuizen et al. 1977; Bruinenberg et al. 1985) and inhibition of biomass synthesis.

When considering biotechnological production of xylitol from xylose, these findings will facilitate the development of a production process in which *Candida* yeasts are used as the host organism. The dual dependency of XR allows more possibilities to control oxygen-limited conditions. In addition, agitation can be used to establish controlled oxygen limitation, and formate can be used for minor adjustments. The dual dependency of *C. tropicalis* XR on both NADPH and NADH is possibly due to only one enzyme as in *Pichia stipitis* (Verduyn et al. 1985). We are currently purifying the XR from both *C. tropicalis* and *C. guilliermondii*.

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References

Bergmeyer J (1985) Methods of enzymatic analysis, 3rd edn, vol 8. Verlag Chemie, Weinheim

Bruinenberg PM, Jonker R, van Dijken JP, Scheffers WA (1985) Utilization of formate as an additional energy source by glucose-limited chemostat cultures of *Candida utilis* CBS 621 and *Saccharomyces cerevisiae* CBS 8066. Arch Microbiol 142:302–306

Deng XX, Ho NWY, Potter AA (1990) Xylulokinase activity in various yeasts including *Saccharomyces cerevisiae* containing the cloned xylulokinase gene. Appl Biochem Biotechnol 24–25:193–199

Dijkhuizen L, Groen L, Harder W, Konings WN (1977) Active transport of oxalate by *Pseudomonas oxalaticus* OX1. Arch Microbiol 115:223–227

Eliasson A, Christensson C, Wahlbom CF, Hahn-Hägerdahl B (2000) Anaerobic xylose fermentation by recombinant *Saccharomyces cerevisiae* carrying *XYL1*, *XYL2*, and *XKS1* in mineral medium chemostat cultures. Appl Environ Microb 66:3381–3386

Gírio FM, Roseiro JC, Sá-Machado P, Duarte-Reis AR, Amaral-Collaço MT (1994) Effect of oxygen transfer rate on levels of key enzymes of xylose metabolism in *Debaryomyces hansenii*. Enzyme Microb Technol 16:1074–1078

Granström T, Ojamo H, Leisola M (2001) Chemostat study of xylitol production by *Candida guilliermondii*. Appl Microbiol Biotechnol 55:36–42

Hahn-Hägerdahl B, Jeppson H, Skoog K, Prior BA (1994) Biochemistry and physiology of xylose fermentation by yeasts. Enzyme Microb Technol 16:933–943

Horitsu H, Yahashi Y, Takamizawa K, Kawai K, Suzuki T, Watanabe N (1992) Production of xylitol from D-xylose by *Candida tropicalis*: optimization of production rate. Biotech Bioeng 40:1085–1091

Kask S, Laht TM, Pall T, Paalme T (1999) A study on growth characteristics and nutrient consumption of *Lactobacillus plantarum* in A-stat culture, Antonie van Leeuwenhoek 75: 309–320

Kötter P, Ciriacy M (1993) Xylose fermentation by *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 38:776–783

Lachke AH, Jeffries TW (1986) Levels of enzymes of the pentose phosphate pathway in *Pachysolen tannophilus* Y-2460 and selected mutants. Enzyme Microb Technol 8:353–359

Müller RH, Markuske KD, Babel W (1985) Formate gradients as a means for detecting the maximum carbon conversion efficiency of heterotrophic substrates:correlation between formate utilization and biomass increase. Biotech Bioeng 27:1599–1602

Oh D-K, Kim S-Y, Kim J-H (1998) Increase of xylitol production rate by controlling redox potential in *Candida parapsilosis*. Biotech Bioeng 58:440–444

Oura E (1977) Reaction products of yeast fermentation. Proc Biochem 12, 19–21.

Prior BA, Killian SG, du Preez JC (1989) Fermentation of D-xylose by the yeasts *Candida shehatae* and *Pichia stipitis*. Proc Biochem 24:21–32

Slininger PJ, Bolen PL, Kurtzman CP (1987) *Pachysolen tannophilus*: properties and process consideration for ethanol production from D-xylose. Enzyme Microb Technol 9:5–15

Smiley KL, Bolen PL (1982) Demonstration of D-xylose reductase and D-xylitol dehydrogenase in *Pachysolen tannophilus*. Biotechnol Lett 4:607–610

Šmogrovicová D, Augustín J, Halama D (1987) Inhibition of growth of *Candida tropicalis* by formate. Biológia (Bratislava) 42:267–275

Vandeska E, Kuzmanova S, Jeffries TW (1995) Xylitol formation and key enzyme activities in *Candida boidinii* under different oxygen transfer rates. J Ferment Bioeng 80:513- 516

Verduyn C, van Kleef R, Frank J, Schreuder H, van Dijken JP, Scheffers WA (1985) Properties of the NAD(P)H-dependent xylose reductase from the xylose-fermenting yeast *Pichia stipitis*. Biochem J 226:669–677

Verduyn C, Postma E, Scheffers WA, van Dijken JP (1992) Effect of benzoic acid on metabolic fluxes in yeasts: a continuous culture study on the regulation of respiration and alcoholic fermentation. Yeasts 8:501–517

Winkelhausen E, Kuzmanova S (1998) Microbial conversion of D-xylose to xylitol. J Ferment Bioeng 86:1–14