Hydroxylamine as the Sole Nitrogen Source for Growth of Some *Candida* sp.

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Free hydroxylamine (HA) is highly toxic for organisms. Chemical alterations of cytosine resulting in disturbed pairings with guanine [1] and disturbed protein synthesis on the t-RNA level [2] are features of its toxicity pattern at extracellular HA concentrations of the order 10^{-1} and 10^{-5} M, respectively. Consequently, organisms able to maintain growth with HA as the sole nitrogen source are known only as rare exceptions [3-5] even if HA forms an intermediate in the inorganic nitrogen metabolism.[6,7]

A strain of *Candida zeylanoides* isolated from marine environment grew on HA at low concentrations (about 10^{-4} M) and after prolonged lag phase.[8] As more pronounced HA-utilizers might be found among yeasts with similar schemes for C-source assimilation and fermentation, twelve such strains, representing eight *Candida* species, were selected for further studies and obtained by the courtesy of Dr. W. Ch. Slooff, Yeast Division, Centraalbureau voor Schimmelcultures, Delft.

Comparisons were made between growth (expressed as absorbancy at 610 nm, measured in a spectrophotometer Beckman B) at 25° in a glycerol or glucose mineral salt medium [8] (pH 4.3, shake cultures) with, on the one hand, HA at different concentrations (0.325, 1.63, 8.13, and 40.6 mM) as the sole nitrogen source and, on the other hand, ammonium sulphate (0.163, 0.813, and 16.3 mM).

Six strains: *C. conglobata* (2018 *= *Torulopsis conglobata* **), *C. marina* v. Uden et Zobell (5235), *C. norvegensis* (Dietrichson) v. Uden et Farinha (1922), *C. rugosa* (Anderson) Diddens et Lodder (613), *C. valida* (638=*Mycoderma valida* Leberle **), *C. vini* (639=*Mycoderma vini* Desmazières **), did not grow at any of the HA concentrations tested. HA added besides ammonium sulphate (16.2 mM) did nullify growth at all concentrations except the lowest one, where it prolonged the lag phase markedly, however.

Four strains: *C. catenulata* Diddens et Lodder (565), *C. silvae* Vidal-Leiria et v. Uden (5498), *C. zeylanoides* (Cast.) Langeron et Guerra (619, and 5262=*Trichosporon piscium* Siepmann **) grew after a prolonged lag phase, the longer the higher the HA concentration was—*C. zeylanoides* (5262) at HA concentrations up to 8.13 mM, *C. silvae* at 0.325 mM only, and the other two up to 1.63 mM—with slower growth rate and giving reduced maximum growth at the highest HA concentrations permitting growth, *i.e.* giving about the same growth pattern as the previously studied strain of *C. zeylanoides* [8] (see Fig. 1).

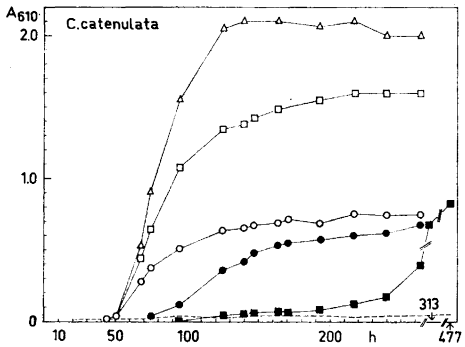

Fig. 1. Growth of *C. catenulata* (565) expressed as absorbancy (A_{610}) after various times (h) of incubation in a glycerol (0.81% w/v) mineral salt medium (10 ml) with different concentrations of hydroxylamine (HA) or ammonium sulphate as the sole nitrogen source. μg N per tube in series with HA (filled symbols) and ammonium sulphate (unfilled symbols): 45.4 (●,○); 227 (■,□); and 4540 (△). Control (no N-addition) ---.

Finally two strains: *C. lipolytica* (Harrison) Diddens et Lodder (599), and *C. lipolytica* var. *deformans* ** (2071), grew without any marked prolongation of the lag phase except at the highest concentration tested, at somewhat slower growth rate but to the same maximum growth, the former on HA concentrations up to the highest one tested (40.6 mM), the latter

* Number within brackets refers to the CBS culture collection number.

** Buckley, H. R. and van Uden in Lodder, J. *The Yeasts*, 2nd Ed., Amsterdam 1970.

Table 1. Hydroxylamine (nmoles) per ml glycerol culture media after various times (h) of growth (A_{610}) of *C. lipolytica* and *C. lipolytica* var. *deformans*. For growth (A_{610}) of *C. lipolytica*, see Fig. 2.

Time	*C. lipolytica*				*C. lipolytica* var. *deformans*					
h	HA	HA	HA	HA	HA	A_{610}	HA	A_{610}	HA	A_{610}
0	325	1 625	8 125	40 625	325		1 625		8 125	
23	60	75	3 000		120	0.18		0.09	6 000	0.05
29	30	0	3 000		80	0.24	1 500	0.15	6 000	0.07
47	20		0	3 300	40	0.38	150	0.28	1 500	0.18
71		0	0	100	40	0.43	300	0.64	0	1.4
119	15	0	1 000	1 000	15	0.48	0	1.33	0	2.1
323									1 400	2.0

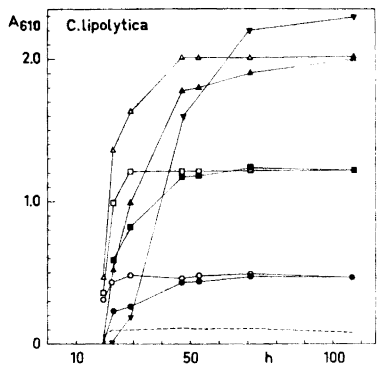

Fig. 2. Growth of *C. lipolytica* (599), expressed as absorbancy (A_{610}) after various times (h) of incubation in a glycerol (0.81 % w/v) mineral salt medium (10 ml) with different concentrations of hydroxylamine (HA) or ammonium sulphate as the sole nitrogen source. μg N per tube in series with HA (filled symbols) and ammonium sulphate (unfilled symbols): 45.4 (●,○); 227 (■,□); 1135 (▲); 5675 (▼); and 4540 (△). Control (no N-addition) ---.

up to the next highest one (8.13 mM), see Fig. 2.

None of the twelve strains did grow with hydrazine as the sole nitrogen source at any of the concentrations tested, the lowest one being 0.325 mM. All strains, however, did grow when ammonium sulphate was added besides hydrazine (0.325 mM).

During growth HA gradually disappeared from the medium (see Table 1), HA was measured by the Czăky procedure.[9] After the stationary phase had been entered, a certain amount of HA reappeared in the medium in series with the highest initial HA concentrations where presumably the C-source had been consumed.

During the reaction of cell-free preparations (*i.e.* the supernatants obtained after centrifugation of washed, HA-grown cells treated in an MSE-sonicator for 45 min) in an HA—NADPH-reaction mixture,[8] HA decreased during the first period, sometimes increased later. Ammonia was formed (measured by Conway micro diffusion technique and nesslerisation, essentially as described by Zucker and Nason [10]). Large quantities of ammonia were, however, formed also from control series without any HA addition, possibly by the reduction of "bound HA", trapped in the treated cells. The presence of "bound HA" could also explain the above-mentioned increase in HA. When tested with ferric chloride for hydroxamates,[11] the cell preparations gave a faintly positive reaction.

A lipase catalysed condensation of fatty acid carboxyl with HA is known from Lipman and Tuttle's work [11] with several types of liver preparations. The reversibility of the lipase system has been shown.[12] A detoxication of HA through the formation of "bound HA" (hydroxamates and oximes of α-ketoacids) in nitrite-grown *Torula utilis* (=*Candida utilis*) has been suggested by Virtanen and Saris.[13] *C. lipolytica* is known as a strong lipase producer.[14] Contrary to all other species it also showed strong lipolytic activity when tested by the plate method of Sierra.[15] The lipase production may contribute to the exceptional ability to utilize HA as the sole nitrogen

source for growth even at concentrations as high as for any other N-source regularly used in growth media. *C. lipolytica* and its variety may detoxicate HA by a lipase catalysed hydroxamate formation, and the hydroxamates formed may successively be hydrolysed and/or reduced by a HA reducing system during growth.

Thanks are due to Miss Birthe Jeppsson for skilful technical assistance.

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Received May 14, 1969.

Comparison of Acid Strengths of Orthophosphoric Acid, Thiophosphoric Acid, Phenylphosphonic Acid, and Monophenyl Phosphate in Aqueous Potassium Chloride Solutions

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In connection with an investigation of reactions of several phosphates in this laboratory, the values of the second dissociation constants of thiophosphoric acid (H_3PO_3S, phosphorothioic acid), phenylphosphonic acid ($PhPO_3H_2$), and monophenyl phosphate ($PhOPO_3H_2$, "phenyl phosphate") in aqueous solutions contain-

Table 1. pK_2 values of the acids at different ionic strengths (25°C).

	$\sqrt{I}$	pK_2
Orthophosphoric acid: H_3PO_4	0.098	7.042
	0.171	6.921
	0.328	6.742
	0.504	6.620
	0.993	6.443
	1.401	6.460
Thiophosphoric acid: H_3PO_3S	0.128	5.611
	0.187	5.517
	0.332	5.363
	0.500	5.228
	0.976	5.044
	1.373	5.053
Phenylphosphonic acid: $PhPO_3H_2$	0.095	7.264
	0.167	7.153
	0.320	6.994
	0.492	6.861
	0.970	6.712
	1.368	6.732
Monophenyl phosphate: $PhOPO_3H_2$	0.105	6.089
	0.145	6.040
	0.247	5.914
	0.333	5.823
	0.511	5.719
	0.715	5.629
	1.005	5.568
	1.418	5.606