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Glucose Metabolism in *Candida* Species

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The information available concerning chemical processes in *Candida* species is somewhat limited. In particular, their behaviour towards glucose does not seem to have been studied in detail. Recently, Lodder & Kreger-van Rij (1952) have brought all species of the genus of asporogenous yeast-like fungi known as *Mycoderma*, into the genus *Candida* under the name *Candida mycoderma* (Reess) Lodder & Kreger-van Rij. For the purposes of our inquiry we obtained from the Delft Collection 12 authentic cultures of *Candida* species, including representatives of the former genus *Mycoderma*. In addition, seven strains were selected from a number of cultures of *C. mycoderma*, isolated at Manchester and described by Walker & Wiles (1952).

The behaviour of these 19 organisms in defined media which contained glucose as sole source of carbon was then studied.

EXPERIMENTAL AND RESULTS

Particulars of the strains. Cultures received from the Centralbureau voor Schimmelcultures, Delft, Holland were labelled: (1) *Candida lipolytica* (Harrison) Diddens & Lodder; (2) *Pseudomonilia albomarginata* Geiger; (3) *Candida monosa* (Kluyver) Diddens & Lodder; (4) *Candida krusei* (A. Cast.) Berkhout (= *Mycoderma bordetii* Kuff); (5) *C. krusei* (A. Cast.) Berkhout (= *Mycoderma chevalieri* Guill); (6) and (7) other strains of *C. krusei*; (8) *Candida rugosa* (Anderson) Diddens & Lodder; (9) *Mycoderma lafarii* Janke; (10) *Mycoderma tannica* Asai; (11) *Mycoderma valida* Leberle; (12) *Mycoderma cerevisiae* Desmazières strain *gallica* (Leberle). The seven strains of *Candida mycoderma* which had been isolated at Manchester were designated each by the letter L followed by a number. Stock cultures were maintained on malt-wort agar containing 1% (v/v) of yeast autolysate. In view of the new classification of yeasts by Lodder & Kreger-van Rij (1952) cultures 3–7 are possibly related and cultures 9–12 also are possibly related.

Media and procedure. All the cultures, with the exception of two of the strains of *C. mycoderma*, developed strongly and formed acid in medium *A*, which consisted of: 2·5 g. of $(NH_4)_2HPO_4$, 2 g. of KH_2PO_4, 1 g. of $MgSO_4,7H_2O$, 0·01 g. of $FeSO_4,7H_2O$, 100 g. of glucose, water to 1 l.; the pH value was adjusted to 6·0. The two remaining strains showed only weak growth in this medium but developed well in medium *B*, which contained: 3 g. of $(NH_4)_2SO_4$, 3 g. of KH_2PO_4, 2 g. of $MgSO_4,7H_2O$, 50 g. of glucose, water to 1 l.; the pH value was adjusted to 6·5. Qualitative trials showed that although in these media all nineteen organisms produced pyruvic acid and ethanol from glucose, accumulation of ethanol was very low in cultures of the majority of the strains of *C. mycoderma* (L series). The latter, except in strains L4 and L6, had very weak fermentative capacities. Oxidative ability in the L series was fairly well developed, gluconic, lactic and acetic acids being detected as metabolites. In contrast to *C. mycoderma* the strains of the other species of *Candida*, particularly those of *C. krusei*, were more strongly fermentative and they also exhibited oxidative activities which varied in degree according to the strain, but such oxidative powers were not compared quantitatively with those shown by the strains of *C. mycoderma*.

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Three strains of *C. krusei* (4–6) and one strain of *C. mycoderma* (L4) were tested for ability to reproduce over a period of time in serial culture in the purely mineral media with glucose as sole source of carbon and energy. They were subcultured with one platinum loopful (3 mm. diameter) into 30 ml. of medium, at weekly intervals, with incubation at 25°. After the sixth serial transfer two of the strains of *C. krusei* grew as vigorously as they had done at their first subculture and the third strain showed a more moderate development. The strain of *C. mycoderma* showed weak development. In all four cases the metabolism solution of the sixth subculture gave positive tests for pyruvic acid, which was detected by the method of Lu (1939) and also by the test described by Caron & Raquet (1942). These reactions were not equally strong but all were quite definite. For purposes of the experiments, the cultures were grown in sets of five 1 l. conical flasks, each charged with 250 ml. of medium *A* or *B*. These flasks had necks of 50 mm. internal diameter and the depth of medium was 30 mm. In a few experiments 250 ml. flasks each charged with 50 ml. of medium were employed. After sterilization, inoculation of each flask was performed with 0·1 ml. of a young culture, which had been developed by inoculation from a malt wort-agar slope into glucose–mineral salts medium of the same composition as that with which the flasks were charged.

Preliminary experiments. The qualitative tests for pyruvic acid were confirmed by isolating it as its *p*-nitrophenylhydrazone and 2:4-dinitrophenylhydrazone, which after purification had m.p. 220° and 218° respectively, undepressed by admixture with the authentic substances. Pure specimens of the 2:4-dinitrophenylhydrazone prepared from cultures of twelve of the strains were combined and the mixture was analysed. (Found: N, 20·7. Calc. for $C_9H_8O_6N_4$: N, 20·9%.) Ethanol was confirmed by oxidation to acetaldehyde, which was characterized as the 2:4-dinitrophenylhydrazone, m.p. 162°. The largest yields of pyruvic acid were given by *C. monosa*, the three strains of *C. krusei* numbered (4), (5) and (6) and strains L3 and L4 of *C. mycoderma*. Appreciable yields of pyruvic acid were obtained also from cultures of *Mycoderma valida*, *M. lafarii* and *M. cerevisiae*, but these strains did not form so much ethanol as was obtained from comparable cultures of *C. monosa* and *C. krusei*.

Quantitative studies

Candida krusei (culture no. 5) in medium *A* and *C. mycoderma* (strain L4) in medium *B* were chosen for detailed studies because the former could set up a vigorous alcoholic fermentation, whereas L4 afforded ethanol in traces only. Glucose was estimated by the method of Willstätter & Schüdel (1918), allowance being made for the pyruvic acid present. The latter was estimated by the method of Case (1932) and ethanol by oxidation with potassium dichromate (Janke & Kropacsy, 1935). The yields shown in the tables are based on the analysis of 100 ml. of fermented medium in each case.

Estimation of accumulated pyruvic acid. Trials were carried out for the purpose of following the course of pyruvic acid formation in cultures of no. 5 and L4 (Tables 1 and 2). In both cases accumulation of pyruvic acid in the metabolism solution was followed by its decline to nil. The fact that in neither instance did this behaviour fall into line with the

Table 1. *Formation of pyruvic acid by* Candida mycoderma *L4, in a medium containing 5 g. of glucose/100 ml.*

Days	Glucose utilized (g.)	Titratable acidity (ml. of 0·1 N-NaOH)			Pyruvic acid (mg.)
		Total	Pyruvic acid, by estimation	Difference, by calculation	
5	0·48	25·5	11·1	14·4	98
7	0·80	36·5	12·5	24·0	110
8	1·02	43·2	11·5	31·7	101
11	1·52	62·4	7·2	55·2	63
14	1·80	67·0	4·3	62·7	38
18	1·99	68·0	Nil	68·0	Nil

Table 2. *Formation of pyruvic acid and ethanol by* Candida krusei *no. 5, in a medium containing 10 g. of glucose/100 ml.*

Days	Glucose utilized (g.)	Titratable acidity (ml. of 0·1 N-NaOH)			Pyruvic acid (mg.)	Ethanol (g.)
		Total	Pyruvic acid, by estimation	Difference, by calculation		
2	0·48	8·8	Trace	8·8	Trace	Trace
4	1·40	27·2	7·8	19·4	69	0·31
6	2·96	42·8	12·4	30·4	109	0·88
7	4·70	59·8	14·1	45·7	124	1·36
8	7·73	70·4	11·4	59·0	100	2·39
9	9·34	52·2	7·7	44·5	68	2·91
11	10·00	45·8	1·4	44·4	12	2·80
14	—	45·0	Nil	45·0	Nil	1·52
27	—	51·0	Nil	51·0	Nil	Trace

figures for titratable acidity shows that in each of these media another acid (or acids) was formed, in addition to pyruvic acid.

The quantity of ethanol formed by no. 5 increased up to a certain limit and then, when other suitable carbon sources were lacking, it decreased, presumably being metabolized by the organism. The residual titratable acid in cultures both of no. 5 and L 4, however, persisted for a long period of time. An account of the acids other than pyruvic acid is reserved for a further communication.

Experiments with nitrates as sources of nitrogen. Wirth & Nord (1942) and Nord & Sciarini (1944) observed accumulation of pyruvic acid in cultures of the mould *Fusarium lini* Bolley on media containing a hexose or a pentose as carbon source. They noted that whereas only traces of pyruvic acid were detectable when $(NH_4)_2SO_4$ was the source of nitrogen its substitution by KNO_3 led to a marked increase in the amount of pyruvic acid accumulating. They considered that this increase might have been caused by nitrite ions (formed by reduction of nitrate) exercising an inhibitory influence on the carboxylase system. In support of this view they obtained positive tests for nitrite in cultures in which the source of nitrogen had been nitrate, and they followed the reduction of the latter to the stage of hydroxylamine. In our experiments, when KNO_3 or $NaNO_3$ was tried as nitrogen source there was very little growth of either no. 5 or L 4, and although tests for pyruvic acid on samples from some of these cultures were positive the quantities present were very small indeed. In no case could the presence of nitrite be detected, although use was made of the very sensitive method of Griess as modified by Blom (1926). When small quantities of KNO_3 were added to the usual media in which $(NH_4)_2HPO_4$ or $(NH_4)_2SO_4$ served as source of nitrogen, the yield of pyruvic acid was not raised and nitrite was not detected subsequently in the metabolism solution.

Effects of addition of sodium nitrite to the media. Trials were made to ascertain the effects produced by addition of $NaNO_2$ to the culture media normally used for no. 5 and L 4, and in Table 3 are shown the results obtained with no. 5 on medium *A* to which nitrite was added before inoculation. It is evident that the added nitrite reduced both the rate of utilization of glucose and the amount of pyruvic acid formed. In L 4, addition of nitrite before inoculation strongly retarded growth and lowered the yield of pyruvic acid. Thus the control (no nitrite) produced 41 mg. of pyruvic acid, whereas in the presence of $NaNO_2$ (5 mg./100 ml.) the yield was 6 mg., and was only 1 mg. when $NaNO_2$ was added at 10 mg./100 ml. In these cases the several extents to which cell proliferation occurred were in the same decreasing order of magnitude as were the yields of pyruvic acid.

Effects of addition of thiamine to the media. The accumulation of pyruvic acid in cultures of no. 5 and L 4 suggested that their cells might be deficient in

Table 3. *Effects of* $NaNO_2$ *on accumulation of pyruvic acid formed by* Candida krusei *no. 5 in media containing* 10 *g. of glucose*/100 *ml.*

	Control without $NaNO_2$			Quantity of $NaNO_2$ added before inoculation: 5 mg./100 ml.			50 mg./100 ml.		
Days	Glucose utilized (g.)	Acid formed (ml. of 0·1 N-NaOH)	Pyruvic acid (mg.)	Glucose utilized (g.)	Acid formed (ml. of 0·1 N-NaOH)	Pyruvic acid (mg.)	Glucose utilized (g.)	Acid formed (ml. of 0·1 N-NaOH)	Pyruvic acid (mg.)
4	2·81	31·1	148	1·99	16·6	76	1·82	13·1	20
8	7·75	56·2	40	3·27	31·2	34	2·67	19·2	26

Table 4. *Effects of thiamine on the behaviour of* Candida mycoderma *L 4 and of* Candida krusei *no. 5 in glucose media*

	L 4					No. 5			
	Control, without thiamine		Thiamine (100 μg./100 ml.) added before inoculation			Control, without thiamine		Thiamine (100 μg./100 ml.) added before inoculation	
Days	Glucose utilized (g.)	Pyruvic acid (mg.)	Glucose utilized (g.)	Pyruvic acid (mg.)	Days	Glucose utilized (g.)	Pyruvic acid (mg.)	Glucose utilized (g.)	Pyruvic acid (mg.)
5	0·50	98	1·20	Trace	4	2·29	113	2·02	9
8	1·08	105	1·54	3	7	3·23	170	4·46	10
11	1·58	60	1·74	Trace	11	6·99	134	9·68	Trace
14	1·85	36	—	—	14	9·95	19	10·0	Nil

carboxylase (cf. Barron, 1943). The effects of thiamine hydrochloride on cultures of no. 5 and of L 4 were therefore studied. Addition of this vitamin as the hydrochloride (100 μg./100 ml.), before inoculation, stimulated growth; in both cases proliferation commenced earlier and resulted in greater quantities of cells as compared with the unsupplemented controls. The quantities of pyruvic acid which accumulated fell, however, to very low values (Table 4), demonstrating that the added thiamine had caused utilization of pyruvic acid at a rate almost as great as that at which it was being formed. Similar effects were observed by Wirth & Nord (1942) after addition of thiamine to cultures of *F. lini* Bolley, such addition nullifying the influence of the nitrite ions produced in the cultures. When thiamine hydrochloride (100 μg./100 ml.) was added to cultures of no. 5, 5 days after inoculation, when some pyruvic acid had already accumulated, the rate of fermentation was enhanced and a higher yield of ethanol was obtained, as compared with the control (Table 5).

Effects of addition of sodium arsenite to the media. Arsenite is known to exercise an inhibitory influence on the oxidative decarboxylation of certain α-keto acids which occur as intermediates in biological systems (Krebs & Johnson, 1937). A concentration of 0·02 M sodium arsenite in medium *A* completely prevented the growth of no. 5, and at 0·004 M it just allowed the formation of a faint surface film. At 0·001 M the inhibitory effect was negligible. The effects produced by addition of arsenite (0·002 M) to cultures of no. 5 at 5 days after inoculation are shown in Table 6.

In the presence of arsenite (0·002 M) less glucose was utilized, and pyruvic acid was present in the medium over a longer period of time than was the case in the control; also a markedly higher yield of ethanol was obtained from the medium containing arsenite.

DISCUSSION

The selection of organisms belonging to the genus *Candida* used in this inquiry includes types regarded as virtually non-fermentative, in addition to those capable of setting up comparatively vigorous alcoholic fermentation. All the strains have been found to accumulate pyruvic acid to a greater or less extent when grown in media containing solely inorganic salts and glucose, and in this respect they resemble certain *Fusarium* species (Nord & Mull, 1945). However, there is a marked difference in the behaviour of the two groups of organisms, since species of *Fusarium* accumulate pyruvic acid in cultures containing nitrate as source of nitrogen, whereas species of *Candida* cannot effectively use potassium nitrate for growth and they accumulate pyruvic acid only when the source of nitrogen is a suitable ammonium salt. According to Wirth & Nord (1942), and to Nord & Sciarini (1944), pyruvic acid was caused to accumulate in the culture media of their *Fusarium* species because the action of the carboxylase of this mould was impeded by the nitrite formed by reduction of nitrate in the

Table 5. *Effects of thiamine on the behaviour of* Candida krusei *no.* 5 *in a medium containing* 10 *g. of glucose*/100 *ml.*

	Control (without thiamine)			Thiamine (100 μg./100 ml.) added on 5th day of incubation		
Days	Glucose utilized (g.)	Pyruvic acid (mg.)	Ethanol (g.)	Glucose utilized (g.)	Pyruvic acid (mg.)	Ethanol (g.)
5	2·53	120	0·189	2·61	129	0·223
7	3·05	175	0·516	4·40	29	1·550
10	4·48	148	1·420	9·78	3	3·120
18	9·91	Trace	1·730	10·00	Nil	0·790

Table 6. *Effects of sodium arsenite on the behaviour of* Candida krusei *no.* 5 *in a medium containing* 10 *g. of glucose*/100 *ml.*

	Control, without sodium arsenite				Sodium arsenite (0·002 M) added on 5th day of incubation			
Days	Glucose utilized (g.)	Acid formed (ml. of 0·1 N-NaOH)	Pyruvic acid (mg.)	Ethanol (g.)	Glucose utilized (g.)	Acid formed (ml. of 0·1 N-NaOH)	Pyruvic acid (mg.)	Ethanol (g.)
5	2·53	22·0	120	0·189	2·49	27·0	137	0·194
7	3·02	38·5	175	0·516	2·79	27·0	129	0·397
10	4·48	50·0	148	1·420	3·27	31·0	93	0·762
18	9·91	42·5	Nil	1·730	5·64	55·5	33	2·310

medium. Since the *Candida* species, on the other hand, accumulated pyruvic acid in absence of nitrite and there was no other substance present known to be an inhibitor of carboxylase, it would seem probable that when *Candida* species are placed in a glucose–inorganic salts medium they are unable to synthesize thiamine at a rate sufficient for optimum development. That their ability to synthesize thiamine in such a medium varies in degree from one species to another was shown by the results of our tests carried out on cultures maintained in serial transfer (see Experimental and Results, paragraph on *Media and procedure*).

In those experiments in which arsenite was added to cultures of *C. krusei* (no. 5) there resulted a slowing down of the rate at which pyruvic acid was metabolized, but this did not serve to increase the quantity of accumulated pyruvic acid. It would seem probable that under normal conditions pyruvic acid is broken down by this organism partly by simple decarboxylation and partly by oxidative decarboxylation, but in the presence of arsenite the latter change is impeded, with the result that simple decarboxylation can occur to a greater extent, thus giving rise to a higher percentage yield of ethanol as compared with the arsenite-free control. Thus after 18 days (Table 6) the medium containing arsenite yielded 2·31 % of ethanol and the control afforded only 1·73 % of ethanol.

SUMMARY

1. Nineteen cultures representing not less than six species of *Candida* grew well in simple media of inorganic salts and glucose, with either ammonium phosphate or ammonium sulphate as source of nitrogen, and all the strains formed: (*a*) pyruvic acid, (*b*) another organic acid (or acids) and (*c*) ethanol, which in some cases was detected in traces only.

2. *Candida* species were unable to utilize nitrates as source of nitrogen, and were restricted in their growth and acid-forming capacity by addition of nitrite to the medium.

3. When thiamine hydrochloride (100 μg./100 ml.) was added to cultures of *C. krusei*, which normally produces an appreciably large yield of ethanol from glucose, the rate of utilization of glucose was increased, accumulation of pyruvic acid was prevented and the yield of ethanol was greater than that afforded by the control without added thiamine. Addition of thiamine hydrochloride (100 μg./100 ml.) to cultures of the very weakly fermentative *C. mycoderma* caused an increase in the rate of utilization of glucose and prevented accumulation of pyruvic acid, but did not promote formation of ethanol.

4. Addition of sodium arsenite (0·002 M) to cultures of *C. krusei* caused the rate of utilization of glucose to fall and the yield of ethanol to be substantially increased.

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*meso*Inositol in the Lens of Mammalian Eyes

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This work was undertaken for two reasons. First to confirm and extend the findings of Krause & Weekers (1938), who reported a high level of free inositol in the lens of three- to four-year-old cattle, considerably higher than that in other tissues of the eye. They used the method of Young (1934) and found the following values (in mg./100 g. of fresh tissue): lens, 126–177; optic nerve, 89–111; cornea, 6–8; iris, 22–35; retina, 13–17; choroid, 29–35; sclera, 10–14; conjunctiva, 6; traces were found in the vitreous humour. The aqueous humour was not analysed. Secondly, the nature of a considerable fraction of the trichloroacetic acid-soluble phosphorus compounds in the lens is unknown (Pirie, van Heyningen & Flanders, 1955). It seemed possible that some of the phosphorus in this fraction might be in the form of inositol phosphates. The only acid-soluble phosphorylated inositol previously found in