ETHANOL TOLERANCE OF SOME YEASTS

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Abstract

By suitable tests ethanol tolerance of twenty-eight strains of yeasts representing seven genera and several species had been determined. In the light of the results recorded, the strains have been classified into three groups, *viz.*, poor ethanol tolerant yeasts, moderately ethanol tolerant yeasts and high ethanol tolerant yeasts.

In 1935 Delano and Menzincesco[1] found that incorporation individually of methanol and ethanol in low concentrations into the medium exerted a favourable influence on the growth and fermentative activity of yeasts. Subsequent work in this field was that of Gray[2-5] who, in a series of extensive experiments, demonstrated the inhibitory effect of alcohol on different genera and species of yeasts. Gray classified these yeasts into five categories on the basis of their alcohol tolerance. In addition, he also found that (1) the utilization of glucose by yeast was progressively inhibited with increasing concentration of alcohol in the medium, and that (2) the alcohol tolerance of yeast varied inversely with lipid content. Troyer[9] reported the limitation of cell population in yeast as a result of the inhibitory effect of ethanol. In continuation of his previous studies Gray and Sova[6] studied the effects of different primary and secondary alcohols on the glucose utilization by yeast and demonstrated a correlation between molecular size and inhibitory action of the different alcohols.

In view of the above observations, it was thought desirable to study the alcohol tolerance of several yeasts in our collection with a view to select, if possible, one or more alcohol tolerant yeasts which on further studies would prove their suitability or otherwise for industrial exploitation. The criterion followed for the alcohol tolerance was the growth of the organism as measured by turbidity.

Materials and Methods

(i) *Material and media.*—Twenty-eight yeast cultures, many of them belonging to different genera and species, were selected for this study. The basal medium prepared, in double strength, contained per litre: Glucose 20 g., KH_2PO_4 5 g., $(NH_4)_2SO_4$ 5 g., $MgSO_4 \cdot 7H_2O$, 0·2 g., $FeSO_4 \cdot 7H_2O$ 0·002 g. and Difco yeast extract 2 g. The pH of the medium was found to be in the range of 4·5–5·0.

95% Ethyl alcohol obtained from commercial sources was refluxed for 6 to 8 hours with magnesium ribbons (rubbed with sand paper and cleaned before use). The refluxed alcohol was distilled using an all-glass distillation set. The alcohol so obtained was absolutely dry and this was rechecked with a Refractometer.

Five ml. aliquots of the double strength medium were pipetted into culture tubes and absolute alcohol by volume was added under aseptic conditions. In the first series of experiments the absolute alcohol was incorporated into the medium in the following arbitrarily chosen quantities (v/v) of 0, 1, 5, 8, 10 and 12%.

Based on observations made on the above ranges, a further series of two experiments were carried out, one involving the incorporation of ethanol from 3 to 10% and the other from 8% to the extent of 14%. In both these experiments, the concentration of alcohol to be incorporated into the medium at the start of the experiment depended upon the capacity of the yeast strain in question to tolerate all the lower ranges. In all these three sets of experiments the total volume of the culture medium in the test-tube was made upto 10 ml. with adequate amounts of sterile distilled water.

(ii) *Preparation of the inoculum.*—Two loopfuls of yeast growth from a 24 to 48 hours agar slant were transferred into 5 ml. of dilute basal medium, the tube was well shaken for a few minutes and allowed to settle. The supernatant was then transferred into another sterile tube and the content of this tube was used as the inoculum, one loopful for each tube. The same loop was used for inoculation in all the subsequent experiments.

TABLE

Ethanol Tolerance

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Yeast species and No. / Ethanol % (v/v)	0			3			4		
Time in Hrs.	24	48	96	24	48	96	24	48	96
Saccharomyces steineri (Y_4) ..	55	160	315	38	72	214	..	67	86
Saccharomyces carlsbergensis (NCYC 73 A)	210	350	355		..			..	
Saccharomyces marxianus (NCYC 111) ..	177	290	360		..		160	284	310
Saccharomycodes ludwigii (NCYC 364 A) ..	166	300	420		..			..	
Torulopsis utilis (NCYC 321) ..	96	157	210	98	154	236	90	140	234
Torulopsis utilis var. *major* (NCYC 359 A)	110	218	375		..			..	
Torulopsis pulcherrima (NCYC 1664) ..	134	260	305		..			..	
Torulopsis datila (Y_2) ..	95	170	270		..		63	101	250
Candida pulcherrima (Y_9) ..	70	158	280		..		64	105	192
Schwanniomyces occidentalis (NCYC 183) ..	152	290	375		..			..	
Hansenula saturnus (NCYC 22) ..	123	177	325	85	130	254	73	120	220

The culture tubes prepared in triplicates were incubated at room temperature (24 to 26° C.) and were handshaken at intervals. At the end of the growth period, the yeast was centrifuged, supernatant poured out and the yeast growth was re-suspended in 10 ml. of distilled water. Turbidity readings were recorded at the end of 24, 48 and 96 hours with a Klett-Summerson Colorimeter using 42 filter. Since growth measurements made upto 48 hours after the incubation were regarded as those of tolerant species, data regarding growths occurring after 96 hours, although were recorded, are not presented in this paper.

Results and Discussion

The results are presented in Tables I and II. The yeast strains used in the present study could be classified broadly into three following categories on the basis of their capacity to grow in presence of low or high concentrations of ethanol.

(i) *Poor Ethanol tolerant yeasts* (between 3 and 6%) were: *Saccharomyces steineri, Torulopsis pulcherrima, Candida pulcherrima* and *Hansenula saturnus.*

(ii) *Moderately Ethanol tolerant yeasts* (between 6 and 10%) were: *Saccharomyces carlsbergensis, Saccharomyces marxianus, Saccharomyces turbidans, Saccharomycodes ludwigii, Torulopsis utilis, Torulopsis utilis* var. *Major, Torulopsis*

I

of Some Yeasts

Readings)

5			6			7			8			9			% Ethanol v/v tolerated
24	48	96	24	48	96	24	48	96	24	48	96	24	48	96	
..	25	55	..	..	..		..			..			..		5·0
118	340	390	..	262	375	..	190	375	..	37	274	..	..	85	8·0
152	256	310	103	230	310	55	135	305	..	..	230		..		7·0
	..		78	246	330	..	140	218	..	..	182		..		7·0
66	118	210	62	162	192	28	67	170	..	12	57	..	..	10	8·0
64	178	330	..	135	310	..	59	252	..	42	45		..		8·0
	..		..	29	155		..			..			..		6·0
32	125	220	45	98	192	..	57	155	..	..	28	..	..	8	7·0
22	90	185	11	32	125	..	..	57		..			..		6·0
	..		65	210	315	..	152	253	..	..	195		..		7·0
..	58	155		..			..			..			..		5·0

TABLE

Ethanol Tolerance

(Klett

Yeast species and No.	Ethanol % (v/v)	0			8			9		
	Time in Hrs.	24	48	96	24	48	96	24	48	96
Saccharomyces cerevisiæ (Standard strain)	..	268	296	330	125	260	276	50	206	244
Saccharomyces cerevisiæ (Y_1)	..	270	345	360		..			..	
Saccharomyces cerevisiæ (Y_3)	..	100	264	375		..		23	88	220
Saccharomyces cerevisiæ (Y_{10})	..	236	278	305	150	256	290	90	240	266
Saccharomyces cerevisiæ (Y_{13})	..	112	240	355	72	190	320	55	158	290
Saccharomyces cerevisiæ (Y_{16})	..	92	274	†	65	195	268	35	195	†
Saccharomyces species (Y_{12})	..	116	242	340	83	140	310	50	163	246
Saccharomyces species (Y_{15})	..	125	244	320	..	80	128		..	
Saccharomyces italicus (NCYC 108)	..	390	430	430		..			..	
Saccharomyces turbidans (NCYC 124)	..	274	400	450	..	188	405	..	65	405
Schizosaccharomyces pombe (NCYC 132)	..	140	320	380		..			..	
Schizosaccharomyces octosporus (NCYC 131)	..	27	87	224	..	..	85	..	..	40
Zygosaccharomyces priorianus (NCYC 176)	..	310	365	415	..	158	305	..	..	108
Zygosaccharomyces barkeri (NCYC 170)	..	90	204	2:8	..	32	163	..	22	114
Candida guilliermondia (Y_8)	..	95	162	250	52	148	234	..	103	210
Candida tropicalis (Y_{14})	..	142	244	305	..	..	137		..	..
Saccharomyces ellipsoideus (NCYC 94)	..	305	420	450		..			..	

†Tube broken by accident, hence readings not taken.

dattila, Schwanniomyces occidentalis, Schizosaccharomyces octosporus, Zygosaccharomyces priorianus, Zygosaccharomyces barkeri, Candida tropicalis and *Saccharomyces species* (strains Y_3 and Y_{15}).

(iii) *High Ethanol tolerant yeasts* (between 10 and 13%) were: *Saccharomyces cerevisiæ* (Standard strain), *Saccharomyces cerevisiæ* (Strains Y_1, Y_{10}, Y_{13} and Y_{16}), *Saccharomyces* species (Y_{12}), *Saccharomyces italicus, Schizosaccharomyces pombe, Candida gulliermondia* and *Saccharomyces ellipsoideus.*

In general, it is of interest to note here that whereas the lowermost limit of alcohol tolerance recorded after 48 hours of incubation, in this instance for *Saccharomces steineri* (Y_{14}) and *Hansenula saturnus* (NCYC 22) is 5% and the highest

II

of Some Yeasts

Readings)

10			11			12			13			14			% Ethanol v/v tolerated
24	48	96	24	48	96	24	48	96	24	48	96	24	48	96	
17	176	224	..	37	185		..			..			..		11·0
12	90	244	..	12	78		..			..			..		11·0
..	35	168		..			..			..			..		10·0
17	170	256	..	42	186	..	..	28		..			..		11·0
25	137	†	..	78	195	..	25	82		..			..		12·0
..	180	238	..	65	162	..	..	40		..			..		11·0
35	138	224	..	98	188	..	70	137	..	..	20		..		12·0
	..			..			..			..			..		8·0
..	260	375	..	90	355	..	..	290		..			..		11·0
	..			..			..			..			..		9·0
	..		..	24	176	..	..	44		..			..		11·0
	..			..			..			..			..		7·0*
	..			..			..			..			..		8·0
..	..	32		..			..			..			..		9·0
..	69	147	..	..	10		..			..			..		10·0
	..			..			..			..			..		7·0*
	..		..	150	350	..	20	305		..			..		12·0

* Determined in the first series of experiments not reported in detail here.

limit for three yeasts, *viz.*, *Saccharomyces cerevisiæ* (Y_{13}), *Saccharomyces* species (Y_{12}) and *Saccharomyces ellipsoideus* (NCYC 94) is 12% (see Tables I and II), those recorded by Gray and Sova[6] were of the order of 4·73 to 11·58% respectively. But it must be emphasized that these limits were arrived at by adopting different criteria for alcohol tolerance. It is also interesting to note that, generally speaking, only *Saccharomyces* and *Schizosaccharomyces* constitute the more tolerant of the genera put to test for ethanol tolerance.

It is of considerable interest to report here that one of the high tolerant yeasts, *viz.*, *Saccharomyces cerevisiæ* (Y_{13}) isolated originally by Mody and Bhat[7] from pine-apple, produced only 3·75% (v/v) alcohol under aerated conditions and that

it is extremely well suited for the production of yeast by exploitation of sugarcane molasses. It would appear therefore that this very yeast would be suitable for alcohol production from molasses but this would be a subject of a future communication.

The experimental methods and the analysis described above can give rise only to qualitative evaluations as to the capacity of a particular strain to tolerate increasing concentrations of ethanol. Since, according to Gray[2] the determination of alcohol tolerance of a particular strain offers a reliable criterion for the selection of a suitable strain for a particular fermentation process, these studies offer some promise for selection of suitable strains for fermentation industry. In this connection, it would be relevant to mention that a potent yeast strain yielding high concentration of alcohol (10 to 14%) in the distillery washes had already been developed and patented in this laboratory.[8] The present studies merely indicate that Y_{13} and Y_{12} tolerate high concentration of ethanol, and that further studies on acclimatization of these yeasts to high concentrations of glucose and subsequently alcohol would perhaps help to improve their ethanol tolerance further and render their suitability for industrial exploitation better.

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