EFFECT OF NUTRITIONAL STATE AND OTHER CONDITIONS ON ULTRAVIOLET RESISTANCE AND PHOTOREACTIVATION IN YEAST¹

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Starvation of protozoans increases their sensitivity to ultraviolet radiations and affects the photoreversibility of ultraviolet injury by visible light (Giese and Reed, 1940; Giese *et al.*, 1954; Brandt *et al.*, 1955). The present study on the variation with nutritional state of the sensitivity of yeast to ultraviolet and its photoreversal was initiated with the aim of relating the specific nutrients or enzyme systems involved in the changed ultraviolet sensitivity resulting from starvation. In addition the effects on ultraviolet injury and its photoreversal of some factors which affect the physiological state of the yeast, especially oxygen tension and selective enzyme poisons, were investigated; findings which emerged are presented below.

MATERIALS AND METHODS

The R strain of the yeast, *Saccharomyces cerevisiae*, originally isolated at the University of Toronto, was grown on 1 per cent yeast extract Difco, 3 per cent glucose, 0.05 M KH_2PO_4 made up in Reader's salt medium (which contains $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , NaCl and $\text{Ca}(\text{NO}_3)_2$; Reader, 1927) in a culture room at 30 C on a shaker to insure growth of the culture under aerobic conditions. For experiments, the suspension harvested by centrifugation was washed 3 times in Reader's medium or KH_2PO_4 (0.05 M) and diluted to give approx 5×10^6 cells/ml. Yeast in the logarithmic phase (up to 8 hr) was used for some experiments but generally out-of-log cultures 18 hr old were used because previous tests had shown them to have more consistently demonstrable characteristics than others.

Anaerobiosis was achieved by flushing the culture of yeast (3 ml sample per 15 ml quartz Warburg vessel) with nitrogen (99.7 per cent pure) which was passed through two wash bottles containing pyrogallol. A suspension of luminous bacteria in nutrient medium was

pipetted into the center well of the quartz vessel containing the yeast to test for the presence of oxygen, and flushing was continued until luminescence ceased, usually after 30 min (Harvey, 1940). To achieve a high oxygen tension, the vessel was flushed with oxygen for 30 min. In either case, the yeast could be irradiated with ultraviolet and subsequently illuminated with visible light without transfer. Comparisons of the effect of different gases upon ultraviolet resistance of yeast were made using aliquots of the same suspension of yeast irradiated at different times in the same quartz flask. In other cases yeast was irradiated in open petri dishes swirled during the irradiation period, and the contents of separate petri dishes were pooled prior to subsequent treatment.

A Westinghouse Sterilamp was the source of ultraviolet, the intensity of which, determined with a Hanovia ultraviolet meter at the start of each experiment, was about 40 ergs/mm²/sec in most experiments. A dosage of 2,500 ergs/mm² was used upon the suspension of yeast in most cases; in a few instances this was raised to 3,000 ergs/mm². To photoreactivate ultraviolet injury to yeast, cultures were exposed to two 90 watt GE daylight fluorescent lamps at a distance of 30 cm for 30 min, an exposure found optimal for suspensions of the density used.

Tests for the effects of various treatments were made primarily by colony counts. One ml of the suspension to be tested was diluted to a degree determined by preliminary trials to give about 100 colonies per plate after three days growth at 30 C in the dark. The nutrient plates contained 2.5 per cent agar, 1 per cent yeast extract, 3 per cent dextrose, and 0.05 M KH_2PO_4 . In earlier experiments broth cultures were used, the same nutrients being supplied as in the agar plates, and counts of the population were made with a haemocytometer until a population of 10^8 cells/ml was present. (The nutrients present were capable of supporting a population ten times larger.)

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Figure 1. Effect of nutritional state on the sensitivity to ultraviolet (UV) and on photoreversal by visible light of UV injury to yeast. Ultraviolet dosage, 2,500 ergs per mm². Data from colony counts.

Effect of starvation on resistance to ultraviolet. Yeast is probably at its optimal nutritional state when it is growing at a maximal (logarithmic) rate at a given temperature, as long as nutrient is not limiting. Yeast in the logarithmic phase was also found to be more resistant to ultraviolet than that taken from any other state tested (figure 1A). As nutrient becomes limiting, the rate of division declines. A culture just out of logarithmic phase declines in resistance to ultraviolet (figure 1B). Sensitivity of yeast to ultraviolet does not increase progressively with length of starvation and is not striking for yeast starved only for carbohydrate (in Reader-buffer medium). It is, however, very marked for yeast starved for nitrogen (in glucose-buffer), survival falling to less than 1 per cent after a single day of starvation for nitrogen. It is quite clear that lack of nitrogen is of greater significance than lack of carbohydrate in lowering the resistance of yeast to ultraviolet (table 1A).

While decline in resistance to ultraviolet of yeast just out of logarithmic phase might be interpreted as a result of nutritional deficiency of the yeast, it is also possible that toxic by-products of the metabolism of yeast are of some moment. Thus yeast washed free of organic nutrients and aerated for 17 hr has a greater resistance to ultraviolet than a culture just out

of logarithmic phase (figure 1C), indicating that starvation alone is not necessarily deleterious.

Effect of addition of nutrients on resistance to ultraviolet of starved yeast. If lack of nutrient lowers the resistance of yeast to ultraviolet, supplying the nutrient might reverse the process. It is at once apparent that addition of glucose to carbohydrate-starved yeast (table 2A) increases its resistance to ultraviolet by some 5-fold after 3 hr, after which little further change occurs in another 3 hr.

Furnishing inorganic nitrogen to nitrogen-starved yeast by immersion in buffered Reader's solution does not ameliorate the high sensitivity to ultraviolet of nitrogen-starved yeast. On the other hand, a supply of yeast extract increases the resistance of yeast to ultraviolet some 10-fold in 6 hr. Even more protection against ultraviolet results from supplying both carbohydrate and yeast extract to nitrogen-starved yeast (last column, table 2A), a 27-fold increase in resistance to ultraviolet being observed in 6 hr after addition of nutrients. No increase in number of cells per ml occurs in the controls during this time. Similar results come from a determination of the ability of yeast, supplied for 6 hr with nutrient after 6 days starvation, to form buds and to reduce methylene blue. From table 3 it is apparent that of a population of yeast starved 6 days for carbohydrate, 63 per cent are budding after 6 hr in glucose medium on the sixth day, and show a viability of almost 90 per cent. However, nitrogen-starved yeast recovered after 6 hr in medium containing inorganic nitrogen only to the extent that 10 per cent of the cells are budding and the viability is only about 60 per cent. A sojourn in yeast extract for 6 hr on the sixth day led to a recovery of the yeast to the extent that 63 per cent of the cells budded. Nitrogen-starved yeast kept for 6 hr in medium containing yeast extract and glucose shows a more marked recovery; 77 per cent budded and the viability rose to almost 75 per cent.

Effects of variation in oxygen tension. As may be seen from the data in table 4A, yeast appears to be no more readily killed in oxygen than in air. Also, reduction of the oxygen tension obtained by gassing a culture with purified nitrogen does not appear to protect yeast from ultraviolet, anaerobic cells being killed as readily as those in air. Data collected from broth cultures are

TABLE 1

Exp. No.	Days Starved for Carbohydrate (in Reader's Buffer Medium)				Days Starved for Nitrogen (in Glucose-Buffer Medium)				Control Cells/Ml
	0	1	2	6	0	1	2	6	
A. Survival (in per cent of control) of ultraviolet-treated* yeast after starvation									
1	0.0865	0.0382	0.065	0.0277	0.0832	0.00056	0.000922	0.00451	1.37×10^6
2	0.0182	0.0192	0.0183	0.0175	0.0365	0.00795	0.000353	0.00108	1.6×10^6
3	0.0414		0.0242	0.177	0.062		0.000394	0.0129	1.98×10^6
4	0.11	0.265	0.43	0.43	0.0775	0.00118	0.00147	0.0039	1.96×10^6
Average...	0.0640	0.0806	0.1344	0.1630	0.0645	0.00267	0.00078	0.00559	
B. Survival (in per cent of control) of ultraviolet-treated* starved yeast after treatment with visible light									
1	15.0	8.17	3.08	0.735	19.0	0.0191	0.0191	0.429	1.37×10^6
2	10.9	4.33	2.52	0.158	10.0	0.119	0.0074	0.248	1.60×10^6
3	11.4		2.84	1.88	12.6		0.0471	0.298	1.98×10^6
4	14.6	13.1	3.86	3.5	15.5	0.0312	0.144	0.335	1.96×10^6
Average...	12.9	8.53	3.07	1.57	14.3	0.042	0.0544	0.33	

* 2,500 ergs/mm² at λ 254 m μ .

equally negative, little difference existing between yeast exposed to ultraviolet in pure oxygen, in air, or in nitrogen. This is in agreement with observations of others (Bie, 1905; Wells, 1929; Kaplan, 1953).

Effects of respiratory poisons on ultraviolet injury. No significant difference was observed, either in plate determinations or in broth cultures between yeast treated with cyanide (0.001 M or 0.01 M KCN) at the time of irradiation, then washed free of cyanide, and yeast given the same dosage of ultraviolet in the absence of respiratory poison (table 5). Even in the presence of excess oxygen, the respiratory inhibitor does not protect yeast from ultraviolet injury; in fact, an additive injury is suggested.

Attempts were also made to study the effect of azide on ultraviolet sensitivity, but since azide readily inhibits growth of yeast, its effect on photoreversal of ultraviolet damage was difficult to test. When concentrations which did not inhibit growth were employed, no essential difference in ultraviolet resistance was noted between yeast treated with azide and that without. Since the data add nothing of interest, they are not documented here.

A few experiments were tried with fluoride, but

extensive experimentation was not possible because growth is inhibited by this poison. When yeast cultures treated with fluoride are capable of growth, no essential difference was observed between those treated with ultraviolet in the presence and in the absence of fluoride. When fluoride affected ultraviolet sensitivity, it was always deleteriously, possibly additively.

Spontaneous recovery of yeast without nutrient from ultraviolet injury. When irradiated yeast is kept suspended in phosphate buffer solution in the absence of external organic nutrients, a considerable degree of recovery from ultraviolet injury is observed. The experiment was performed by exposing to ultraviolet one aliquot of a given suspension of yeast in buffer solution and then storing this in the dark, either in buffer solution or after plating it on an agar plate. Aliquots from those suspended in buffer were then plated periodically. As seen in figure 2, the ultraviolet-treated aliquot progressively recovers when kept in buffer at 28 C, maximal recovery having been reached in about 24 hr. The presence of glucose in the buffer solution does not prevent dark recovery from occurring.

The fact that we are here dealing with a thermal process is indicated by keeping an

aliquot of the yeast in buffer-salt solution at 5 C. Yeast so treated showed no spontaneous recovery whatsoever (figure 2).

Photoreactivation of ultraviolet injury. Visible light to a considerable extent reverses ultraviolet

injury to yeast as shown by an approximate 100- to 600-fold increase in the number of colonies on a sample exposed to visible light as compared to an aliquot kept in the dark (table 5, lower half).

Photoreactivation occurs only if the sample of

TABLE 2

Hours after Addition of Nutrient	Exp. No.	Starved 6 Days for				Control Cells/MI
		Carbohydrate	Nitrogen (N)			
			Nutrient added:			
		Glucose	Inorganic N (Reader's solution)	Yeast extract	Yeast extract + glucose	

A. Effect of supplying nutrient on survival (in per cent of control) of starved yeast exposed to ultraviolet

0	1	0.183	0.0011	0.0174		1.98×10^6
	2	0.59	0.005	2.30		9.26×10^4
	3	0.54	0.0046	1.90	1.3	1.96×10^6
Average.		0.438	0.0032	1.406		
3	1	0.574	0.00017	4.3		1.98×10^6
	2	3.40	0.0051	1.4		9.26×10^4
	3	5.00	0.0016	6.9	9.62	1.90×10^6
Average.		2.99	0.0023	4.2		
6	1	1.03	0.0041	3.61		1.98×10^6
	2	2.70	0.0012	19.3		9.26×10^4
	3	5.00	0.00174	26.1	35.6	1.96×10^6
Average.		2.91	0.00235	16.34		

B. Effect of supplying nutrients on survival (in per cent of control) of ultraviolet-treated* starved yeast subsequently exposed to visible light

0	1	1.16	0.985	9.84		1.98×10^6
	2	6.5	1.0	15.8		9.26×10^4
	3	2.86	0.474	23.8	26.1	1.96×10^6
Average.		3.51	0.819	16.48		
3	1	5.29	1.4	6.3		1.98×10^6
	2	7.2	1.8	12.5		9.26×10^4
	3	16.2	0.22	18.3	27.3	1.96×10^6
Average.		9.56	1.14	12.4		
6	1	13.3	0.555	13.9		1.98×10^6
	2	23.0	1.2	39.8		9.26×10^4
	3	22.3	0.421	38.8	33.5	1.96×10^6
Average.		19.5	0.725	30.8		

* 2,500 ergs/mm² at λ 254 m μ .

TABLE 3

Budding and viability of yeast (tested by methylene blue reduction) suspended in nutrient medium for 6 hours after 6 days starvation for the nutrient indicated

Criterion	Exp. No.	Starved for Carbohydrate	Starved for Nitrogen (N)		
		Nutrient added:			
		Glucose	Inorganic N (Reader's solution)	Organic N (Yeast extract)	Organic N + glu- cose
Per cent budding	1	57.0	14.0	53.0	77.0
	2	65.8	12.8	67.5	
	3	66.6	4.2	69.3	
Average.....		63.1	10.3	63.3	
Per cent nonviable	1	7.0	41.0	40.0	25.6
	2	18.9	47.4	33.0	
	3	6.7	31.3	30.8	
Average.....		10.9	39.9	34.6	
Per cent viable (Average).....		89.1	60.1	65.4	74.4

TABLE 4

Data are colonies per ml of suspension

Exp. No.	Control (Colonies)	Oxygen (100% O ₂)	Air (20% O ₂)	Control	Nitrogen (0% O ₂)	Air
A. Effect of oxygen tension on resistance of yeast to ultraviolet (2500 ergs/mm ²)*						
1	6.7×10^5	170	10	5.5×10^5	30	6
2	4.8×10^5	110		5.3×10^5	10	13
3	5.8×10^5	80	33	6.0×10^5	10	20
4	11.0×10^5	31	53	5.0×10^5	3.3	40
5	7.9×10^5	240	130	5.9×10^5	13	17
6	6.0×10^5	26	20	5.9×10^5	450	470
7	5.0×10^5	36	40	4.7×10^5	13	16
8				4.7×10^5	20	86
Average.....	6.5×10^5	99	47.6	5.4×10^5	68.7	83.5
B. Effect of oxygen tension on photoreversal of ultraviolet injury to yeast						
1	6.7×10^5	8,300	1,300	5.5×10^5	2,000	4,300
2	4.8×10^5	4,600	4,300	5.3×10^5	460	300
3	5.8×10^5	2,600	3,600	6.0×10^5	25,000	14,000
4	11.0×10^5	47,000	69,000	5.0×10^5	20,000	560
5	7.9×10^5	120,000	100,000	5.9×10^5	37,000	4,000
6	6.0×10^5	23,000	14,000	5.9×10^5	68,000	20,000
7	5.0×10^5	39,000	660	4.7×10^5	14,000	4,600
8				4.7×10^5	27,000	2,500
Average.....	6.5×10^5	34,930	27,551	5.4×10^5	25,408	6,283

* Except in experiments 1 and 2 in which a dosage of 3,000 ergs/mm² was used.

TABLE 5
Effect of cyanide on ultraviolet injury (2,500 ergs/mm²) to yeast and its photoreversal

Exp. No.	Hours in Broth, after Inoculation, to Reach 10 ⁶ Cells/ml							
	Unirradiated		Ultraviolet treated			Ultraviolet + white light		
	No KCN	0.01 M KCN	No KCN	0.01 M KCN	+ O ₂ + 0.01 M KCN	No KCN	+0.01 M KCN	+ O ₂ + 0.01 M KCN
1	8.5		24.5	31.0	26.2	20.0	21.2	22.0
2	8.5	8.5	28.5	27.5	28.0	17.5	22.2	20.2
3	9.5	7.5	26.5	23.0	27.5	17.0	16.0	17.5
Average...	8.8	8.0	26.5	27.2	27.2	18.2	19.1	19.9

Number of colonies per ml (plate counts)								
1a	5.6 × 10 ⁵		45	10	15	31,000	14,000	7,500
2b	5.7 × 10 ⁵	7 × 10 ⁵	400	320	220	94,000	120,000	88,000
3c	6.0 × 10 ⁵	5.3 × 10 ⁵	20	26	30	84,000	46,000	54,000
Average...	5.8 × 10 ⁵	6.0 × 10 ⁵	150	119	88	69,700	60,000	33,170

yeast is not supplied for too long with all the required organic nutrients before treatment with visible light. If irradiated cells are plated on nutrient agar kept at 28 C and periodically illuminated, photoreversal progressively declines. When the culture of ultraviolet-treated yeast plated on nutrient agar is kept at 5 C instead of at 28 C, photoreversibility declines much more slowly over the 30-hr span tested (figure 2). This suggests a thermal reaction involved in the "fixation" of ultraviolet injury (Kelner, 1949, 1950).

Photoreversal of ultraviolet injury to yeast occurs under anaerobic conditions, even in the absence of external nutrient, as shown by the data in table 4. The degree of photoreversal from ultraviolet injury seems to be relatively independent of oxygen tension (table 4), there being a suggestion of greater photoreactivation in oxygen, but because of variability, the experiments are not statistically significant.

Photoreversal of ultraviolet injury to yeast is not significantly affected by the presence of cyanide in the yeast suspension (table 5). Azide in a concentration which does not suppress growth also has no effect on photoreversal of ultraviolet injury to yeast.

The photoreactivation of ultraviolet injury in a strain of yeast containing an excess of riboflavin was tested because Bowen (1953), on the basis of the action spectrum of photoreactivation, has suggested that riboflavin might be the pigment

which mediates the reactivating action of visible light. In the presence of so much riboflavin a greater degree of photoreactivation might be expected. However, no marked difference was observed between the amount of photoreactivation in this strain and in the R strain used in most of the experiments. Unfortunately the data cannot be considered conclusive because both ultraviolet and visible light destroy riboflavin. Furthermore, individuals of this strain of yeast form chains which were not broken up by any of the procedures tried. Assumption of a similar number of cells per chain after the various treatments was not justified; therefore, the data have a low order of reliability.

Starvation of yeast for carbohydrate (table 1B) had relatively little immediate effect on its capacity for photoreactivation, but after 6 days of starvation a considerable change is observable, photoreactivation being only about one-tenth that at the beginning. Even more striking is the effect of withholding a source of nitrogen from yeast, glucose being supplied (table 1B). In this case photoreactivation is reduced to 0.3 per cent of that at zero time. Some improvement comes with continued starvation, but after 6 days, photoreactivation is still only 2 per cent of that at zero time of starvation.

Supplying glucose to yeast starved for carbohydrate for several hours before illumination increases photoreactivation on the average by about 5-fold (table 2B). Supplying inorganic

Figure 2. Effect of ultraviolet (UV) (2,500 ergs per mm^2), photoreactivation of yeast and spontaneous recovery of yeast from UV injury. The labels on each curve indicate the treatment accorded the yeast used in each experiment. Thus: "UV (5 C) + WL (white light), plated" means that the yeast in buffer was exposed to a dosage of 2,500 ergs per mm^2 of UV, then placed at 5 C and samples were periodically plated and incubated at 28 C. Note that in A spontaneous recovery occurs when the yeast is kept at 28 C in buffer (UV(28 C), plated), not when it is kept at 5 C (UV(5 C), plated). In B note that when the culture is plated and kept at 28 C and then illuminated (UV, plated (28 C), + WL), the degree of photoreactivation gradually declines.

nitrogen or yeast extract for a few hours before illumination of yeast deprived for several days of a nitrogen source, on the other hand, has little effect on its capacity for photoreactivation (table 2B). Apparently the adverse effect produced by lack of nitrogen is not easily mended.

DISCUSSION

Physiological and nutritional state of yeast has a marked effect upon its resistance to ultraviolet, yeast being most resistant in the logarithmic phase, and least resistant after prolonged

starvation for nitrogen. That the greater resistance of the yeast in the logarithmic state is not due merely to a more reduced state is suggested by the lack of any difference between resistance to ultraviolet of anaerobic and aerobic yeast and the unchanged sensitivity of yeast to ultraviolet in which the aerobic side of the respiratory chain is blocked by cyanide, although increased resistance to ultraviolet is reported in other microorganisms (Mefferd and Matney, 1952; Berger *et al.*, 1953). One possibility which suggests itself to explain the increased sensitivity of yeast starved for nitrogen is unbalanced growth in cells just past the logarithmic phase. Such yeast actually increases in size yet markedly decreases in its resistance to ultraviolet. Under such circumstances growth, as measured by cell mass, may continue after irradiation but may be unbalanced (Cohen and Barner, 1954). Yeast kept without a nitrogen source in the experiments reported here is, however, not decrepit, since addition of glucose led to active exogenous respiration comparable to that of controls within 10 min.

Correlation of physiological state with ultraviolet sensitivity has been noted by other workers using microbes (Heinmetz and Kathan, 1954; Thompson *et al.*, 1951; Heinmetz *et al.*, 1954; Heinmetz, 1953; Hollaender, 1952), especially the effect of various added metabolizable substrates which protect against ultraviolet, presumably by producing a favorable reduced condition in the cell. Such protection has been found especially against X-ray injury (Hollaender, 1952), but to some degree against ultraviolet effects as well. In contrast with yeast, *Escherichia coli* was found to be more sensitive to ultraviolet in the logarithmic phase of growth than in the out-of-log growth phase (Hollaender and Claus, 1936; Demerec and Latarjet, 1946; Witkin, 1951).

Spontaneous reactivation of yeast in absence of organic nutrients and the finding of its temperature dependence is similar to the phenomenon observed by Roberts and Aldous (1949), Anderson (1951) and Charles and Zimmerman (1956) on *E. coli*. It implies a potential in the cell for recovery if growth is suspended for a period of time, thus preventing unbalanced growth. Recovery from ultraviolet injury by illumination with visible light is greater than the spontaneous dark recovery. That the same systems are

probably involved in spontaneous recovery and photorecovery is suggested by the finding that spontaneously recovered yeast cells, subsequently subjected to an amount of visible light which causes maximal photoreactivation, never show greater recovery than the maximum after photoreactivation. If the two were of different nature one might expect them to be additive in an algebraic way. This suggests that the ultraviolet lesion, which is slowly repaired spontaneously, is repaired very quickly by visible light.

Since photoreactivation of ultraviolet injury to yeast occurs under anaerobic and aerobic conditions, it is perhaps not surprising to find that the various poisons such as cyanide and azide do not measurably affect the process in yeast, which may mean that the cytochrome oxidase system is not involved. These results confirm earlier conclusions on phage (Delbecco, 1950) and *E. coli* (Johnson *et al.*, 1950). However, the somewhat greater photoreactivation in a gaseous nitrogen atmosphere (table 4B) suggests the importance of a reduced condition for this purpose. In only one of the eight series of experiments was reactivation somewhat greater in an air than in a nitrogen gaseous atmosphere.

Unfortunately the basic reactions in photoreactivation remain elusive. What they are not is easier to determine than what they are. However, the present study circumscribes the area for further experimentation.

SUMMARY

The effect of various environmental variables upon inhibition of growth of the yeast, *Saccharomyces cerevisiae*, by ultraviolet radiations was studied.

The time in the growth cycle affects the resistance of yeast to ultraviolet. Yeast in the logarithmic phase of growth is much less sensitive than that just past the logarithmic phase. Yeast starved for nitrogen becomes much more sensitive to ultraviolet than when starved for glucose alone.

Supplying glucose to yeast which has been deprived of carbohydrate for a time increases its resistance to ultraviolet. Supplying yeast extract to yeast which has been deprived of a nitrogen source for several days greatly increases its resistance to ultraviolet, while supplying an inorganic nitrogen source does not.

In the absence of oxygen yeast is about as

sensitive to ultraviolet as in air. In pure oxygen, also, its sensitivity to ultraviolet changes little. Cyanide does not protect yeast against ultraviolet injury, whether in an air or in an oxygen atmosphere.

Irradiated yeast recovers from ultraviolet injury to a considerable extent in the dark if suspended in phosphate buffer solution at 28 C, but not at 5 C; but recovery is greater following illumination (photoreactivation) with white light.

Photoreactivation declines soon after plating yeast on nutrient agar and much more rapidly at 28 C than at 5 C. Photoreactivation occurs equally well under anaerobic conditions.

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