FUNGI CAPABLE OF GROWING IN STRONGLY ACID MEDIA AND IN CONCENTRATED COPPER SULFATE SOLUTIONS

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It is well established that the bacterium *Thiobacillus thiooxidans* is capable of growing in very strong mineral acid solutions and of producing, from elementary sulfur, sulfuric acid solutions more concentrated than 1 N and a reaction considerably below pH 1. This organism had been considered unique in its tolerance to acid until Starkey and Waksman (1943) demonstrated that certain fungi, *Acontium velatum* Moore, and an unidentified dematiaceous mold were able to develop in a medium initially as acid as 2.5 N sulfuric acid and in a normal sulfuric acid solution saturated with copper sulfate.

We were able to confirm the tolerance to extreme acidities of some fungi with three isolates and tolerance to copper sulfate with two of them. The first isolate, no. 7752, was obtained from a bottle of N/5 sulfuric acid reagent, the second, no. 9010, from soil rubbish picked up from an athletic running track, and the third, no. 9024, from another sample of standard sulfuric acid reagent.

Numbers 7752 and 9024 are possibly the same as Starkey and Waksman's unidentified dematiaceous mold. It has been tentatively identified as *Trichosporon cerebriforme* (Kambayashi) Ota by Dr. R. L. Bouthilet. Difficulties in getting authentic cultures for comparison or original descriptions published in Japan have made it impossible for him to make an absolute identification as yet. The third isolate died out before it was identified.

The first experiment demonstrates that when *T. cerebriforme* (which breaks up readily into what we interpreted as arthrospores, but Starkey and Waksman as chlamydospores) is inoculated, in amounts so small as to be invisible in the culture, into a medium consisting of 1, 1.5, or 2 N solutions of sulfuric acid plus 0.1 per cent glucose and 0.1 per cent peptone, a growth results easily visible as dark, almost black, globose masses of hyphae. Growth was evident in about 10 days with the 1 N acid solution, but prolonged incubation was necessary to obtain the maximum yields. The incubation was at room temperature.

The second experiment was in part a confirmation of the first. Narrow-mouthed bottles of about 400-ml capacity, half filled with media, were used. Some were sealed with glass stoppers; others were plugged with cotton wool. The media in the second experiment were essentially the same as in the first, except that some were more strongly acid, and some were not autoclaved. The normalities of the media were determined before and after incubation. Following

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the prolonged incubation the viability of the cultures was tested by plating on 4 petri plates, using potato glucose agar, 1-ml portions of water blanks, each blank containing 4 loopfuls of the thoroughly shaken culture. Such small inocula do not prove that the mold was no longer alive in those cultures in which it was

TABLE 1

Growth of two isolates of molds in acid media

NUMBER OF ISOLATE	NORMALITY OF SULFURIC ACID	AGE OF GROWTH	AMOUNT OF GROWTH
		days	
7752	1.0	67	Fair
7752	1.47	67	Fair
7752	2.04	103*	Light
7752	2.04	103*	Fair
9024	1.0	58	Heavy
9024	1.47	58	Heavy
9024	2.04	58	Light

* No visible growth in 67 days.

TABLE 2

Growth and viability of molds on prolonged incubation in acids

CULTURE NUMBER	STOPPER	AUTOCLAVED	NORMALITY OF ACID		INCUBATION	GROWTH	PLATES POSITIVE
			Initial	Final			
					days		
7752	Cotton	+	1.00	1.46	678	+	4
7752	Cotton	+	1.47	2.32	678	+	4
7752	Cotton	+	2.0	2.92	678	+	0
7752	Glass	−	2.04	2.20	713	+	0
7752	Cotton	+	2.49	3.52	678	−	0
7752	Glass	−	2.56	2.76	724	+	0
9010	Cotton	+	1.0	1.94	522	+	4
9010	Cotton	+	1.47	4.23	522	+	1
9010	Glass	−	2.0	2.05	569	+	3
9024	Cotton	+	1.0	2.25	669	+	0
9024	Cotton	+	1.47	—	669	+	0
9024	Cotton	−	2.04	4.93	669	+	0
9024	Cotton	+	2.04	3.98	669	+	0
9024	Cotton	−	2.49	4.58	669	−	0
Control	Cotton	+	2.0	2.84	678	−	0
Control	Cotton	+	2.49	3.51	678	−	0
Control	Glass	−	1.0	1.01	679	−	0
Control	Glass	−	1.47	1.52	679	−	0
Control	Glass	−	2.0	2.07	679	−	0

not found, but do demonstrate that it was still viable in those which yielded positive results.

Table 2 shows that one of the three strains produced a visible growth in 2.5 N sulfuric acid, and all three did so in 2.0 N sulfuric acid. It was demonstrated that

there was a tendency for the isolates to die out in prolonged incubation, especially where the acidity was allowed to increase by evaporation.

A single experiment with 1 N hydrochloric acid with the addition of 0.1 per cent each of glucose and peptone indicated that cultures of *T. cerebriforme* grew in other mineral acids of high titer.

It was further found that a solution of 280 grams of $CuSO_4 \cdot 5H_2O$, 1 gram of glucose, and 1 gram of peptone per liter of 1 N sulfuric acid allowed good development of both isolates of *T. cerebriforme*. This also confirms the amazing findings of Starkey and Waksman, findings which, incidentally, have been doubted by certain individuals.

Although pH determinations were made, they are not reported. The great inaccuracies of measurements with the glass electrode of hydrogen ion activities at the pH values which were less than 1, and indeed in many cases less than 0

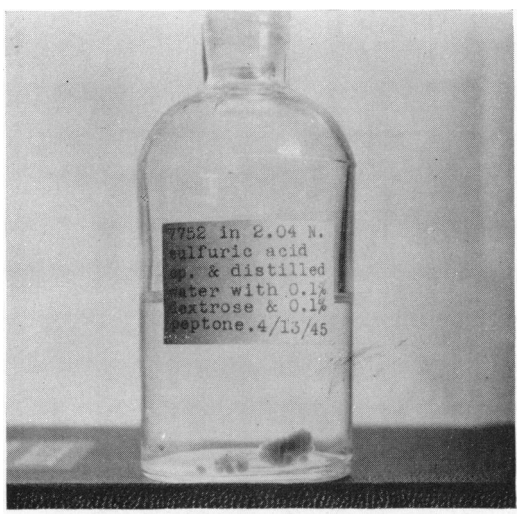

Figure 1. Growth of mold in 2 N sulfuric acid solution.

are such that determinations of normalities in these poorly buffered solutions are more meaningful.

SUMMARY

A fungus provisionally identified as *Trichosporon cerebriforme* was found to grow in 1, 1.5, 2, and 2.5 normal sulfuric acid fortified with the addition of 0.1 per cent glucose and 0.1 per cent peptone. Another isolate of the same species and an unidentified mold grew at normalities of 1, 1.5, and 2. Both isolates of *T. cerebriforme* grew in a solution of 280 g $CuSO_4 \cdot 5H_2O$, 1 g glucose, and 1 g peptone per liter of 1 N sulfuric acid.

REFERENCE

Starkey, R. L., and Waksman, S. A. 1943 Fungi tolerant to extreme acidity and high concentrations of copper sulfate. J. Bact., **45**, 509–519.