Penicillin Production by a Superior Strain of Mold

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The figures given in this note represent a small part of the data obtained during the course of an investigation of factors influencing fermentation time and yield in penicillin production in submerged cultures, the results of which will be published in more detail elsewhere. It is the purpose of this note to summarize briefly the results obtained when a number of penicillin-producing mold cultures were tested in 80-gallon tanks.

The cultures were isolated by laboratories at Stanford University (Department of Biology), the University of Minnesota (Division of Plant Pathology and Botany), and the Carnegie Institution of Washington (Department of Genetics). These laboratories, as well as our own, carried on penicillin research projects as a part of a program supported by the Office of Production Research and Development of the War Production Board. The testing of the most promising of these strains in tank fermentations at Wisconsin was a part of this program. The tanks used were of 80-gallon capacity and contained 220 liters of medium, 20 liters of which was inoculum grown in a smaller tank under aeration and agitation. The tanks were supplied with air at the rate of 200 liters per minute. Sterile air was admitted through pipe spargers containing 54 holes, each 1/32 in. in diameter. The agitators used were 12-in. propellers having two flat blades, set at a 30° angle, and revolving at 270 r.p.m. The tanks were held at gage pressure of 20 pounds per square inch during the fermentation period. The temperature was held at 23° C.

TABLE 1

HIGHEST PENICILLIN YIELDS OBTAINED FROM VARIOUS CULTURES*

Culture number	Source of culture	Highest yield obtained	Age at maximum yield	Comparable yield from culture 1951–B25†
		Units/ml.	Hours	Units/ml.
1951–B25	NRRL	245	64	...
832	NRRL	99	71	214
15–U1	Minnesota	222	68	245
R–38	Minnesota	135	55	172
R–1139	Minnesota	96	74	214
R–1205	Minnesota	191	69	214
1982	Stanford	119‡	64	103
25099	Stanford	244	62	184
35217	Stanford	275	84	245
35347	Stanford	255	72	245
45417	Stanford	229	69	214
X–1612	Carnegie	557	72	214

* The medium contained 4 per cent corn steep liquor (dry basis), 3 per cent lactose, and 1 per cent calcium carbonate.
† Because the yield on the reference cultures varied, the yield on the culture tested should be compared with the reference culture yield given in this column, which was determined at the same period under the same conditions.
‡ No calcium carbonate was used. The medium was adjusted to pH 6.5 with NaOH.

In Table 1 are listed the highest yields obtained with each of the cultures tested in tanks. Highest yields on two cultures obtained from the Northern Regional Research Laboratory of the U. S. Department of Agriculture are included for comparison, since these have been widely used and may be regarded as reference cultures. The last column of the table gives yields obtained on one of the reference cultures under conditions comparable with those under which the new culture was tested. This column is included because of the presence of a substantial degree of uncontrolled variability, due to changes in the composition of the corn steep liquor used and to unknown factors.

As may be seen from the table, only one of the new cultures, No. X–1612, gave a yield greatly in excess of that obtained with culture No. 1951–B25. Culture X–1612 is an X-ray mutant from *Penicillium chrysogenum* culture No. 1951–B25 of the Northern Regional Research Laboratory. This X-ray mutant was produced at the Carnegie Institution and after preliminary tests was forwarded for further testing to the University of Minnesota, where its performance in shaken flask cultures resulted in its being included with a group of promising Minnesota cultures forwarded to the University of Wisconsin for larger-scale tests.

TABLE 2

EFFECT OF NUTRIENT LEVEL ON YIELD OF CULTURE X–1612*

Composition of medium				Yield	Age at maximum yield
Corn steep liquor (solids)	Lactose	$CaCO_3$	Salts†		
Per cent	Per cent	Per cent		Units/ml.	Hours
2	2	0.2	+	239	54
4	3	1	–	480	64
6	3	1	–	636	67

* The fermentation conditions were those described in Table 1.
† The salts added per liter of medium were 0.25 gram KH_2PO_4, 0.12 gram $MgSO_4 \cdot 7H_2O$, and 1.5 gram $NaNO_3$. At a low steep liquor concentration the use of an auxiliary nitrogen source is desirable.

The penicillin yield obtained with this culture is, of course, greatly influenced by the conditions of nutrition, aeration, and agitation under which it is grown. Illustrative data on the effect of nutrient level at a high aeration rate are given in Table 2. It will be noted that in the presence of excess lactose (unfermented sugar remained at the end of each of the

fermentations) the penicillin yield is a function of the corn steep liquor level used.

Addendum: It is regretted that publication delay has caused this preliminary report to lose some of its significance during the interval since the manuscript was submitted on 30 July 1945. Since that time a number of papers describing work in which Culture X-1612 was used have appeared in this journal and elsewhere, from this and from other laboratories.

The Evolution of Oxygen From Illuminated Suspensions of Frozen, Dried, and Homogenized Chloroplasts

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The evolution of oxygen from suspensions of chloroplasts in solutions containing ferric oxalate and potassium ferricyanide was discovered by Hill and Scarisbrick (*5*). The possible relation of this photochemical and enzymatic reaction to the process of oxygen evolution by green-leaf photosynthesis and further details of the reaction have been discussed by various authors (*1, 2, 3, 4, 6, 7, 8*). It is the purpose of this note to summarize some data on the activity of chloroplast preparations that have been treated in various ways.

The significance of this work lies in the fact that chloroplast preparations are shown to be amenable to some treatments, such as would be necessary for the separation of their active components. By the use of similar preparations it may be possible to study some of the enzymes involved in a reaction which is initiated by light absorbed by chlorophyll and which results in oxygen evolution.

The participation of an enzyme system in the reaction appears to be indicated from the thermolability of the preparation and also from the inhibition by low concentrations of enzyme poisons such as NH_2OH, Duponol, NaF, and NaN_3. The Hill reaction is considerably less sensitive to NaN_3 than is the catalytic decomposition of H_2O_2 by chloroplasts.

Concomitant with the oxygen evolution there is also a production of H^+ which affords a simple means of measuring the reaction velocity. We have used a constant pH titration procedure for many of the activity measurements. In order to obtain quantitative results it is necessary to use a red filter to prevent the effects of light on the reagents themselves at high light intensity. The pH optimum was found to be at pH 7.0 or 7.5, depending on the conditions of measurement. Higher rates and a greater total evolution of oxygen were found if nitrogen was used instead of air in the manometer vessels. Temperatures of about 10° are most suitable for the measurement of this reaction, since the activity is rapidly lost at room temperature. Chloroplasts suspended in solutions of potassium ferricyanide alone were found to produce oxygen when illuminated, but at a lower rate than when ferric oxalate was also present. Supersonic irradiation breaks up the chloroplasts to give solutions which, when centrifuged for ¾ hour at *ca.* 20,000g, are green and appear clear but show the Tyndall scattering effect in an intense beam of light. The effects of various treatments on the activity of preparations of chloroplasts in ferric oxalate solution with ferricyanide are shown in Table 1.

TABLE 1

THE INFLUENCE OF VARIOUS TREATMENTS ON THE PHOTOCHEMICAL O_2 EVOLUTION BY CHLOROPLASTS IN A SOLUTION CONTAINING FERRIC OXALATE AND POTASSIUM FERRICYANIDE

Original material	Treatment	Per cent of original activity retained after treatment
Suspension of intact chloroplasts in 0.5 M sucrose	Frozen 15 hr.	72
"	Disintegrated in blender	54
Suspension of intact chloroplasts washed with water	Lyophilized	69, 31
"	Dried with molten $Na_2SO_4 \cdot 10\ H_2O$	78, 69
Suspension of intact chloroplasts in 0.5 M sucrose	Supersonic 1 min.	118
"	Supersonic 1 min. followed by ½ hr. centrifugation at 800g. Supernatant used after 15 hr. at 0°	81
Suspension of chloroplasts in water	Supersonic 1 min., centrifuged at 20,000g for ½ hr. Supernatant green solution used.	134
"	Sediment from the above centrifugation.	0
Fragments of leaves after 5 min. in blender strained, collected by centrifugation for ½ hr. at *ca.* 20,000g	Washed by suspension in water, recentrifuged, precipitate used.	57
	Above plus boiled water extract of leaves.	100
	Precipitated with ⅓ saturation $(NH_4)_2SO_4$	94, 85, 54, 55, 67