EFFECT OF CERTAIN ORGANIC ACIDS ON THE UTILIZATION OF MANNOSE AND FRUCTOSE BY THE FILAMENTOUS WATERMOLD, *ALLOMYCES MACROGYNUS*[1]

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The Burma 1Da strain of *Allomyces macrogynus* does not grow in minimal synthetic medium (Machlis, 1953*a*, *b*) with mannose or fructose replacing glucose as the source of carbon and energy except after a long and variable lag phase (Machlis, 1953*c*; Sistrom and Machlis, 1955). Vigorous growth with mannose or fructose, approaching that with glucose, takes place when these sugars are supplemented with a small amount of glucose. It was reported at the same time that autolyzed spores initiated vigorous growth with mannose, indicating the existence of unknown substances effective in very low concentrations—much lower than the concentration of glucose required for the same response. The purpose of the present investigation was to identify substances, other than glucose, capable of initiating vigorous growth with mannose and fructose.

MATERIALS AND METHODS

The materials and methods used were the same as described earlier (Machlis, 1953*b*; Sistrom and Machlis, 1955). Briefly, 50 ml of medium contained in 125-ml Erlenmeyer flasks were inoculated with approximately 25,000 motile mitospores. The cultures were then incubated at 25 C on a shaker. Each treatment consisted of 5 to 20 replicates. Growth was measured by compositing the replicates and drying the plant material to constant weight at 70 C. The sugars (together with the $MgCl_2$ and $CaCl_2$) were autoclaved separately from the rest of the medium, thus eliminating from consideration reactions between sugars and amino acids known to occur at elevated temperatures.

The mitospores were obtained by placing young sporophytic plants grown in minimal synthetic medium (with glucose) in a dilute salt solution for approximately 24 hr. (During this period sporangia develop and then release the motile spores.)

The minimal medium contained mineral salts, glucose, thiamin, and methionine, with nitrogen supplied as an ammonium salt. The dilute salt solution used to obtain spores for inoculum was a 1:10 dilution of the inorganic components of the minimal medium not including the trace elements.

The growth data were plotted as the cube root of the dry weight against time. For this organism, under the conditions used, a straight line resulted from this method of graphing the data (Machlis, 1957). Where this line intersected the horizontal axis at a level of 6 mg dry weight marked the end of the lag phase. The subsequent growth period was called the cubic growth phase.

For convenience of presentation, the long-delayed, sporadic growth with mannose or fructose was treated as a failure to grow, i. e., no growth.

EXPERIMENTAL RESULTS

The activating substances. Table 1 shows that low concentrations of substances present in yeast extract and casamino acid preparations make possible growth with mannose but not with fructose. If such an experiment is continued for longer incubation periods, no changes in the results occur until after 11 or more days, when an occasional culture with mannose or fructose alone or in combination with inactive supplements begins to grow.

The results indicate amino acids to be effective in making possible growth with mannose but not with fructose. In a parallel study (Machlis, 1957) it was shown that the amino acids related through the ornithine cycle and acetate reduce the lag phase of growth with glucose. Glutamic acid was most effective in evoking this response.

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TABLE 1

The effect of various additions to the nutrient medium on the utilization of mannose and fructose

Treatment	Incubation Period			
	94 hr	120 hr	144 hr	168 hr
	Dry weight per flask			
	mg			
Mannose, 0.5%		0	0	0
Plus vitamins*		0	0	0
Plus yeast extract, 10 ppm		38	71	80
Plus casein hydrolyzate, 10 ppm		7	42	69
Plus peptone, 5 ppm, and casein hydrolyzate, 5 ppm		60	77	72
Plus nucleic acids, 10 ppm†		0	0	0
Mannose, 0.45% plus glucose, 0.05%§		59	73	73
Fructose, 0.5%	0			
Plus casein hydrolyzate, 10 ppm	0			
Plus yeast extract, 10 ppm	0			
Fructose, 0.45% plus glucose, 0.05%§	23‡			
Glucose, 0.5%	26‡			

* Folic acid, myoinositol, *p*-aminobenzoic acid, riboflavin, nicotinamide, pyridoxine HCl, and calcium pantothenate, 50 μg per L each; and biotin 25 μg per L.

† Deoxyribonucleic acid, 5 ppm, plus ribonucleic acid, 5 ppm.

‡ Growth continues to completion with additional incubation.

§ Growth with 0.05% glucose alone does not exceed 10 mg per flask (Machlis, 1953*c*).

For this reason, L-glutamic acid was directly tested for its effect on mannose utilization, with the results shown in figure 1. About 50 μmoles per L effects the maximum response obtainable. The growth with mannose supplemented by this concentration of glutamic acid has the same lag phase and growth rate as that with glucose alone.

Glutamic acid, however, reduces the lag phase with glucose. Table 2 shows that as the concentration of L-glutamic acid is increased the lag phase with mannose approaches that with glucose containing the same concentration of glutamic acid. At still higher concentrations (figure 2) the two growth curves are almost identical.

The following amino acids and Krebs cycle intermediates were tested for activity in initiating growth with mannose at a concentration of 200 μmoles per L: DL-α-alanine, L-arginine, L-aspartic acid, L-citrulline, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, DL-norleucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine, citrate, malate, succinate, acetate, fumarate, α-ketoglutarate, isocitrate, *cis*-aconitate, and pyruvate. Of these only arginine, aspartic acid, citrulline, ornithine, and proline were active. As shown in table 3, glutamic acid is most active, followed closely by aspartic acid and then ornithine. The less active amino acids—for example, proline—can elicit responses equal to the maximum effected by glutamic acid, if supplied at higher concentrations (table 4).

The effect of acetate. It was to be expected that the Krebs cycle intermediates, except for acetate, would have no effect because considerable evidence is available that they do not penetrate the mycelium. They do not serve as sources of carbon (Machlis, 1953*c*), nor do they reduce the lag phase of growth with glucose except at quite high concentrations (Machlis, 1957). On the other hand, they become labeled within the mycelium when the mold is fed radioactive CO_2 (Lynch and Calvin, 1952) or radioactive glucose (Bonner, 1956). Further, the mycelium yields highly active mitochondria (Bonner, 1956).

Acetate, however, is an acceptable carbon source and does reduce the lag phase with glucose, although it is not oxidized by the mitochondria. It was, therefore, tested for effects on mannose utilization up to a concentration of 25,000 μmoles per L. The results were negative

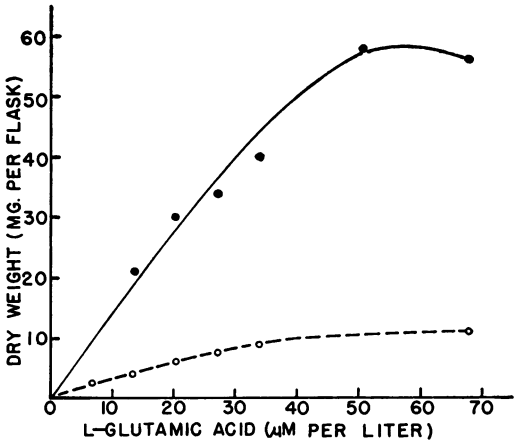

Figure 1. The effect of L-glutamic acid on growth with mannose. The broken curve is after 120 hr incubation and the solid curve after 151 hr.

TABLE 2

The effect of L*-glutamic acid on the lag phase of growth with glucose and with mannose*

L-Glutamic Acid	Glucose	Mannose	Difference
μmoles per L	*Hr*	*Hr*	*Hr*
0	88	0*	—
25	65	113	48
50	56	90	34
100	50	79	29
500	41	65	24

* The first of the replicate cultures began growing after a lag phase of 240 hr. See section on Materials and Methods for designating this lag phase as zero.

except for concentrations of 16,680 and 25,000 μmoles per L. At these levels and with no difference in the effect of the two concentrations, uniform growth among replicate flasks was obtained. The lag phase, however, was reduced below that with mannose alone only slightly more than the same concentration of acetate reduces the lag phase with glucose (figure 2). Thus, in contrast to the effect of glutamic acid, it was not possible to make the growth with mannose equal to that with glucose, both supplemented with the same concentrations of acetate.

Because acetate is a source of carbon, it should be noted that growth with acetate alone does take place as shown in figure 2 (see also Machlis, 1953*c*). This growth has a short lag phase but is soon terminated, the latter because the medium rapidly becomes highly alkaline. The significant fact is that the mannose inhibits this limited utilization of acetate. This is more strikingly evident at slightly lower levels of acetate where no growth occurs at all for 11 or more days, whereas with the acetate alone growth is prompt.

The utilization of fructose. The factors controlling the utilization of fructose are clearly different from those involved in mannose utilization. Some indication of this was obtained during the earlier study (Sistrom and Machlis, 1955), when growth was obtained with both mannose and fructose by supplying simultaneously a small amount of glucose. The balls of plant material in a mannose culture are soft and open, as are those grown with glucose. In contrast, balls of material grown with fructose are dense and compact. It has already been shown that fructose utilization cannot be initiated by amino

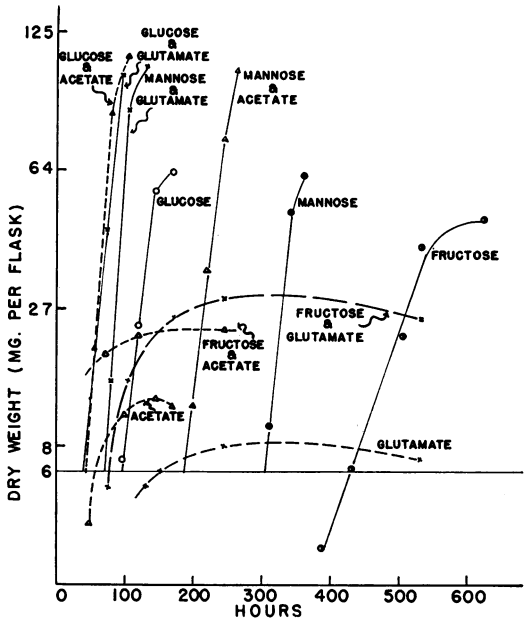

Figure 2. The effect of a high concentration of DL-glutamic acid (6,060 μmole per L) and of acetate (25,000 μmoles per L) on the utilization of mannose and fructose. The curves for growth with mannose and fructose alone are approximations. By inoculating a large number of replicate cultures, a few are obtained which begin growing more or less together. These few constitute the sample from which the growth curve data were obtained. With each sugar, the depicted curve represents the earliest growth observed in a large number of experiments.

acids as is mannose utilization. Further evidence in the same direction is included in figure 2. In the presence of high concentrations of glutamate and acetate, but not low ones, growth with fructose begins promptly; however, it stops very soon when the dry weight is more than that obtained with the acetate or glutamate alone but only about one-fifth of that possible were the fructose fully utilized.

The fate of the inoculum with mannose. When cultures containing unsupplemented mannose are inoculated no visible growth occurs for many days. Such cultures and comparable petri dish and depression slide cultures were observed microscopically as described in detail by Machlis (1957). With glucose, 24 hr after inoculation the spores have a well-developed rhizoidal system (see Emerson, 1941, 1955, for developmental history). During the next 48 hr the primary

TABLE 3

The relative effectiveness of amino acids in initiating growth with mannose

Amino Acid*	Dry Weight*
	mg per flask
L-Glutamic acid	67
L-Aspartic acid	57
L-Ornithine	43
L-Arginine	22
L-Citrulline	10
L-Proline	1

* Each amino acid was supplied at a concentration of 50 μmoles per L, and the incubation period was 157 hr.

TABLE 4

The effect of L-proline on initiation of growth with mannose

Concentration	Dry Weight*	
	L-Proline	L-Glutamic acid
μmoles per L	*mg per flask*	*mg per flask*
17	0.5	36
34	3.0	43
51	23.0	41
68	31.0	41
136	39.0	43

* 148 hr incubation.

hyphal tube develops and begins to dichotomize, giving rise to the mycelium.

With mannose, a rhizoidal system also develops during the first 24 hr, but then nothing more happens. The plants remain at this stage until growth is resumed, approximately 11 days at the minimum, and, at the maximum in excess of 26 days, the longest period tested.

With glucose and with rich yeast extract media, 25 per cent of the spores inoculated into a culture survive and develop into plants. With mannose the survival is a little over 4 per cent.

DISCUSSION

The utilization of the sugars, mannose and fructose, by the Burma 1Da strain of *A. macrogynus* under the specified nutritional and environmental conditions is seen to be governed by a number of factors.

Growth occurs with either sugar, provided each is supplemented with a small amount of glucose. Still lower concentrations of certain amino acids make possible growth with mannose but not with fructose. Mannose appears to inhibit the utilization of acetate, and finally, very high concentrations of acetate or glutamate make possible growth with fructose; this growth, however, terminates abruptly long before the nutrients are exhausted or an unfavorable pH develops. These results are physiological observations. Their interpretation depends on biochemical information which is not available. It may not be too presumptive, however, to indicate that the earlier interpretation of the role of glucose in initiating the utilization of mannose and fructose, that is, as a supply of carbon and energy for induced enzyme synthesis, is still tenable with respect to mannose. The amino acids which initiate growth with mannose can, presumably, supply both carbon and nitrogen. Thus, both energy and amino acids would be provided for the synthesis of enzymes. The failure of acetate to function would appear to be caused by the fact that mannose somehow prevents either its entrance or its metabolism. Certainly, if it entered and were metabolized, as it is when fed alone to the organism or together with glucose, it could serve both as an energy source and as the carbon skeleton for amino acid synthesis from ammonia.

Fructose utilization is an enigma. Because a small amount of glucose permits growth with fructose, whatever the organism needs for fructose utilization it can make from glucose. Very high concentrations of glutamate—much higher than those needed for mannose utilization—and still higher concentrations of acetate initiate early growth with fructose. Under these conditions, however, the nature of the metabolism is self-inhibitory and soon causes growth to cease. It is not known if toxic products are excreted into the medium. One might speculate that fructose interferes with the uptake of the amino acids until such interference is overcome by very high concentrations.

Although these results are not now explicable in biochemical terms, certain general and other specific conclusions may be made. Lilly and Barnett (1953), in concluding their discussion of an extensive study of sugar utilization by fungi, suggested that such studies should include the effects of mixed sugars and of the other constituents of the medium, such as nitrogen sources. The present study shows that both factors can critically shape the answer to the question: "Which sugars are utilized by a given species?"

There is some evidence of similar responses with other fungi. The effects of mixed sugars (see Lilly and Barnett, 1951, 1953, and citations therein) on the growth of fungi appear, at least in part, to be like the interaction of glucose with mannose and fructose as described above. Several examples of amino acids and more complex nitrogenous substances affecting the course of metabolism are known among the bacteria and fungi. Most closely related to the present study is that of Friedman (1954), who found amino acids necessary for the utilization of high concentrations of fructose by the luminous bacterium, *Achromobacter fischeri*.

The results obtained in the present study clarify the conflicting reports of Machlis (1953*c*) and Ingraham and Emerson (1954) on the utilization of mannose by the Burma 1Da strain of *A. macrogynus*. Since the latter investigators included glutamic acid in the test medium, they reported growth with mannose. Machlis, omitting the glutamic acid, obtained no growth.

Reference has been made to the fact that with mannose alone growth ultimately takes place after a long lag period, the lag varying from a minimum of about 11 days to periods in excess of 26 days. In such cultures, the survival of the spores added as inoculum is a little over 4 per cent. It seems likely that the long delayed growth and its sporadic appearance can be explained as the result of the appearance in the medium of traces of amino acids due to autolysis of the bulk of the inoculum. If this explanation is correct, then the autolyzed spores presumably release other substances, which in traces, initiate the delayed, sporadic growth with fructose.

SUMMARY

The Burma 1Da strain of the watermold, *Allomyces macrogynus* grows vigorously in minimal synthetic medium with glucose as the carbon and energy source, but not with mannose or fructose, unless these are supplemented with a small amount of glucose. Vigorous growth with mannose but not with fructose is also initiated by small amounts of L-aspartic acid, L-arginine, L-citrulline, L-glutamic acid, L-ornithine, and L-proline, but by no other amino acid nor by any of the Krebs cycle acids. Glutamic acid is the most active and elicits a maximum response at a concentration of 50 μmoles per L. Very high concentrations of acetate initiate growth with mannose, which, however, has a long lag phase, indicating that mannose inhibits the normal growth with acetate, which has a short lag phase. With very high concentrations of both acetate and glutamate, but not with low concentrations, growth with fructose begins promptly but then terminates rapidly before the medium is exhausted of nutrients or an inhibitory pH develops.

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