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An Organism Able to Metabolize 3-Methylglucose and 3-Methylfructose

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SUMMARY: An organism was isolated from soil, which grew in a basal mineral medium with either 3-methylglucose or 3-methylfructose as sole source of carbon. The bacterium is a member of the *coli-aerogenes* group, intermediate type 1. Grown in presence of either methyl-sugar, under suitable conditions, washed suspensions of the organism oxidize either 3-methylglucose or 3-methylfructose. Under anaerobic conditions, the two methyl-sugars are broken down with the formation of hydrogen, carbon dioxide and acid. The ability to attack 3-methylglucose or 3-methylfructose was acquired by growth in presence of either of the two methyl-sugars but not in the presence of glucose.

When 3-methylglucose is fed or administered to rats it is largely excreted in the urine unchanged (Campbell, 1949). The present investigation was undertaken in an attempt to discover whether micro-organisms exist which are able to attack and utilize both 3-methylglucose and 3-methylfructose, and whose existence might affect the use of these sugars in feeding experiments.

METHODS

Growth media. A basal mineral medium was prepared as follows: $(\text{NH}_4)_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 4.0 g.; KH_2PO_4 , 0.4 g.; NaCl, 1.0 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.7 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g.; distilled water to 1.0 l.; pH adjusted to 7.0. The basal medium was distributed in 9 ml. portions in miniature Roux bottles and sterilized by autoclaving (110°). Solutions (10 %, w/v) of 3-methylglucose or 3-methylfructose were sterilized by filtration through Seitz filters. One ml. of one or other of these solutions was added to each Roux bottle; for anaerobic growth the medium was distributed in flasks.

Preparation of washed suspensions of bacteria. After 36 hr. incubation at 30° cultures were centrifuged; the cells were washed once in distilled water and made up into a suspension in water. The dry weight of cells in such suspensions was determined on the Hilger Spekker absorptiometer previously calibrated by dry-weight determinations.

Respiration experiments. Oxygen consumption was measured in Warburg manometers containing washed cell suspension, phosphate buffer (pH 7.0) and 3-methylglucose or 3-methylfructose; NaOH solution and filter-papers were placed into the centre cups to absorb CO_2 . The production of CO_2 was determined in parallel experiments by omitting NaOH from the centre cup and releasing retained CO_2 at the end of the experiment by tipping H_2SO_4 from a second side-bulb. Rates of oxidation are expressed as Q_{O_2} .

Fermentation experiments. The anaerobic breakdown of the methyl-sugars was studied in Warburg manometers. Gas production was measured in mano-

meters filled as above but containing nitrogen gas; CO_2 retained was measured by the acid-tip method as above; manometers were set up in duplicate, one of each pair containing NaOH in the centre cup to absorb CO_2 if produced. Acid and gas production were measured in manometers containing washed suspension, NaHCO_3 and 3-methylglucose or 3-methylfructose; the manometers were filled with a gas mixture containing 95 % (v/v) N_2 and 5 % CO_2 . Acid production was calculated from the decrease in NaHCO_3 concentration determined after the experiment by the usual acid-tip method. Gas production in the bicarbonate buffer system is expressed in terms of CO_2 .

Isolation of organism utilizing methyl-sugars

Several bottles each of the two media containing the respective methyl-sugars were inoculated with garden soil and aerobically incubated at 25° . In all cases the medium became turbid within 24–48 hr. During the first series of experiments, four serial subcultures were made into fresh medium at 48 hr. intervals. In a second series of experiments, four subcultivations were made at 24 hr. intervals. At the end of these periods the cultures were plated on the two media solidified with 3 % of agar.

No consistent growth was obtained in the experiments using 3-methylglucose, the fourth subcultivation giving a slight amount of growth which proved to consist of a mixture of many different organisms. These experiments were discontinued, since an organism utilizing 3-methylglucose was isolated from the cultures containing 3-methylfructose.

The cultures grown with 3-methylfructose grew heavily and proved to consist of a mixture of four or five morphologically distinct organisms. In the culture series which had been subcultivated at 48 hr. intervals the predominant organism was isolated in pure culture and identified as a species of *Pseudomonas*. This organism, however, proved unable to grow in liquid medium with 3-methylfructose as sole source of carbon. This *Pseudomonas* species was not predominant on plates of cultures subcultivated at 24 hr. intervals, and four different colonies were selected from the plating of this series for further investigation. Organisms from these colonies were grown in 3-methylfructose media and then replated until pure cultures were obtained. At the same time the organisms were inoculated into Roux bottles containing 150 ml. 3-methylfructose medium, incubated at 30° for 36 hr., harvested on the centrifuge, washed once in distilled water, resuspended in water and tested for ability to oxidize 3-methylfructose. Growth was poor with one strain (not further studied) but heavy with one and moderate with the remaining two. The respiration experiments indicated that the three organisms oxidized 3-methylfructose at different rates. The organism which gave heavy growth in 36 hr. at 30° had Q_{O_2} (3-methylfructose) = 12–24 in preliminary experiments; this organism was used for the studies reported in this paper.

Identification of organism

The organism was identified as *Bacterium coli* intermediate type 1 (Wilson, Twigg, Wright, Hendry, Cowell & Maier, 1935). The bacterium has the morphological and cultural characteristics of *Bact. coli* intermediate type 1,

with an optimal growth temperature range of 25–30°. This organism belongs to the type which is one of the commonest of all coliform bacteria found in soils of this country (Bardsley, 1934). The species with closest resemblance to our organism listed in *Bergey's Manual* (1948) is *Escherichia freundii* (Braak) Yale.

The biochemical characteristics of our strain can be summarized as follows: acid and gas formation, under aerobic conditions, took place in peptone broth after 18 hr. with arabinose, glucose (also at 37°), 3-methylglucose, fructose, 3-methylfructose, galactose, mannitol, sucrose, maltose and cellobiose. Acid and traces of gas after 3 days with rhamnose and lactose; acid but no gas with glycerol after 40 hr.; neither acid nor gas from dulcitol, raffinose, inulin or starch. Under anaerobic conditions (N_2/CO_2 or H_2/CO_2) acid and gas in peptone broth or Stephenson's medium with glucose, fructose, 3-methylglucose or 3-methylfructose (Stephenson, 1949).

Other biochemical reactions: nitrite formed from nitrate but no N_2 liberated; hydrogen sulphide positive; indole negative; methyl-red positive; Voges-Proskauer negative; citrate utilization positive; urease negative; catalase positive; gelatine liquefaction negative after 14 days' incubation.

Metabolism of 3-methylfructose and 3-methylglucose

The growth tests indicated that the organism could utilize either 3-methylfructose or 3-methylglucose for aerobic or anaerobic growth. The organism was therefore grown on a larger scale for metabolic investigations in the defined basal medium containing the appropriate methyl-sugar. The ability of washed suspensions to oxidize the methyl-sugars was first tested and experiments were

Table 1(a). *Metabolism of 3-methylfructose by washed suspensions of cells grown in the presence of 3-methylfructose*

(Organism grown for 36 hr. at 30° in basal medium + 0.5 % (w/v) 3-methylfructose. Suspension density: 22 mg. dry-weight cells/ml. For oxygen consumption: main compartment contained 1.0 ml. 0.1 M phosphate buffer (pH 7.0) and 1.0 ml. cell suspension; 0.5 ml. 0.01 M 3-methylfructose (or water in controls) in side bulb, and 0.2 ml. 10 % NaOH with filter-papers in inner cup. For CO_2 production, conditions as above and in addition 0.5 ml. 2N- H_2SO_4 in side bulb 2 but without NaOH in inner cup; air.

For fermentation experiments: main compartment 1.0 ml. washed suspension + 1.0 ml. 0.03 M- $NaHCO_3$; 0.5 ml. 0.01 M 3-methylfructose in side bulb 1, and 0.5 ml. 2N- H_2SO_4 in side bulb 2; 95 % N_2 + 5 % CO_2 ; all values in μ l.)

Atmosphere		Cells + substrate (μ l.)	Cells alone (μ l.)	Difference (substrate) (μ l.)
Air	Q_{O_2} :	12.9	1.8	11.1
	Oxygen consumption (completion)*:	790	363	427
Air	Q_{O_2} :	23.6	4.6	19.0
	Oxygen consumption (completion)*:	699	206	493
	CO_2 production (completion)*:	695	245	450
95 % N_2 + 5 % CO_2	Gas production, as CO_2 :	307	24	283
	Acid production, as CO_2 :	208	0	208

* Theory for complete oxidation = 840 μ l.

Table 1(b). *Metabolism of 3-methylglucose by washed suspensions of cells grown in presence of 3-methylglucose*

(Conditions similar to Table 1(a) with 3-methylglucose substituted for 3-methylfructose.)

Atmosphere		Cells + substrate (μ l.)	Cells alone (μ l.)	Difference (substrate) (μ l.)
Air	Q_{O_2} :	24.5	8.7	15.8
	Oxygen consumption*			
	150 min.:	624	299	325
	Completion, 12 hr.:	1184	728	456
Nitrogen	Hydrogen production:	227	17	210
	CO ₂ production:	233	31	202
	Q_{H_2} :	7	0	7
95 % N ₂ + 5 % CO ₂	Gas production, as CO ₂ :	399	16	383
	Acid production, as CO ₂ :	159	0	159

* Theory for complete oxidation = 840 μ l.

continued until the rate of oxygen consumption in presence of the given methyl-sugar had fallen to the rate in manometers without substrate. Table 1, *a* and *b*, shows that for both methyl-sugars the oxidation continued until the oxygen uptake (allowing for blank respiration) had reached a value approximately 55 % of that required theoretically for complete oxidation of substrate. The CO₂ output was approximately equal to the oxygen consumption. When washed suspensions were incubated anaerobically in N₂ or N₂ + CO₂ atmospheres, evolution of gas took place. With 3-methylglucose in N₂, the gas evolved consisted of approximately equal volumes of hydrogen and carbon dioxide. Experiments in bicarbonate buffer indicated (Table 1*a* and *b*) that, under anaerobic conditions, acid was also produced. Allowing for the fact that the gas produced contained hydrogen, the acid formed was approximately equivalent to the CO₂ formed during the fermentation.

Effect of presence of substrate during growth

The organism was grown in basal medium with 3-methylfructose, 3-methylglucose or glucose as sole carbon source. Washed suspensions were prepared and the ability to oxidize the methyl-sugars tested. Table 2 shows that neither

Table 2. *Effect of growth substrate on ability to oxidize 3-methylglucose and 3-methylfructose*

(Conditions similar to those given in Table 1(a). Rate of oxygen uptake studied over 1 hr. and expressed as Q_{O_2} = μ l. oxygen taken up/hr./mg. dry-weight cells. Figures in () are those for endogenous respiration blanks.)

Carbon source in growth medium	Respiration substrate		
	Glucose Q_{O_2} (μ l.)	3-Methylglucose Q_{O_2} (μ l.)	3-Methylfructose Q_{O_2} (μ l.)
Glucose	32.5 (0.5)	1.0 (0.5)	0.0 (0.5)
3-Methylglucose	—	73.6 (9.5)	65.8 (9.5)
3-Methylfructose	—	69.2 (13.2)	64.5 (13.2)

3-methylfructose nor 3-methylglucose was attacked by cells grown in glucose but that both methyl-sugars were attacked by cells grown in the presence of either. It appears probable that the primary breakdown of the methyl-sugars requires the presence of an adaptive enzyme evoked by the presence of either 3-methylglucose or 3-methylfructose.

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