I.—A STUDY OF THE SYMBIOTIC FUNGUS FROM THE MYSORE LAC INSECT.

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Lac insects are normally associated with intracellular organisms, ellipsoidal and yeast-like in appearance. The organism is a fungus and its presence is a sign of vigorous health in the insect. Similar symbiotic associates have been recorded from time to time but the physiological function of the symbionts has not been the subject of much study. Keeble has suggested that they utilise the excretory nitrogen of the host while Portier ascribes to them a more important role, since he found that they produce reactive ketones and aldehydes on fermentation.

The organism was first isolated from the Mysore lac-insect on prune-agar medium, the reaction of which was distinctly acid to litmus. It was then grown in three series of artificial media; the first series consisting of carbohydrates, the second of nitrogenous compounds, while in the third a study was made of the necessary inorganic constituents.

In the carbohydrate series, various sugars were employed in equivalent proportions, as also starch, inulin and gum arabic. Various nitrogen compounds, amino-acids, amides, purines, nitrates etc. were used in the second series as the source of nitrogen, in such quantities that the nitrogen-content of the medium was kept constant. With regard to the inorganic constituents, the table explains itself.

After eight weeks' growth the solutions were analysed in the following manner. The solution was made up to the original volume and then filtered through a bed of previously washed and ignited kieselguhr. The residue was washed repeatedly with hot water to free it from the gummy medium and the precipitate together with the kieselguhr bed was dried in the hot air oven at 90° to constant weight, ignited and again weighed. The difference was taken as the weight of the fungus crop.

The filtrate, of which the first portions were rejected, was used for determining rotation and viscosity, the latter being measured with an Ostwald's viscosimeter at 25°, taking the usual precautions. The viscosity has been taken as a measure of the gum-content of the medium.

TABLE I.

Carbohydrate Series.

No.	Source of carbon	Yeast crop in grams	Rotation in 10 cm. tube	Viscosity at 25°
1	No Sugar ...	...	...	1·30
2	Glucose ...	74·6	...	1·38
3	Fructose ...	56·4	...	1·43
4	Sucrose ...	75·4	.	1·47
5	Maltose ...	118·0	...	1·46
6	Lactose ...	40·0	+ 0·25	1·33
7	Arabinose ...	24·8	...	1·38
8	Xylose	51·6	...	1·38
9	Starch	25·0	?	2·37
10	Inulin	7·4	−0·81	1·96
11	Gum arabic ...	127·6	−0·25 (Initial, + 1·05)	1·86

From the above it follows that among the sugars, maltose yields the highest crop: gum arabic, however, serves as well if not better. Maltose and sucrose are hardly distinguishable in yielding the highest gum-content as shown by the greatest viscosity. Lactose is not quantitatively utilised by the organism, the galactose portion of the molecule perhaps being left untouched; unfortunately the galactose medium became contaminated and this point has not yet been made clear. Inulin is not utilised by the organism, while only a part of the gum arabic appears to be attacked, the laevo-component being left untouched or appearing as a result of excretion; further work alone can decide this interesting point.

TABLE II.

Nitrogen Series.

No.	Source of nitrogen	Yeast crop in grams	Viscosity at 25°	Rotation in 10 cm tube
1	No nitrogen (not inoculated) ...	...	1·32	+ 0·95°
2	No nitrogen ...	25·5	1·36	+ 0·50
3	Glycine	130·0	2·51	+ 0·19
4	Alanine	63·4	2·10	+ 0·34
5	Leucine	56·3	2·13	+ 0·43
6	Tyrosine ...	112·3	2·15	+ 0·21
7	Asparagine ...	204·0	2·18	...
8	Acetamide ...	313·4	3·25	...
9	Urea	169·2	3·10	...
10	Uric acid ...	323·6	3·33	...
11	Hippuric acid ...	73·5	2·20	+ 0·29
12	Nitrate	153·0	2·51	+ 0·10
13	Ammonium sulphate	267·0	3·10	...

Uric acid and acetamide yield the heaviest crops of yeast, ammonium sulphate coming next. When such a diversity of compounds serve as sources of nitrogen it is difficult to draw any definite conclusion. Sugar is quantitatively utilised in the presence of asparagine, acetamide, urea, uric acid and ammonium sulphate. The greatest quantity of gum is produced with uric acid and the nitrogen series generally yield a higher gum value. The utilisation of uric acid is interesting in view of Keeble's suggestion that a function of the organism is to remove the excretory nitrogen of the host.

TABLE III.

Inorganic Constituents Series.

No.	Medium	Yeast crop in grams	Rotation in 10 cm. tube	Viscosity at 25°
1	Normal	156·8	...	1·41
2	Nitrogen free ...	25·5	+0·50	1·32
3	Phosphorus free ...	5·8	+0·93	1·31
4	Calcium free ...	168·6	...	1·35
5	Magnesium free ...	266·8	...	1·42
6	Potassium free ...	149·0	+0·12	1·37
7	Sodium free ...	166·8	...	1·39
8	Calcium excess ...	136·0	+0·16	1·43
9	Magnesium excess ...	110·6	...	1·33
10	Normal (not inoculated) ...	...	+0·95	1·30

The magnesium-free medium yields the highest crop of the fungus and, with the medium containing excess of calcium, has the highest gum-content. The absence of nitrogen, phosphorus or potassium affects the carbohydrate metabolism and the sugar is not quantitatively utilised.

A large scale fermentation was carried out to study the products, the medium being composed of:—Potassium nitrate, 20 gms., hydrogen potassium phosphate, 10 gms., sodium chloride, 5 gms., magnesium sulphate, 5 gms., glucose (commercial), 100 gms., water, 2 litres.

The time allowed for fermentation was two months. The fermented mash was filtered through a kieselguhr bed and the clear filtrate distilled on a sand-bath. The first 200 c.c. were collected and found to have the following properties :—It reduces ammoniacal silver nitrate but not Fehling's solution; it answers to the iodoform reaction and with phenylhydrazine gives a yellow precipitate. All these tests point to the presence of an aldehyde or ketone, but further work is necessary to identify the constituents.

The kieselguhr fungus crop was ground up with quartz sand and extracted with ether. A light yellow oil has been obtained, which has the property of drying, or absorbing oxygen from the air. A micro-analysis of the product will throw some light on the nature of the oil. Lastly it may be stated that the comparative bio-combustion experiments carried out with the Mysore symbionts as well as *S. Cereviseæ* showed that the carbon dioxide produced by the former was only one-third that given out by the latter.

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