THE SLIME OF *DEMATIUM PULLULANS*.

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During the investigation into the etiology of the gum-flux of the peach and almond, a mould was isolated which, pending further examination, was called a dematium-yeast.* Upon saccharose media, it appeared as a raised glistening growth which eventually became dull. The culture had an appearance which suggested the presence of a small quantity of slime which was most noticeable when the growth was raised from the surface of the medium. It then appeared to be attached to the agar by a slimy substance.

Dematium pullulans has been credited with the production of a gummosis (? gum-flux) of the plum by Massee†, and Wortmann‡ has claimed that it causes a viscosity in musts and wines. It is known§ to produce a slimy or ropy consistency of unhopped beer-wort.

If the examination of the cultures of the mould should reveal the presence of a gum or slime, and if this should prove to consist of arabin or metarabin, then the rôle of the organism in contributing to the gum-flux of the peach, almond and other fruits would require consideration.

When time permitted, I investigated the mould and found that it undoubtedly was *Dematium pullulans*.

A quantity of the mould was obtained by growing it upon the surfaces of plates of saccharose-potato agar. Furthermore, flasks

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* *Antea*, p. 129.

† Kew Bulletin, 1899.

‡ See Meissner, Cent. f. Bakt. 2, v. 232.

§ See Lindner, Cent. f. Bakt. iii. 750, and Betriebskontrolle in den Gärungsgewerben (1898), 218.

of saccharose nutrient fluid* were infected. After having stood in a cupboard for some months, these contained a number of films of a tough slimy consistency that had formed on the surface and had fallen to the bottom of the culture fluid. The fluid itself was somewhat viscous, although it could not be called ropy, and the viscosity was most pronounced when the medium contained chalk.

A portion of the agar-culture suspension was boiled with 1 % sodium hydrate. The emulsion contracted to a curd, leaving a clear fluid. The addition of alcohol to the fluid produced no precipitation, showing that dilute alkali was powerless to extract any gum carbohydrates from the growth. Another portion was boiled with 1 % hydrochloric acid. The suspended matter distributed itself throughout the acid in floccules and did not contract into a curd as with dilute soda. The acid filtrate after neutralisation gave a partly flocculent and partly stringy precipitate upon the addition of alcohol. The nature of the precipitate showed that a gummy body had been extracted by the dilute acid, and this solvent was accordingly used in the further treatment of the suspension.

The remainder of the suspended growths was diluted with water, and hydrochloric acid was added to make a 1 % solution, after which it was heated on the water-bath for some hours. The fluid was then strained through calico and filtered. Further treatment of the insoluble matter was found to be unnecessary, as everything soluble in acid and precipitable by alcohol had been removed. The filtrate, after neutralisation, was evaporated to small volume and treated with alcohol. A glutinous precipitate was thrown out of solution. The alcohol was removed by straining through calico and pressing the precipitate. Treatment with water showed that the solid consisted of at least two constituents, one soluble in water, the other insoluble. Both were repeatedly precipitated by alcohol from aqueous solution and suspension. Finally, both solution and suspension were tested and found to be free from reducing sugars.

* Saccharose 50, peptone 5, potassium chloride 3, sodium phosphate 2, water 1000 grms.

The gum-like substances were boiled with 5 % sulphuric acid in flasks provided with aërial condensers for five hours. The soluble portion was completely hydrolysed by the acid, as was shown by the presence of reducing sugars and the absence of a precipitate upon adding alcohol to a small neutralised portion. The water-insoluble gum had not been attacked, and the boiling was continued for 20 hours. Still no reducing sugars could be detected. From the resistance to hot dilute acid and from the solubility in acid and alkali, it was probable that the slime would eventually be shown to be a pararabin.* The hydrolysis with concentrated acid was deferred.

The acid in the solution of the hydrolysed water-soluble portion was removed by barium carbonate. Traces of the precipitate that passed through the filter were eliminated with aluminium hydrate and the clarified solution was evaporated to small volume. Phenylhydrazine acetate solution was added, and the whole was heated on the water-bath for two hours. The osazones that separated out upon cooling the fluid were filtered off, moistened with alcohol and extracted with ether, which removed much impurity. The semi-pure osazones were heated with water, which dissolved a trace of galactosazone and a vitreous yellow substance comparatively easily soluble in hot water. The portion insoluble in water was dissolved in hot alcohol and cooled. There separated out a yellow crystalline precipitate which melted at 200° and which was eventually separated into galactosazone (m.p. 193°) and glucosazone (m.p. 205°). The cold alcohol filtrate from the osazone (m.p. 200°) upon evaporation yielded glucosazone.

The soluble substance extracted from the cultures of *Dematium pullulans* by dilute acid thus hydrolysed to galactose and a glucose.

These results were confirmed by the examination of a fluid culture. Much the same method of procedure was adopted in

* It may be noted here that Skerst (Cent. f. Bakt. 2, iv., 864) found that *Dematium pullulans* produced a characteristic skin of a gelatinous consistency with sugars such as saccharose, dextrose and levulose, especially when these were present in nutritive fluid to the extent of 10 %. He found that the slime or gum was not attacked by nitric and hydrochloric acids, zinc chloriodide, iodine, alcohol, petroleum-ether, ether, chloroform or potassium hydrate. It was attacked by concentrated sulphuric acid.

this case. The culture was evaporated to smaller volume and heated after hydrochloric acid had been added to make a 1 % solution. The acid filtrate was made alkaline, whereupon the difficultly hydrolysable carbohydrate was precipitated, while the easily hydrolysable constituent remained in solution. The latter was purified by repeated precipitation with alcohol, the former by solution in acid and precipitation with alkali until all reducing sugars had been eliminated. As in the former case, the soluble substance was completely hydrolysed to galactose and a glucose, while the insoluble carbohydrate was not attacked.

What the nature of the glucose was, I did not endeavour to determine. The object of the research was to prove the presence or absence of arabin or metarabin among the products of *Dematium pullulans*. The absence of arabinose among the sugars of the water- and alkali-soluble portion showed that neither of these gums is a product of the organism.

Remembering that Kossel* had shown that the nucleic acid of yeast when boiled with dilute hydrochloric acid gives a mixture of a glucose and a pentose, it occurred to me that the glucose and galactose that I had obtained had in all probability been derived from the nucleic acid of the fungoid nucleoproteid. That they had been so derived was shown by the absence of carbohydrates, soluble in dilute alkali, in the viscous filtrate from a culture of the mould in a saccharose-peptone fluid which contained chalk. It can therefore be accepted that the glucose and galactose had been derived from the proteids of the *Dematium* and should not be considered as having been derived from the slime products of the organism.

Upon finding that the constituent insoluble in dilute alkali could not be hydrolysed, the dilute acid solution was evaporated down upon the water-bath until it charred. At this stage it showed traces of reducing sugars when tested with Fehling's solution. The dark-coloured liquid was diluted with water to the original volume and boiled for some hours under an aërial condenser. After removal of the sulphuric acid and concentra-

* Lafar, Technical Mycology II., i., 162.

tion of the fluid, the osazones were prepared. The crude osazone, after treatment with ether, yielded a yellow crystalline constituent soluble in warm water, with a melting point of 160° (arabinosazone) and another which melted at 194° (galactosazone). The quantity of the former that was obtained was just enough to determine the melting point. The latter had the characters of galactosazone. When these results are considered in conjunction with the solubility of the carbohydrate in acid, the insolubility in dilute alkali and the resistance of the hydrolytic action of boiling 5 % sulphuric, it is apparent that the carbohydrate is a pararabin.

The pararabin is a kind which when once in the insoluble condition is not easily made soluble. As obtained by growing the mould upon solid media, the slime did not dissolve to any extent upon digesting a suspension of the culture in the autoclave, and on this account dilute acid was used as a solvent. In contrast with this behaviour, the pararabin formed by *Bact. pararabinum* was easily dissolved by the autoclave treatment. Still some of the Dematium slime is dissolved, and it is probably simply a question of time or of temperature in order that the carbohydrate may be completely altered to the soluble modification. Upon the evaporation of most of the water, a solution of the slime became gelatinous. Drops of this gelatinous solution when tested with drops of reagents gave white curdy precipitates with basic and ammoniacal lead acetate; faint white precipitates with baryta water, silver nitrate and phosphotungstic acid; pale blue precipitates with Schweitzer's and Fehling's solutions; and no reaction with neutral lead acetate, ferric chloride, copper sulphate, iodine or the alkalies.

Summary.—A race of *Dematium pullulans* was separated from specimens of the peach and almond affected with gum-flux. When grown upon or in media containing saccharose, it produced a pararabin. Neither arabin nor metarabin was obtained, and therefore *Dematium pullulans* has no influence in the production of the gum-flux of these fruits.