

### Occurrence of Antifungal Substances in *Brassica rapa*, *Brassica oleracea* and *Beta vulgaris*

SINCE roots and tubers contain different substances which are easily assimilable by many micro-organisms, the question arises whether the mechanical protection by the few outer cell layers of the cortex is sufficient to prevent infections from the surrounding soil, or whether the plant produces antibacterial and antifungal substances to increase its resistance against microbial invasion.

Antifungal substances have been isolated from the tubers of *Brassica rapa rapifera*, *Brassica oleracea gongylodes* and the fleshy roots of *Beta vulgaris*. In the first two cases, the presence of the active substances was strictly limited to the superficial cell-layers, whereas the interior parts were practically free from it; this seems to point to the hypothesis of antimicrobial protection referred to earlier.

In the case of *Brassica rapa* the substance, which we have called 'rapine', could be isolated by disintegrating the peelings in a blender and subjecting the mass to steam distillation. From the distillate a yellow oily substance could be extracted with ether; the yield amounted to 4 mgm. per 100 gm. of fresh peelings. The substance had the characteristic penetrating smell of turnips. The solubility in water was low and, mixed with malt agar, a concentration of 1:10<sup>5</sup> was sufficient to prevent the growth of different fungi and yeasts. At this concentration, rapine did not possess any antibacterial activity against *Escherichia coli*, *Micrococcus aureus* and *Bacillus subtilis*. This specific antifungal activity of rapine was used to check so-called pure cultures of phytopathological fungi for the presence of bacteria, which otherwise is sometimes rather difficult.

As *Trichophyton rosaceum*, a typical parasite of man and various animals, is readily inhibited by rapine, the pharmacological activity of this substance will be a matter of further examination. The following fungi were tested: *Aspergillus niger*, *Aspergillus oryzae*, *Botrytis* sp., *Ceratostomella pififera*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium notatum*, *Rhizopus nigricans*, *Trichophyton rosaceum* (growing on Sabouraud agar), *Verticillium cinnabarinum*. Of these, *Fusarium oxysporum* proved to be the most susceptible. The yeasts tested were: *Saccharomyces cerevisiae*, *Saccharomyces lactis* Dom-browski, *Schizosaccharomyces Pombe*, *Cryptococcus dattila* and *Cryptococcus utilis*, all of which were readily inhibited by a concentration of 1:10<sup>5</sup> of rapine.

The active principle from *Brassica oleracea gongylodes* was prepared in the same way as rapine. It proved to be a yellowish, solid substance, also slightly soluble in water and with an intense smell of Swedish turnips. It inhibited the growth of the same micro-organisms as did rapine; its activity, however, seemed to be somewhat less pronounced, as in the cases of *Penicillium notatum* and of *Fusarium oxysporum* the critical concentration was 1:10<sup>4</sup>.

From *Beta vulgaris* a crude preparation was made which was especially inhibitive against *Fusarium* sp. This substance was not volatile with steam, was not deteriorated by boiling, was soluble in water and insoluble in ether. It could be extracted from the cortex as well as from the inner part of the root.

Further studies in this field are in progress.

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### 'Lactobacillin', an Antibiotic from *Lactobacilli*

A SURVEY of streptococci has already shown that the lactic group are the most powerful antibiotic producers<sup>1</sup>. It was of interest to see whether antibiotics are also produced by lactobacilli, especially since Grossowics, Kaplan and Schneerson<sup>2</sup> have already reported the existence of such substances. Young Gruyère cheese samples from different areas of France were used as one of the sources of these lactobacilli. 40 antibiotic-producing strains, about 3 per cent of the total number of strains tested, have been isolated in pure culture. These gave zones of inhibition 20–30 mm. in diameter when assayed by the cylinder plate method with *Staph. aureus* as the test organism.

One of these organisms, which is homofermentative and resembles *L. helveticus*, is being investigated in further detail. In peptone-glucose broth, containing 0.01 per cent Tween 80, which appears to be essential, supplemented with tomato juice, whey or malt extract, it grows well at 30° C., 37° C. and 43° C.; the lower temperatures are, however, more conducive to good yields of antibiotic. Yeast-glucose broth supports good growth at 43° C., but the yeast extract is inhibitory to growth at lower temperatures, and inhibits production of antibiotic at all temperatures of incubation.

With dilution assays in plain nutrient broth, in which the test organism grows well but the lactobacillus does not grow, titres of 1/1,280 were frequently obtained. Against *Staph. aureus* this culture-fluid appears to be about twenty times more powerful than nisin<sup>3</sup>; it is, however, very heat-labile.

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<sup>1</sup> Hirsch, A., and Wheeler, D. M., *J. Dairy Res.*, 18, 193 (1951).

<sup>2</sup> Grossowics, N., Kaplan, D., and Schneerson, S., *Proc. IV Inter. Congr. Microbiol.*, 137 (Copenhagen, 1947).

<sup>3</sup> Mattick, A. T. R., and Hirsch, A., *Lancet*, ii, 5 (1947).

### Urease Metabolism in *Citrullus*

SINCE the speculations by one of us<sup>1</sup> as to the function of urease in *Citrullus* seeds appear to have aroused interest, we feel we ought to report that further work has shown the original hypothesis (that urease is a reserve protein) to be untenable. We remain unconvinced that the metabolism of urease in *Citrullus* is connected with the metabolism of urea or its derivatives; but it does not appear to be a