of this to observed differences in varietal susceptibility of sugar-cane to cane-fly.

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MICROBIOLOGY

Serotypes of *Candida albicans*

The classification of *Candida albicans* into two serological groups on the basis of agglutination tests was first reported by Hasenclever and Mitchell[1], and both in the original and subsequent papers[2–4] these authors and their colleagues referred to group *A* and group *B* strains. The findings were confirmed by me[5,6] using a double-diffusion method, and, for the sake of conformity, similarly using the word 'group' with reference to the serological division within the species. Nevertheless, it must be apparent that this terminology is not consistent with that relating to the serological classification of other microbiological species such as *Pneumococcus* and *Haemophilus influenzae*, where the word 'type' is employed, and that confusion may arise if the word 'group' is used to describe not only an antigen (group antigen) which is common to more than one species of a particular genus, but also the serological division within a species.

There is reason, therefore, for hoping that in any future contributions to this subject the word 'type' or 'serotype' will replace the word 'group'.

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Inhibitory Effect of Dipicolinic Acid on the Anaerobic Oxidation of Glucose by the Cell-free Extract from Vegetative Cells of *B. subtilis*

It has been reported that the dipicolinic acid content of several species of bacterial spores ranges from 5 to 10 per cent of the dry weight of spores[1,2]. The biological actions of dipicolinic acid in spores have been discussed in a few reports. Harrell[3] suggested that the release of dipicolinic acid from spores has some relation to the ability of the organism to oxidize glucose. Harrell, Doi and Halvorson[4] have further shown that dipicolinic acid has a stimulatory effect on glucose oxidation of the cell-free extract of *B. cereus* spores. The relationships of dipicolinic acid content to the heat stability[5] and to the stainability[6] of spores have also been studied.

We wish to report that dipicolinic acid has an inhibitory effect on the anaerobic oxidation of glucose by the cell-free extract from the vegetative cells of *B. subtilis*.

B. subtilis (PCI 219) was cultivated on meat extract agar at 37° C for 20 h. Sporulation is known to begin immediately after the 20th h of cultivation under the conditions employed. The cells were gathered in M/50 phosphate buffer (*p*H 7·2), washed several times with sterilized phosphate buffer, and finally suspended in M/10 phosphate buffer. The bacterial suspension was then oscillated in a 10-kc/s sonic oscillator (Kubota 'KMS-100') for 30 min at 2°–3° C. After rupture the vegetative cell brei was centrifuged at 6,000 r.p.m. for 20 min. The supernatant cell-free extract was stored at −20° C before use in enzymatic studies.

The anaerobic oxidation of D-glucose was assayed by measuring the dehydrogenase activity using the Thunberg technique. The side-arm cap contained 0·5 ml. of the cell-free extract containing 2–4 mg protein/ml., 0·1 ml. of 1 M phosphate buffer (*p*H 7·2), 0·2 ml. of a neutralized solution of dipicolinic acid in varying concentrations, and 0·2 ml. water to make up a total volume of 1·0 ml. The concentrations of dipicolinic acid used were 0·5 M, 0·05 M and 0·005 M. The side-arm caps were incubated for 60, 120 or 180 min at 37° C in order to ensure enough contact of dipicolinic acid with the enzyme preparation before the reaction of the latter with the substrate. The main tubes contained 1·2 ml. 0·025 M glucose, 1·0 ml. 0·01 per cent methylene blue in 1/10 M phosphate buffer (*p*H 7·2), 0·5 ml. 1 mg/ml. NAD and 0·3 ml. 1 M phosphate buffer (*p*H 7·2). At the end of the pre-incubation period the tube was thoroughly evacuated, the contents were mixed, and the tube was again incubated at 37° C. The time for complete decolorization of methylene blue was then recorded. In each experiment two different types of control runs were made: (1) dipicolinic acid was omitted, and (2) dipicolinic acid was added to the main tube instead of to the side-arm. The latter type of control experiment was designed to determine the effect of a simultaneous contact of the cell-free extract with both dipicolinic acid and the substrate. The results obtained are summarized in Table 1.

From the results shown in Table 1, it is apparent that dipicolinic acid has an inhibitory effect on the anaerobic oxidation of glucose catalysed by the cell-free extract of *B. subtilis* in the presence of NAD and methylene blue. Two important features of the inhibition noted are: (*a*) the inhibitory effect is dependent on the concentration of dipicolinic acid (Expts. 1–3), a pronounced inhibition being observed at a concentration of dipicolinic acid as high as 0·1 M in the pre-incubation mixture; (*b*) the degree of inhibition is markedly increased by a longer contact of dipicolinic acid with the enzyme preparation before incubation with the substrate (Expts. 3–5).

We have further examined whether the inhibitory effect could be found only with dipicolinic acid or with a variety of pyridine mono- and di-carboxylic acids. The compounds tested were quinolinic acid (pyridine-2,3-dicarboxylic acid), lutidinic acid, isocinchomeronic acid (pyridine-2,5-dicarboxylic acid), phthalic acid, nicotinic acid, and isonicotinic acid. The experimental conditions were the same as those described here except that 0·2 ml.

Table 1. Inhibitory Effect of Dipicolinic Acid on the Anaerobic Oxidation of Glucose by the Cell-free Extract from Vegetative Cells of *B. subtilis*

The cell-free extract was added to the side-arm cap, and glucose, methylene blue and NAD were added to the main tube

Experiment No.	Side-arm cap: Pre-incubation (min)	Side-arm cap: Dipicolinic acid (μmoles)	Dipicolinic acid in main tube (μmoles)	Complete decolorization of methylene blue (min)
1	60	1·0	0	80
	60	0	0	28
	60	0	1·0	46
2	60	10	0	87
	60	0	0	34
	60	0	10	47
3	60	100	0	>150*
	60	0	0	37
	60	0	100	110
4	120	100	0	>150*
	120	0	0	22
5	180	100	0	>150*
	180	0	0	22
	180	0	100	65

* Only partial decolorization was noted after 150 min.