A culture of *P. vulgaris* (Macdonald College Culture No. 261) was obtained from the Department of Bacteriology and the experiment with the DDT-containing medium was repeated with this organism. Morphological and biochemical tests showed no significant differences between the gut isolate which produced the DDD and *P. vulgaris* from the laboratory culture. This species is associated with some diseases of animals and is one of the primary invaders of animal tissues after death[10]. Analysis of extracts of the media after incubation with the laboratory culture showed DDD to be the main metabolite present.

Total DDD production in the gut isolate cultures decreased in inverse proportion to the logarithm of the time of incubation, as shown in Table 1. The decrease in the total quantity of DDD produced suggests that this material is further metabolized to another product which has not yet been adequately identified.

Table 1. THE QUANTITY OF DDD PRODUCED FROM 5·45 MG OF DDT AFTER DIFFERENT PERIODS OF INCUBATION AT TWO TEMPERATURES

Time of incubation	DDD in mg 30° C	37° C
6 days	0·355	—
10 days	—	0·395
15 days	0·270	0·355
20 days	0·243	0·300

P. vulgaris was also grown on a medium containing 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE) but no DDD was found in extracts of these preparations. Thus as in DDT detoxification by yeasts[3], DDE does not appear to be an intermediate in the production of DDD from DDT.

We conclude that the bacterium *P. vulgaris*, commonly found in the gut of mice and other animals, has the capacity of metabolizing DDT to DDD and can be assumed to be an agent, if not the sole one, in the conversion of DDT to DDD in animals which have been killed by DDT poisoning.

We thank Dr. A. C. Blackwood, Dr. R. Knowles and Dr. J. Tanner for their help.

P. S. BARKER
F. O. MORRISON

Department of Entomology,

R. S. WHITAKER

Department of Bacteriology,

Macdonald College of McGill University,
Province of Quebec, Canada.

[1] Finley, R. B., and Pillmore, R. E., *Am. Inst. Biol. Sci. Bull.*, **13**, 41 (1963).
[2] Allison, D., Kallman, B. J., Cope, O. B., and Van Valin, C. C., *Science*, **142**, 958 (1963).
[3] Kallman, B. J., and Andrews, A. K., *Science*, **141**, 1050 (1963).
[4] Barker, P. S., and Morrison, F. O., *Can. J. Zool.*, **42**, 324 (1964).
[5] Ozburn, W. G., and Morrison, F. O., *Nature*, **196**, 1009 (1962).
[6] Bligh, E. G., and Dyer, W. J., *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
[7] Evans, W. H., *Analyst*, **87**, 569 (1962).
[8] Kauffman, F., *Enterobacteriaceae*, second ed. (Ejnar Munksgaard, Copenhagen, 1954).
[9] Breed, R. S., Murrey, E. G. D., and Smith, N. R., *Bergey's Manual of Determinative Bacteriology*, seventh ed. (Williams and Wilkins, Baltimore, 1957).
[10] Wilson, G. S., and Miles, A. A., in *Topley and Wilson's Principles of Bacteriology and Immunology*, fourth ed., 1, 737 (Edward Arnold, Ltd., London, 1955).

Isolation of the Crystalline Parasporal Bodies of *Bacillus thuringiensis*

THE method of Angus[1] for the isolation of parasporal bodies (crystals) from a mixture of crystals and spores has been modified so that only two treatments instead of five with fluorocarbon are necessary, and the separation can be completed in approximately 6 h. It dispenses also with the need for preliminary germination of spores.

The crystal-spore suspension is obtained by washing a crude sample as described by Angus[2] and is adjusted to contain 0·05 g wet wt./ml. 10 ml. of the suspension is lightly shaken for 15 min in a 1-oz. McCartney bottle containing five drops of tri-*n*-butyl citrate and 5-g glass beads (2·0–2·5 mm diameter) by means of a Microid flask shaker (Griffin and George). The shaken suspension is transferred to the blender bottle of an MSE homogenizer containing 80 ml. 1 per cent Na_2SO_4 solution, 1 ml. tri-*n*-butyl citrate, and 10 ml. trifluorotrichloroethane and stirred for 2·5 min at 7,700–8,000 r.p.m. The mixture is immediately transferred to a cylindrical separating funnel and allowed to stand for 15 min, when the organic phase separates out. The organic phase is discarded and the aqueous phase is centrifuged at 3,000 r.p.m. for 20 min in an MSE bench centrifuge. The deposit is taken up in a minimum of water and re-subjected to treatment with the fluorocarbon, including the shaking with glass beads. After centrifuging the separated aqueous phase, the deposit contains more than 99 per cent crystals and less than 1 per cent spores.

The deposit can be stored as an aqueous suspension or evaporated to dryness over silica gel under vacuum at room temperature, when a yield of approximately 0·2 mg/ml. of original suspension is obtained.

J. B. BATESON

Department of Agriculture,
University of Leeds.

[1] Angus, T. A., *J. Insect Pathol.*, 1, 97 (1959).
[2] Angus, T. A., *Canad. J. Microbiol.*, 2, 416 (1956).

Invertase Content of Yeast Protoplasts

IN 1958 three of us reported[1] that a large part of the invertase is liberated in a soluble state from living yeast cells (*Saccharomyces cerevisiae*) by the action of snail digestive juice. This was observed independently by Friis and Ottolenghi[2] and later confirmed by a number of other authors[3,4] although there remained some disagreement as to the extent to which protoplasts retain invertase activity.

Burger, Bacon and Bacon[5], who were concerned primarily with establishing the soluble nature of yeast invertase in opposition to the views held at that time by Myrbäck[6], stated without giving experimental data that "protoplasts isolated after 20 h (of snail juice action) and washed by centrifuging in 0·2 M lactose liberated only traces of invertase when lysed in water". Millbank[4], on the other hand, like Friis and Ottolenghi[2] and Sutton and Lampen[3], demonstrated that nearly a third of the invertase of the original cells was retained in the protoplasts. Furthermore, the protoplasts obtained from Millbank's yeast strains metabolized sucrose, without lag, at rates comparable with those of the intact cells.

A quantitative examination of the results on which these statements are based shows that no real discrepancy exists. The total invertase activity of the culture of yeast used by Burger *et al.* was almost ten times that used by Millbank. Variations of such magnitude are quite common[7]. Table 1 gives the results of several unpublished experiments by Burger *et al.* which show that the invertase activity of the protoplasts was in all cases of the same order as those of Millbank. However, in relation to the total activity they represented usually only 3–5 per cent 'in-

Table 1. INVERTASE ACTIVITY OF PROTOPLASTS PREPARED FROM *R XII* YEAST BY THE ACTION OF SNAIL CROP JUICE

Exp.	Total invertase activity* of original yeast	Invertase activity* of protoplasts produced in presence of 0·2 M lactose	Percentage of invertase activity retained in protoplasts
1	276	7	2·5
2	208	9	4·3
3	256	12	4·7
4	157	16	9·6
5	146	6	4·1

* Expressed as units/g wet pressed yeast; one unit of enzyme liberates 1 mg reducing sugar/min at 20°. Full details of all procedures have been given previously[5].

© 1965 Nature Publishing Group