only instead of a leaf (for example, *Avena sativa*[2] and *Lolium perenne*[3]).

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BACTERIOLOGY

A Growth Factor for *Haemophilus* Species secreted by a Pseudomonad

THE clustering of bacterial colonies of one species around those of another—satellitism—was first described for *Haemophilus influenzae* by Grassberger in 1897[1]. The interrelationship is probably due to the supply of critical nutrients by one micro-organism to the other. While the special nutritional requirements of *Haemophilus* species have been studied and some compounds essential for growth identified[2], the nature of bacterial substances responsible for the satellite phenomenon have not been investigated. In this communication we report on the isolation of such substances.

It is much easier to study excretory products of bacteria in a chemically defined medium than in a complex one. Therefore, only cultures able to grow in simple media were screened for their ability to stimulate growth of *Haemophili* on blood agar or support it on brain heart infusion (Difco Laboratories, Detroit) agar. Test organisms included seven strains of *Haemophilus* of animal origin, four strains of *H. influenzae*, and one strain each of *H. parainfluenzae*, *H. parahaemolyticus* and *H. gallinarum*. The cultures were streaked on test media and crossed with a single streak of the possible 'feeder'. A good 'feeder' was found to be a pseudomonad isolated from an emperor penguin and obtained from Dr. L. A. Page. This bacterium grew well in a solution of histidine (200 mgm. per cent), monobasic potassium phosphate (20 mgm. per cent), mineral mix No. 49 (supplied by Dr. J. H. Hunter, Haskins Laboratories, New York, and containing iron, manganese, zinc, cobalt, copper, vanadium and boron) (8 mgm. per cent) at *p*H 7·5 and 37° C. When grown in such a medium, the organism secreted growth factors essential for *Haemophilus* species, apparently the same as produced in the more complex media.

The penguin pseudomonad was grown in 1 litre of the above medium for 48 hr. After the cells had been removed by centrifugation, the supernatant was Seitz-filtered and lyophilized. The dried material was redissolved in 10 ml. of water and chromatographed, using ascending chromatography with a solvent system consisting of butanol, acetic acid, and water (4 : 1 : 5). Two ultra-violet-absorbing spots were observed under illumination with a 'Mineralight' (2537 *A*) (Ultra-violet Products, Inc., South Pasadena), of which one was much denser than the other. An additional spot was detected after spraying with ninhydrin—probably histidine from the growth medium, as shown by a reference sample.

The ultra-violet-absorbing material (*EP*) contained in the denser spot was eluted from the paper and added to brain heart infusion agar. This medium could then support the growth of *Haemophili*. *EP* was further purified by repeated partition on Whatman No. 1 filter paper and subsequent elution. The solvents, used in sequence, were: monobasic sodium phosphate, 5 per cent; *iso*propanol, ethanol, and water in the ratio of 7 : 2 : 1; and *iso*propanol and 0·2 *M* ammonium hydroxide in the ratio of 3 : 1. Prior to use, the filter paper had been washed for 24 hr. in distilled water.

Purified *EP* had ultra-violet absorption optima at 265 and 275 mμ at *p*H 1·0 and 11·0, respectively. On hydrolysis, with 2 *N* hydrochloric acid at 115° C. for 60 min., a carbohydrate was released which, in three different solvents, had the same R_F as ribose. *EP* was found to be free of phosphorus. In aqueous solution it could be heated at 100° C. for 30 min. and 120° C. for 5 min. without substantial loss of activity; heating at 120° C. for 10 min. resulted in complete inactivation.

Species of *Haemophilus* require for growth either *V* factor—replaceable with diphosphopyridine nucleotide, or its nucleoside[3]—*X* factor—replaceable with hæm, or both. Since most of our animal strains which were stimulated by *EP* showed no hæm requirement, a relationship between *EP* and diphosphopyridine nucleotide was suggested. *EP*, both before and after acid hydrolysis, had a different R_F value from niacin, niacinamide, niacinamide riboside, niacinamide mononucleotide, and diphosphopyridine nucleotide. It also differed from these in ultra-violet absorption spectra. Infra-red absorption spectra do not exclude *EP* from being a derivative of niacinamide.

EP could be replaced as a growth factor for *Haemophilus* by diphosphopyridine nucleotide and niacinamide mononucleotide but not by niacinamide, adenosine triphosphate or the products of acid hydrolysis of ribo- or deoxyribo-nucleic acids. Test organisms grew in brain heart infusion broth with either niacinamide mononucleotide or diphosphopyridine nucleotide in the concentration of 10γ per cent, whereas *EP* was required at the concentration of 1,000γ per cent. While it is possible that this amount reflects the true needs of the test species, the possibility of partial inactivation of the compound during the purification process cannot be disregarded. Dorfman *et al.*[4], working with *Shigella*—which requires nicotinic acid or niacinamide for growth—showed that N-substituents of niacinamide served as growth factors while substitution on the ring inactivated the molecule. In one case where quinolinic acid could be used to promote some growth, its effective concentration was 1,000 times that of niacinamide.

While the data on hand are insufficient to characterize *EP* completely, present information suggests that it might be a niacinamide riboside with unknown substitutions. Its implied biological significance would be consistent with such an interpretation. Work to elucidate further the structure and composition of *EP* is in progress.

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