Virulence of Auxotrophic Mutants of *Erwinia aroideae*

The virulence of a pathogen depends upon numerous factors, including the availability of essential nutrilites from the host. If the host cannot supply all these nutrilites, either in quantity or in a form suitable for assimilation, the pathogen cannot proliferate and produce the characteristic reactions in the host. For example, purine-requiring mutants of *Salmonella typhosa*[1] and of *Klebsiella pneumoniae*[2] were avirulent for mice because the requirement for purine was apparently not satisfied by the host. Garber *et al.*[2] suggested that auxotrophic mutants of pathogens may be useful for purposes of bioassay. Preliminary data from experiments using a plant pathogen, *Erwinia aroideae*, and the potato tuber, var. Red Russet, suggest that this technique may be extended to plant material in certain instances.

Cultures of *E. aroideae* were grown in the *M*-9 synthetic medium[3] 22° C. for 24–48 hr. After 24 hr. in a humid chamber at 22° C., the parental strain, added to the cut surface of a potato tuber, produced a distinctive brown discoloration (and eventually rot). Two auxotrophic mutants of *E. aroideae* were isolated after ultra-violet irradiation: threonine-requiring and tryptophan-requiring. Auxanographic tests indicated that these requirements were apparently specific in that no other amino-acids would satisfy the requirement.

The auxotrophic mutants were grown in a nutrient broth ('Difco') and, throughout the experiments, thoroughly washed prior to use. The threonine-requiring mutant produced the typical discoloration but less markedly than the parental strain. The tryptophan-requiring mutant, however, produced no discoloration. When a few scattered drops of a tryptophan solution were added to the surface of a sliced potato tuber previously inoculated with the tryptophan-requiring mutant, a typical discoloration appeared at the site of each drop. Finally, two mutants which had reverted to prototrophy regained their virulence simultaneously with the loss of the requirement for tryptophan.

These results indicate that the loss of virulence of the tryptophan-requiring mutant is probably correlated with the failure of the mutant population to proliferate, thereby suggesting that tryptophan was not available at the cut surface of the potato tuber. The appearance of the typical discoloration when tryptophan was supplied, and the restoration of virulence in the two strains which had reverted to prototrophy, would indicate this. The maintenance of the virulence of the threonine-requiring mutant suggests that the cut surface of the potato tuber contains the threonine required by this mutant.

Dent *et al.*[4] have reported the presence of threonine and tryptophan in alcohol extracts of potato tuber observed by means of paper partition chromatography. Whether the rapid metabolism of the cut surface of the potato tuber leading to protein synthesis from stored reserves of soluble nitrogen[5] was responsible for depleting the supply of available tryptophan and thereby 'starving' the population of tryptophan-requiring cells is not known as yet. Differences in the kinds or quantities of free amino-acids in different varieties of the potato may also be a factor.

These preliminary data suggest a potential method of bioassay using auxotrophic mutants of pathogens with distinctive pathological reactions in the host to detect the presence or absence of specific substances.

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Palæozoic Plants from New Zealand

Three small collections of Palæozoic plant fossils have recently been examined and will be reported on more fully in the near future. Plant fossils of Palæozoic age have not previously been found in New Zealand.

The Permian collection was made by B. L. Wood (N.Z. Geological Survey) from the Arthurton Group of Pukerau, Gore District, Southland. The plant fossils are associated with marine fossils indicating a Permian (probably Artinskian) age and consist of well-preserved fragments of leaves. Species identified are *Equisetites* sp., *Cladophlebis roylei* Arb., *Sphenopteris* sp., *S. lobifolia* Morris, F. Neuropteridae, cf. *Linguifolium* sp., *Noeggerathiopsis hislopii* (Bunbury). All the plants identified specifically are known from the Bowen Series coal measures of Queensland[1], which are correlated with the Sakmarian to Tartarian stages of the Permian[2]. The above species are usually associated with glossopterids, which have not been recognized in this flora.

The two older collections come from the Te Anau Group, of Rai Valley and Alfred Stream, Nelson Province, and were found by H. W. Wellman and H. J. Harrington (N.Z. Geological Survey), about 30,000 ft. below the Wairoa Gorge Limestone, which contains marine fossils of lower Permian age[3]. The plant fossils are very fragmentary, consisting of a fern-like pinnule, a Sphenopsid stem and two doubtful sporangia. The presence of the fern-like pinnule suggests that this part of the Te Anau Group is younger than Lower Devonian[4].

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