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This Week's Citation Classic

Shewan J M, Hobbs G & Hodgkiss W. A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the *Pseudomonodaceae. J. Appl. Bact.* **23**:379-90, 1960.

This paper defines the methods and determinative criteria appropriate to the study of psychrophilic bacteria. Broad groups are outlined on the basis of a small number of preliminary tests. The further differentiation of motile gram-negative organisms of the genera *Pseudomonas, Aeromonas,* and *Vibrio* is described. [The *SCI*® indicates that this paper has been cited over 190 times since 1961.]

J.M. Shewan
Ministry of Agriculture, Fisheries and Food
Torry Research Station
P.O. Box 31
Aberdeen AB9 8DC
Scotland

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"As a unit of the UK Food Investigation Board (DSIR) one of the specific remits of our work at Torry Research Station was to improve the quality of marine fishery products. In the immediate postwar years the bulk of these products were stored and transported in ice. As most of the undesirable changes which occur under these conditions are due largely to the action of psychrophilic bacteria, the identification of the different types of these spoilage organisms was an important part of our investigations. Qualitative analyses of the bacterial flora of fish undergoing storage tests involved the examination of large numbers of isolates, the identification of which was a time-consuming and difficult procedure owing to the lack of appropriate determinative tests. Furthermore, our task was not made easier by the confusion which existed at that time in the nomenclature and classification of non-enteric gram-negative bacteria.[1] For these reasons we decided to attempt to devise a scheme based upon tests which would be economical in materials and quickly yield results compatible with accepted taxonomic principles Our earliest attempts to differentiate some isolates into 'generic' groups were in part **a** spin-off from experiments on the preservative action of antibiotic ice. This method utilised antibiotic sensitivity tests and introduced the use of the 0/129 compound (2:4-diamino-6:7-isopropyl-pteridine) as a specific test for *Vibrio* spp.[2] John Liston was then a member of our staff and the ptendine experiments started after discussions he attended on the possible use of these bacteriostatic agents to prevent the corrosive action of desulfovibrios. Through the generosity of H.O.J. Collier we obtained a range of pteridine compounds and found no. 0/129 the most effective in our tests. For a long time we depended upon Collier for supplies of this compound as it was not commercially available for many years.

"The 1950s brought other developments which influenced the progress of our work, e.g., the O/F test of Hugh and Leifson[3] and the Kovacs oxidase test.[4] Phase contrast optics became available and simplified our motility tests. Finally, in 1958 the acquisition of an electron microscope enabled us to assess the flagellation of motile cells with accuracy. In addition to our own isolates, we examined cultures from various environments as well as reference strains and consequently felt that we could identify most of them with some certainty The determinative scheme was presented at the Society for Applied Bacteriology 'Symposium on *Pseudomonas* and *Achromobacter'* in 1960 and subsequently published as part of the proceedings.[5] The Symposium was well attended and, as reference to the proceedings shows, there was much interest in methods of studying those organisms. The original scheme has subsequently been modified through changes in nomenclature, but it is pleasing to know that it is widely used and that the basic premises were sound

"We think that the paper has been highly cited because the determinative scheme described therein has proved to be a rapid, economical, and accurate approach to the identification of bacteria isolated from psychrophilic environments. "

1. **Ingram M & Shewan J M.** Introductory reflections in the *Pseudomonas-Achromobacter* group. *J. Appl. Bad.* **23**:373. 1960.
2. **Shewan J M, Hodgkiss W & Liston J.** A method for the rapid differentiation of certain asporogenous bacilli. *Nature* (London) **173**:208-9. 1954.
3. **Hugh R & Leifson E.** The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J. Bacteriology* **66**:24-6. 1953.
4. **Kovacs N.** Identification of *Pseudomonas pyocyanea* by the oxidase reaction *Nature* (London) **178:**703,1956.
5. Symposium on *Pseudamonus* and *Achromobacter. J. Appl. Bact.* **23:**373-537,1960.