The Identity of the Gram-negative Bacterium NCIB 8250 ('Vibrio 01')

By C. A. FEWSON

Department of Biochemistry, University of Glasgow

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SUMMARY

The original strain of Vibrio 01 (Happold & Key, 1932) appears to have been lost. The Bacterium NCIB 8250, which is frequently referred to as 'Vibrio 01', has different properties from those of the original isolate and is a Gram-negative non-motile oxidase-negative coccobacillus.

INTRODUCTION

The Bacterium Vibrio 01 (Happold & Key, 1932) has been quite widely used in studies on the metabolism of aromatic compounds (e.g. Evans, 1947; Dagley, Fewster & Happold, 1952; Cain, 1961). In the course of experiments designed to delineate the metabolic versatility of this organism (Fewson, 1967) a culture was obtained from the National Collection of Industrial Bacteria (NCIB, Torry Research Station, Aberdeen, Scotland). It soon became apparent that the specificity of growth of this strain, NCIB 8250, was different from that originally described for Vibrio 01 by Evans (1947). In subsequent work, which is described in this paper, it also became apparent that the Bacterium NCIB 8250 was basically different from the bacterium isolated by Happold & Key (1932).

METHODS

Stock cultures of the Bacterium NCIB 8250 were maintained in Oxoid cooked-meat medium stored at 4°. Subcultures were made into Oxoid nutrient broth at intervals of approximately 1 month and were also kept at 4°. Inocula required for the various tests were produced by a further subculture into nutrient broth and incubated for 17-24 hr at 30° immediately before use.

Oxidation-fermentation reactions were measured as described by Hugh & Leifson (1953) and Sellers (1964). All other tests were done according to the methods described by Cowan & Steel (1965).

Oxoid media were used for testing growth on nutrient agar (CM3), MacConkey agar (CM7) and Koser's citrate medium (CM65) and also for following acid production from sugars (Andrade Peptone Water, CM61). Other reagents were the best quality available from British Drug Houses Ltd., Poole, Dorset.

Incubation was at 30° in all cases.

RESULTS

The Bacterium NCIB 8250 appeared as Gram-negative non-motile coccobacilli about 1 μ wide, frequently arranged in pairs and occasionally as short chains, but never as tetrads. No capsule was detected. The cell size of the organism was apparently related

to the conditions of growth: in general the faster the growth the larger were the organisms.

Numerous experiments failed to demonstrate oxidase activity by Kovacs test. No urease was detected with either Christensen's or SSR media although the Bacterium NCIB8250 was shown to grow on urea as sole source of nitrogen (Fewson, 1967) and can therefore presumably form urease under some conditions. Nitrate reduction was not detected in the standard assay but the production of nitrite from nitrate was shown when nitrate was the sole source of nitrogen in a succinate-salts medium (Fewson, 1967). There was vigorous catalase activity.

No acid was produced from glucose, sucrose, lactose or dulcitol and the Hugh-Leifson and the Sellers media showed no action on glucose, either aerobically or anaerobically.

There was good growth on nutrient agar, MacConkey agar and Koser's citrate medium. Peptone water cultures gave diffuse growth with a thin pellicle.

Tests in appropriate media for the production of indole, acetoin and hydrogen sulphide, for methyl red reaction and for the utilization of malonate were all negative. There was no liquefaction of gelatin. Some ammonia was formed in peptone medium.

DISCUSSION

Vibrio 01 was isolated by Happold & Key in 1932. It was stated to be a "gram negative vibrio" with an oxidase system, without action on any of the common sugars and not liquefying gelatin. Presumably the organism was motile, in view of the designation 'vibrio' and the fact that it was stated to resemble *Vibrio tyrosinatica* (Happold & Key, 1932) and *V. cuneatus* (Evans, 1947; Kilby, 1951). There seems little doubt, therefore, that *Vibrio* 01 was a member of the *Pseudomonas-Vibrio* group, although a precise classification cannot be arrived at from the information available. Early experiments on the metabolism of aromatic compounds were done with this organism (Evans & Happold, 1939; Evans, 1947; Prof. W. C. Evans, personal communication) but the original strain appears to have been lost. Most subsequent work apparently done with *Vibrio* 01 has, in fact, been done with the Bacterium NCIB8250, which is clearly a different organism. Bacterium NCIB8250 is neither like the original *Vibrio* 01 nor characteristic of the genus *Vibrio* since, amongst other things, it is a non-motile oxidase-negative coccobacillus. Evans (1947) found that *Vibrio* 01 grew on *m*-hydroxybenzoate but not on *o*-hydroxybenzoate: Bacterium NCIB8250 shows the opposite pattern (Fewson, 1967). It is also noteworthy that Dagley *et al.* (1952) found that 'Vibrio 01 originally isolated by Happold & Key' grew on phenylalanine as carbon source, whereas later it was reported (Chapman & Dagley, 1960; 1962; Fewson, 1967; Prof. S. Dagley, personal communication) that Bacterium NCIB8250 showed little or no growth with phenylalanine as carbon source. Bacterium NCIB8250 does, however, resemble the original strain of *Vibrio* 01 in its failure to grow on the common sugars and in its general ability to metabolize aromatic compounds. Previous reports describing experiments carried out with *Vibrio* 01 should obviously be treated with care unless it can be clearly established whether the original *Vibrio* 01 or the Bacterium NCIB8250 was used.

Davis & Park (1962) placed Bacterium NCIB8250 in the genus *Comomonas* because one of the authors observed 'a few motile organisms' and 'occasional organisms bearing

one or two flagella'. Motility was never observed in the present work and this has been confirmed (W. Hodgkiss, personal communication). Davis & Park (1962) also gave conflicting results for the oxidase activity of *Bacterium* NCIB8250 but it is of interest that the report of motility was made on a culture which was stated to be, by Kovacs test, oxidase-positive and to show no growth on pyruvate or lactate, which are compounds supporting good growth of *Bacterium* NCIB8250 (Fewson, 1967). *Bacterium* NCIB8250 is now listed by the National Collection of Industrial Bacteria (1964) as an *Achromobacter* sp. but cannot be retained in this genus if the proposals of Brisou & Prévot (1954) and Steel & Cowan (1964) are followed. Sebald & Véron (1963) identified *Bacterium* NCIB8250 as *Moraxella lwoffii* (*Acinetobacter lwoffii*, Steel & Cowan, 1964), apparently very largely on the basis of the G+C content of the DNA of the organism. G+C base composition, although a useful tool in classification, cannot be the sole criterion of taxonomy since, in view of the small range of G+C contents possible, it is inevitable that quite different organisms will be found to have similar gross DNA analyses (De Ley, 1964). It seems likely, however, that Sebald & Véron (1963) were substantially correct, since the characteristics of the *Bacterium* NCIB8250 reported in the present paper are typical of those of the *Acinetobacter*-*Moraxella* group of bacteria (Steel & Cowan, 1964; Henderson, 1965). Doctors Stanier & Doudoroff and their co-workers have come to similar conclusions (Dr R. Y. Stanier, personal communication). Work will have to be done on a large number of strains before the question of nomenclature can be finally settled since it has been found by the present author that the *Bacterium* NCIB8250 differs from *Acinetobacter lwoffii* NCTC5866 and from *Acinetobacter anitratus* NCTC7844 in its ability to grow on a number of aliphatic and aromatic compounds.

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