On Sporulation in Sulphate-reducing Bacteria

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SUMMARY

Enrichment cultures of mesophilic sulphate-reducing bacteria were readily obtained from soils, mud and corrosion products which had been heated to 90°, yet pure cultures of *Desulfovibrio desulfuricans* were killed by heating to 60° even when absorbed on dried sterilized soil. Examination of the populations from heated samples revealed exclusively organisms resembling *Desulfovibrio orientis*; unheated samples contained either *D. orientis* alone or both *D. orientis* and *D. desulfuricans*. The belief that *D. desulfuricans* sporulates is questioned.

INTRODUCTION

Starkey (1938) observed that, though laboratory cultures of mesophilic sulphate-reducing bacteria were killed by heat, such organisms could nevertheless be obtained from soils which had been heated to 90° for ten minutes. Since he was able to demonstrate sporulation among the thermophilic sulphate-reducing bacteria, and since he supported the now obsolete belief that the mesophiles were adapted variants of the thermophiles (see Postgate, 1959*a*, for references), Starkey concluded that the mesophilic species *desulfuricans* formed spores in natural conditions but lost this ability after passage in laboratory media. Pochon & Chalmagnac (1952) described a strain which showed such a change on microscopic observation. Starkey proposed the use of the generic name *Sporovibrio* on this account, but the oblique nature of the evidence for sporulation inhibited wide acceptance of this designation, *Desulfovibrio* being generally preferred. Prévot (1948), however, retained the name *Sporovibrio* and, in the third edition of his manual (Prévot, 1957), gave reasons for continuing to retain this generic name and for not accepting the exclusion of the sporulating thermophiles. A mesophilic species showing unequivocal sporulation was isolated by ourselves (Adams & Postgate, 1959) and named *Desulfovibrio orientis*. This species is readily differentiated from *D. desulfuricans* by two additional characters: (i) one or two polar or near-polar flagella (Adams & Postgate, 1959); (ii) absence of a fluorescence reaction typical of *D. desulfuricans* (Postgate, 1959*b*); it is distinguished from the thermophile *Clostridium nigrificans* by its mesophily and by the shape of its spores. It is possible that the heat resistance and sporulation earlier attributed to *D. desulfuricans* was due to the unrecognized presence of *D. orientis*; the present report provides evidence in favour of this view.

METHODS

Organisms and cultivation. *Desulfovibrio desulfuricans*, strain Hildenborough National Collection of Industrial Bacteria (NCIB) 8308, Essex 6 (NCIB 8307) and Beckton (NCIB 8319) were of fresh water origin; strains Canet 41 (NCIB 8398) and El Agheila Z (NCIB 8380) originated in saline environments and their culture media were supplemented with 2.5 % (w/v) NaCl. Stocks were kept in a freeze-dried condition; cultures for routine use were subcultured weekly in medium C of Butlin, Adams & Thomas (1949b) at 30° under H₂ 99 % (v/v) + CO₂ 1 % (v/v). *D. orientis*, strain Singapore (NCIB 8382), was maintained in a similar manner, but sodium thioglycollate (0.01 %, w/v) was added to its culture media.

Enrichment cultures of sulphate-reducing bacteria from natural samples were prepared in medium C supplemented with 0.01 % (w/v) sodium thioglycollate and 0.05 % (w/v) ferrous ammonium sulphate, pH 7.4 ± 0.3, in 1 oz. (c. 25 ml.) stoppered bottles filled to overflowing to exclude air. The thioglycollate ensured that *Desulfovibrio orientis* would grow if present (Adams & Postgate, 1959) and it also facilitated growth of *D. desulfuricans*, being below the concentration which showed inhibitory effects (Grossman & Postgate, 1958).

Diagnostic tests. Enrichment cultures were decanted to give bacterial suspensions reasonably free from FeS and the fluorescence test for *Desulfovibrio desulfuricans* (Postgate, 1959b) was performed on the decanted material. Flagella were observed microscopically after Casares-Gil staining. Thermophiles were sought by incubation at 55°. Motility was observed in wet preparations under phase contrast. Heat resistance was tested by sealing 1 ml. culture or 1 g. soil in a Durham tube and wholly immersing this in water at 90° for 10 min. Sterility of treated soils was tested by incubating portions aerobically and anaerobically in the glucose + peptone + yeast extract + salts medium of Postgate (1958).

Pure cultures were not in general isolated from the enrichment cultures. After 8–6 days at 30°, when the enrichment cultures were well blackened and rich in H₂S, the populations were assigned to *Desulfovibrio desulfuricans* or *D. orientis* groups according to the following criteria: *D. desulfuricans*, positive fluorescence test, vibrio form, single flagellum, translational motility, failure of subculture to grow at 55°; *D. orientis*, negative fluorescence test, fat vibrio form, more than one flagellum, motility absent or oscillatory, failure of subculture to grow at 55°.

RESULTS

We have repeatedly confirmed Starkey's observation that soils may be heated to 90° and still yield enrichment cultures of sulphate-reducing bacteria. On the other hand, the five pure strains of *Desulfovibrio desulfuricans* mentioned above were all killed when their cultures were heated to 60° or above for 10 min.; *D. orientis* usually survived heating to 90°. To see whether soil exerted a protective effect on *D. desulfuricans*, samples which had yielded sulphate-reducing bacteria after heating were sterilized (by autoclaving or by exposure to ethylene oxide at 4° for 30 min.), dried and then damped with cultures of the Hildenborough strain. The organisms were nevertheless killed when the damped soil was heated to 60° for 10 min. Since commensal spore-formers might have induced heat resistance in the manner that variants

of *Clostridium sporogenes* enhance the heat resistance of *Clostridium welchii* (*Welchia perfringens*; Prévot, Raynaud & Tataki, 1951), three colonial types of sporulating anaerobe were isolated from heated enrichment cultures and mixed with the Hildenborough strain of *D. desulfuricans*. None conferred resistance to 10 min. at 60° upon it. Desiccation conferred a slightly increased heat resistance: a freeze-dried ampoule of the Hildenborough strain as supplied by the NCIB survived heating to 70° for 10 min., though others were killed at 80° and 90°.

It seemed likely that the heat-resistant types of sulphate-reducing bacteria were not of the species *Desulfovibrio desulfuricans*. Nineteen specimens of natural materials likely to contain these bacteria were collected and enrichment cultures made from them before and after heating for 10 min at 90°. All yielded flourishing cultures of sulphate-reducing bacteria. These were then assigned to the *D. desulfuricans* or *D. orientis* groups according to the criteria mentioned above. Although the cultures were not pure, and non-sulphate reducing bacteria were present, all the enrichments contained organisms which fell unequivocally into one or the other groups; intermediate types were not found.

Seven samples yielded cultures of the *Desulfovibrio desulfuricans* group when enriched without heating, but after 10 min at 90° they yielded only the *D. orientis* group. The samples tested comprised: corrosion products from a cooling system and a tubercle within a water pipe (cf. Butlin, Adams & Thomas, 1949*a*); a sewage sample from the Northern Outfalls Sewage Works, Beckton, London; scrapings from a mild steel plate held 1 ft. deep in the Thames at central London; mud samples from the Thames at Teddington and from Byron's pool at Cambridge; a soil sample from Pontardulais, Wales. Twelve specimens yielded the *Desulfovibrio orientis* group both before and after heating. All these were soil samples originating from: the laboratory grounds (2); a local compost heap; the Roman site at Catterick, Yorkshire (3); Singapore; Buenos Aires; Tanganyika; Hawaii; India (2). No soils of marine or brackish origin were tested. None of the enrichments grew on subculture at 55°.

DISCUSSION

These observations suggest that the heat-resistant sulphate-reducing bacteria in soils resemble *Desulfovibrio orientis* rather than *D. desulfuricans*. Consequently, the belief that the latter species sporulates in nature is probably mistaken. The relevance of these considerations to the inclusion of the species *desulfuricans* in a genus named *Sporovibrio* is evident.

We should like to draw attention to the fact that the *Desulfovibrio orientis* group was present in all specimens tested; in a majority of them it was the exclusive type of sulphate-reducing organism. This observation has little statistical value since the number of soils tested was small and their origins were not randomly distributed; nevertheless, it shows an unexpectedly high incidence. We attribute this to the use of thioglycollate in the enrichment media, without which *D. orientis* often fails to grow, whereas growth of *D. desulfuricans* is merely delayed. It seems likely that the *D. orientis* organism is more ubiquitous than was believed at the time of its isolation.

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REFERENCES

- ADAMS, M. E. & POSTGATE, J. R. (1959). A new sulphate-reducing vibrio. *J. gen. Microbiol.* **20**, 252.
- BUTLIN, K. R., ADAMS, M. E. & THOMAS, M. (1949*a*). Sulphate-reducing bacteria and internal corrosion of ferrous pipes conveying water. *Nature, Lond.* **163**, 26.
- BUTLIN, K. R., ADAMS, M. E. & THOMAS, M. (1949*b*). The isolation and cultivation of sulphate-reducing bacteria. *J. gen. Microbiol.* **3**, 46.
- GROSSMAN, J. P. & POSTGATE, J. R. (1953). The estimation of sulphate-reducing bacteria (*D. desulphuricans*). *Proc. Soc. appl. Bact.* **16**, 1.
- POCHON, J. & CHALVIGNAC, M. A. (1952). Sur l'instabilité des caractères d'une souche de *Sporovibrio*. *Ann. Inst. Pasteur*, **82**, 399.
- POSTGATE, J. R. (1953). On the nutrition of *Desulphovibrio desulphuricans*: a correction. *J. gen. Microbiol.* **9**, 440.
- POSTGATE, J. R. (1959*a*). Sulphate reduction by bacteria. *Annu. Rev. Microbiol.* **13**, 505.
- POSTGATE, J. R. (1959*b*). A diagnostic reaction of *Desulfovibrio desulfuricans*. *Nature, Lond.* **183**, 481.
- PRÉVOT, A.-R. (1948). *Manuel de classification et de détermination des bactéries anaérobies*, 2nd ed. Paris: Masson et Cie.
- PRÉVOT, A.-R. (1957). *Manuel de classification et de détermination des bactéries anaérobies*, 3rd ed. Paris: Masson et Cie.
- PRÉVOT, A.-R., RAYNAUD, M. & TATAKI, H. (1951). Recherches sur la thermorésistance des *Cl. sporogenes* et le phénomène d'entraînement des espèces peu résistantes. *Ann. Inst. Pasteur*, **80**, 553.
- STARKEY, R. L. (1938). A study of spore formation and other morphological characteristics of *Vibrio desulfuricans*. *Arch. Mikrobiol.* **9**, 268.