Differences in Colony Morphology and Carbohydrate Fermentation of *Clostridium sporogenes*

By T. J. T. PRINCEWILL*

Department of Microbiology, The University, Leeds LS2 9JT

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INTRODUCTION

The first detailed description of *Clostridium sporogenes* was given by Metchnikoff (1908) who isolated the organism from faeces of normal individuals and from diarrhoeal material of patients with chronic colitis. Subsequently, different names were given to organisms with descriptions similar to that of *C. sporogenes*. Thus, organisms labelled 'Reading' bacillus (Donaldson & Joyce, 1917; Donaldson, 1918; Harris, 1919); *C. parasperogenes* (McIntosh, 1917); *C. tyrosinogenes* (Hall, 1921, cited by Kahn, 1924); *C. flabelliferum* (Sturges & Reddish, 1925); *C. sporogenes* var. *A. P. Marie* (Marie, 1925); and *C. sporogenes* var. *equine* (Prévot, 1938) are now classified in the *C. sporogenes* complex.

In the present study colonial morphology and sugar fermentation properties of *C. sporogenes* were examined to throw light on the variation found in the taxon.

METHODS

Cultures. Details of the 84 strains of *C. sporogenes* and their sources are given in Table 1. Lyophilized cultures were reconstituted, grown in cooked meat medium (CMM; Southern Group Laboratory, Hither Green Hospital, London SE13) and plated on fresh horse blood agar. After incubation at 37 °C for 48 h, single colonies were isolated and their identity was checked by routine methods.

Colony morphology. The test strains were grown on fresh blood, chocolate and egg-yolk agars and the colonial characters were recorded after incubation at 37 °C for 48 h.

Carbohydrate fermentation. Strains were examined for their ability to ferment glucose, glycerol, lactose, maltose, salicin, sucrose and starch in a modified MRS (de Man *et al.*, 1960) medium, in which the peptone content was decreased to 1.25 mg ml⁻¹, the pH indicator was phenol red and the pH was re-adjusted to 7.2. The medium was dispensed into test tubes containing Durham tubes and the appropriate Seitz-filtered carbohydrates were added to the sterile medium to give a final concentration of 1 %. Inocula were prepared by growing strains for 24 h at 37 °C in MRS medium without peptone. The seeded sugars were incubated in anaerobe jars at 37 °C and examined for fermentation reactions after 48 h.

RESULTS

Colony morphology

The colony morphology of strains grown on fresh blood agar varied considerably. Generally, the colonies were large and flat with a rhizoid margin and a raised centre which was usually smooth and translucent and varied from pinpoint to 2 to 3 mm in diameter. The rest of the colony was dry and rough (Fig. 1*a*). Some of the colonies were evenly smooth and flat with crenated edges and a matt surface showing a greyish to creamy white colour, but very occasionally small creamy white colonies with radial ridges and crenated edges or smooth translucent colonies with entire edges were seen (Fig. 1*b, c*).

Colonies on chocolate agar were similar to those on fresh blood agar but there was a

* Present address: Nigerian Institute for Trypanosomiasis Research, Vom, Via Jos, Nigeria.

Fig. 1. *Clostridium sporogenes*: (a, b, c) colonies on fresh blood agar of types A (strain 2) and B (strains 59 and 60), respectively; (d, e, f) colonies on egg-yolk agar of types A (strain 2) and B (strains 59 and 60), respectively.

Table 1. *Correlation between colony type and fermentation of sucrose and salicin by Clostridium sporogenes*

	Colony type	
	A	B
Total no. of strains*	66	18
No. of strains producing acid and gas in:		
Salicin	1	17
Sucrose	1	14
Glucose	66	18
Maltose	66	18
Glycerol	61	18
Starch	7	1
Lactose	0	0

* Of the 84 strains of *C. sporogenes*, 7 were from this laboratory; 6 from the National Collection of Industrial Bacteria (NCIB), Torry Research Station, Aberdeen; 37 from L. S. McClung, Indiana University, U.S.A.; 9 from Wellcome Research Laboratories, Beckenham; 16 from A. Prévot, Pasteur Institute, Paris, France; 4 from the late H. Meisel, Serum Research Institute, Warsaw, Poland; and 5 from G. C. Mead, Food Research Institute, Norwich.

greater tendency towards rhizoid formation and the production of a brown pigmentation, the latter intensifying under aerobic conditions at room temperature.

Morphologically, the colonies on egg-yolk agar were of two main types with type A colonies more frequent than type B (Table 1). The type A colonies were flat, rhizoid, usually circular, with a feathery edge and a small, raised, somewhat dome-shaped or irregular centre; the rhizoid or arborescent appearance gave the impression of a tendency to spread (Fig. 1*d*). In contrast, the type B colonies were entire with a raised centre and a very short rhizoid or feathery periphery. The central portion sometimes looked like a plateau with a raised margin and the body of the colonies occasionally showed radial ridges particularly when the raised centre was pyramidal (Fig. 1*e, f*).

Carbohydrate fermentation

All of the strains fermented glucose and maltose, most fermented glycerol (> 90 %) but not starch, and none attacked lactose (Table 1). The results with sucrose and salicin were the most interesting. Thus, only one (strain 17) of the 15 strains that fermented sucrose and one (strain 19) of the 18 strains that fermented salicin did not produce a colony of type B (Table 1). Of the 18 strains which gave rise to type B colonies, 14 fermented both sucrose and salicin, three fermented salicin but not sucrose and only one failed to ferment either salicin or sucrose. The 64 strains with no action on sucrose and salicin and strains 17 and 19 all gave type A colonies on egg-yolk agar.

DISCUSSION

The colonial morphology of *C. sporogenes* on both fresh blood and chocolate agars is not sufficiently stable to be used in classification and identification. The work of Reed (1945) on the interconversion of smooth and rough forms vividly illustrates the futility of using colonial morphology alone in taxonomy (see Fig. 1); some workers (Holdeman & Moore, 1975) have gone further by not using colonial characters at all in the classification of clostridia. McClung & Toabe (1947) were the first to use egg-yolk agar as a differential medium, with which they achieved presumptive identification of some species of *Clostridium*. In the present study, the *C. sporogenes* strains exhibited two types of colony, A and B, on egg-yolk agar and this separation was supported by the results obtained in the

fermentation of salicin and sucrose. Nakamura *et al.* (1977), using blood agars supplemented with glucose and brain heart infusion, found a correlation between colony type and deoxyribonucleic acid homology in a study of *C. sporogenes* and *C. botulinum* strains. Their types I and II colonies compare with types A and B.

In the present investigation, no attempt was made to study the relationship between strains of *C. sporogenes* and the proteolytic strains of *C. botulinum*. However, in a study on the specificity of somatic antisera for detecting *C. botulinum*, Lynt *et al.* (1972) included *C. sporogenes* strains and, although they observed differences in the physiological characteristics of the two groups of organisms, the differences were confined to the fermentation of a few sugars and colonial morphology on liver-veal-egg-agar. These workers concluded that there was no regular pattern by which *C. sporogenes* could be separated from *C. botulinum* on the basis of sugar fermentation and colony morphology.

Fermentation reactions are helpful in the taxonomy of *Clostridium*. In the present study, all the *C. sporogenes* strains produced acid and gas from glucose and maltose but did not ferment lactose. However, these results are not in complete agreement with those of Holdeman & Moore (1975) who recorded a weak fermentation of glucose and a negative or weak reaction in maltose by most strains of *C. sporogenes*. Most *C. sporogenes* strains fail to ferment sucrose and salicin and produce type A colonies on egg-yolk agar and can be differentiated from those that ferment the sugars and exhibit type B colonies (Table 1). Sucrose and salicin fermentation are also important in the differentiation of other *Clostridium* species (McIntosh, 1917; Robertson, 1918); *C. septicum* ferments salicin and not sucrose whereas *C. chauvoei* does the reverse (Moussa, 1956; Princewill, 1969).

The basal medium used in the fermentation tests gave reproducible results. *Clostridium sporogenes* is a strongly proteolytic organism and so turns protein media alkaline due to deamination of the amino acids formed during proteolysis. Excessive proteolysis will therefore tend to mask acid production. By decreasing the peptone concentration to a minimum, acid production from sugars has been readily detected using phenol red as indicator. Measurement of culture pH with a pH meter would probably be more accurate but is not a practicable proposition in routine laboratories where large numbers of cultures have to be examined.

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