A Study of the Electrokinetic Properties of Some Actinomycete Spores

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SUMMARY

Microelectrophoresis of spores of the actinomycete genera *Micromonospora*, *Nocardia*, *Streptomyces* and *Thermoactinomyces* at various pH values and after various modifying treatments indicated that *Micromonospora* and *Streptomyces* spores had amino and carboxyl surface groups. *Thermoactinomyces* spores and the bacillary or coccoid elements of *Nocardia* had carboxyl groups but not amino groups; their responses to lipase and sodium dodecyl sulphate suggested presence of lipid. All strains studied showed changes in mobility after treatment with lysozyme.

INTRODUCTION

Microelectrophoresis has provided information on the surface properties of many types of microbe. The bacteria have received most attention, among those studied being the genera *Aerobacter* (Plummer & James, 1961), *Bacillus* (Douglas & Parker, 1957, 1958*a*, 1958*b*), *Micrococcus* (Dyar, 1948; Dyar & Ordal, 1946), *Rhizobium* (Marshall, 1967) and *Streptococcus* (Hill, James & Macted, 1963*a*, 1963*b*). A number of fungal spores have also been examined, including those of *Penicillium* species, *Mucor* and *Fusarium* (Douglas, Collins & Parkinson, 1959) and *Neurospora* (Somers & Fisher, 1967). A wide range of fungal species was studied by Fisher & Richmond (1969).

As far as we know, the technique has not been applied to spores of actinomycetes. Wall composition of many genera of actinomycetes appears to have some taxonomic significance (Becker, Lechevalier & Lechevalier, 1965; Lechevalier & Lechevalier, 1965; Yamaguchi, 1965). Therefore, we studied spores of a number of genera by microelectrophoresis to enable comparison with wall composition data and with the surface properties of bacteria and fungi.

METHODS

Strains and their culture. The organisms used were *Micromonospora* sp., *Nocardia rubra* (CBS), *Streptomyces* sp. (F1), *S. griseus* (Waksman, 3475), *S. finlayi* (ISP 5218), *S. viridochromogenes* (CBS) and *Thermoactinomyces vulgaris* (CBS specifies Centraal-

bureau voor Schimmelcultures, Baarn, Netherlands; ISP denotes code number in International Streptomyces Project).

The Micromonospora and Nocardia had readily wetted spores, and Streptomyces and Thermoactinomyces had relatively hydrophobic spores. Two Streptomyces, strain F1 and *S. griseus*, had unornamented spores, *S. viridochromogenes* had spiny spores and *S. finlayi* had hairy spores.

The streptomycetes and Micromonospora were grown on oatmeal agar (Waksman, 1961). *Nocardia rubra* and *Thermoactinomyces vulgaris* were grown on Oxoid nutrient agar. All were incubated at 25° for 4 weeks except *T. vulgaris*, which was grown at 45° for 4 days.

For certain experiments indicated, *Streptomyces viridochromogenes* was also grown on starch-casein medium (Küster & Williams, 1964) and yeast extract-malt extract agar (Shirling & Gottlieb, 1966). The influence of age on surface properties was studied using 1 to 7 week cultures of *S. griseus* and *S. viridochromogenes*.

Preparation of suspensions for electrophoresis. Spores (or bacillary and coccoid elements in the case of *Nocardia rubra*) were removed from plate cultures into deionized water. After homogenization for 15 min. the suspensions were centrifuged and washed five times with deionized water before introduction into prepared buffer solutions. Damage to spores during this procedure was checked by examining samples with a Stereoscan electron microscope (Williams & Davies, 1967).

The main buffer system used was sodium acetate+veronal+HCl (Michaelis, 1930), which covered the pH range 2.5 to 9.0, with NaCl added to adjust the ionic strength (*I*) to 0.05. Lower pH levels were obtained using HCl+NaCl, *I* = 0.05. For pH 9.5 to 10.5, glycine+NaOH and for values over 10.5, NaOH+NaCl, both at *I* = 0.05, were used.

Treatment with surface modifying agents. Sodium dodecyl sulphate (SDS) was used at 1 mM in Michaelis buffer at pH 7.0 (*I* = 0.05). Washed spores were mixed with it for 15 min. at 25° before examination. Controls were spores in buffer at pH 7.0. *p*-Toluene sulphonyl chloride (PTSC), recrystallized from ether, was used in saturated aqueous solution; washed spores were left in it for 2 h. at 5° and then removed and washed three times in deionized water. They were then resuspended in Michaelis buffer at pH 7.0 for examination. Controls were spores left at 5° in distilled water and then washed and resuspended in the buffer. Di-sodium tetraborate (DST) was prepared as a 25 mM (*I* = 0.05) solution and washed spores were suspended in it for 2 h. at 25°. The solution, buffered at pH 9.2, was used directly for mobility determinations. Controls were obtained by suspending washed spores in Michaelis buffer at the same pH for the same time. Lipase (0.5 mg./ml.) and lysozyme (0.05 mg./ml.) were added to suspensions of washed spores in Michaelis buffer at pH 7.0 which were then incubated for 2 h. at 37°. Controls were comparable suspensions kept at 5° and untreated spores suspended in the buffer for 2 h. at both temperatures.

Determination of electrophoretic mobilities. The electrophoresis cells used were of the pattern described by Douglas (1955, 1957), having a channel of rectangular section, 10 mm. wide and 0.2 mm. deep. This was mounted on the stage of a phase contrast microscope capable of 600-fold magnification, illuminated by a 6 V, 100 W ribbon filament lamp with a heat filter between the lamp and the cell. The apparatus was housed in a thermostatically controlled room at 25°.

The cell, after cleaning with permanganic acid, distilled water and ethanol, was

filled with octadecyl alcohol in ethanol for about 10 min. The solvent was then evaporated off in a gentle air current, leaving a layer of octadecyl alcohol over the glass surfaces, which ensured an electrochemically symmetrical channel. After filling with the appropriate buffered suspension the cell was mounted vertically on the microscope stage, the instrument being used horizontally.

The channel depth was determined microscopically using a micrometre gauge fixed to the barrel with its probe bearing against the stage. The two stationary levels (i.e. the levels in the channel where electro-osmotic and return flows negate each other's effects) were calculated as fractions of the total depth from the formula of van Gils & Kruyt (1936).

Movement of the spores over a distance of 78 μm . was timed with a stop-watch reading to 0.1 sec. The current applied was adjusted to give a timing of 8 to 11 sec. and was measured with a Cambridge Unipivot d.c. microammeter. Spores were timed at both stationary levels and travelling in both directions by current reversal. At least 32 observations were made to determine the mean velocity of a spore population (standard error of the mean being no more than 5 %).

The specific conductivities of suspensions were determined with a Mullard conductivity cell and a Wayne-Kerr B 221 bridge; mobilities were calculated and expressed as $\mu\text{m.}/\text{sec.}/\text{V}/\text{cm}$.

RESULTS

Effects of homogenization on spores. No damage to spore surfaces was detected when suspensions were examined with the scanning electron microscope after homogenization and washing. Ornaments were still present on spores of *Streptomyces finlayi* and *S. viridochromogenes*, indicating that the outer sheath was still present.

Effect of media and culture age on electrophoretic mobility. Use of the three different media to produce spores of *Streptomyces viridochromogenes* had no significant effect on their pH mobility pattern, which ranged from about 0.4 at pH 3.0 to 1.8 at pH 10.0, despite wide differences in the composition of these media.

The pH mobility patterns of spores from 1 and 7 week cultures of *Streptomyces viridochromogenes* were similar, a decrease in net negative charge occurred with age (Fig. 1). Comparison of the mobilities at pH 7.0 of *S. griseus* and *S. viridochromogenes* spores from cultures aged between 1 and 7 weeks showed no decrease in net negative charge in the former species.

pH mobility of different species. These results are given in Fig. 2. Patterns for the variously ornamented spores of *Streptomyces* species and *Micromonospora* were the same: after a progressive rise in mobility up to about pH 6.0, the net negative charge remained constant between pH 6.0 and 8.0. Above 8.0, a slight increase in mobility occurred in *Micromonospora* which was barely detectable in *Streptomyces* species. Isoelectric points occurred at about pH 2.5 with a net positive charge at pH 2.0. These patterns indicated mixed carboxyl-amino surfaces.

The patterns for *Nocardia* and *Thermoactinomyces* were somewhat different. Although mobility increased up to pH 6.0, a gradual rise continued above 6.0. Spores had a small net positive charge at pH 2.0, but no steepening of the curves occurred above 8.0. These patterns indicated the presence of carboxyl groups but few or no amino groups on the surface.

Effect of surface-modifying treatments on mobility. These results are summarized

in Table 1. Again, responses of all *Streptomyces* species and *Micromonospora* were similar. Treatment with PTSC, which reacts with primary and secondary amines (Cohen, 1945), produced small increases in net negative charge at pH 7.0, again suggesting the presence of amino groups. None of these strains responded to treatment

Fig. 1. pH mobility curves of *Streptomyces viridochromogenes* spores from 1 week (+ — +) and 7 week (x — x) cultures.

Fig. 2. pH mobility curves for spores of *Streptomyces* sp. (FI) (□ — □), *S. viridochromogenes* (■ — ■), *S. griseus* (+ — +), *S. finlayi* (x — x), *Micromonospora* sp. (▲ — ▲), *Nocardia rubra* (△ — △) and *Thermoactinomyces vulgaris* (⊠ — ⊠).

with SDS, which reacts rapidly with lipid alkyl chains (Schulman, Pethica, Few & Salton, 1965); consistent with absence of accessible lipid was their lack of response to lipase. No changes was induced by DST, which reacts with 1·2 *cis*-hydroxyl groups associated with most monosaccharide subunits; this indicated that the outer layers were not composed of carbohydrate. However, amino sugars, which have trans-hydroxyl groups, would not react with DST. Treatment with lysozyme produced quite large decreases in net negative charge at pH 7·0, suggesting the presence of glycosidic bonds between the amino sugars acetyl glucosamine and acetyl muramic acid.

Table 1. *The influence of various surface-modifying treatments on the electrophoretic mobility of actinomycete spores*

Strains	% Changes in mobility to anode after treatment as compared with controls				
	PTSC at pH 7·0	SDS at pH 7·0	DST at pH 9·2	Lysozyme pH 7·0, 37°	Lipase at pH 7·0, 37°
<i>Streptomyces finlayi</i>	+10 %	0	0	-43 %	0
<i>S. griseus</i>	+9 %	0	0	-22 %	0
<i>S. viridochromogenes</i>	+15 %	0	0	-29 %	0
<i>Streptomyces</i> sp. (F1)	+7 %	0	0	-21 %	0
<i>Micromonospora</i> sp.	+10 %	0	0	-20 %	0
<i>Nocardia rubra</i>	0	+9 %	0	-38 %	-12 %
<i>Thermoactinomyces vulgaris</i>	0	+16 %	0	-40 %	-27 %

For code initials of surface modifying agents, see Methods.

Responses of *Nocardia* and *Thermoactinomyces* were again different from those of the above strains. No reaction occurred with PTSC, suggesting that amino groups were absent from the surface; lysozyme produced a decrease in net negative charge, indicating the presence of amino sugars. Again no reaction with DST occurred. Increases in net negative charge were produced by SDS, and the presence of lipid was further suggested by a reduction in mobility after lipase treatment.

DISCUSSION

The actinomycetes studied here were grown on solid medium and spores examined were produced above this. Presumably this is why we observed no effects of medium and culture age. Such effects among bacteria have usually been detected in liquid cultures, where the cells are more directly influenced by the composition of the medium (Douglas & Parker, 1958*b*; Plummer & James, 1961; Plummer, James, Gooder & Maxted, 1962).

Our results suggest the presence of hexosamine-peptide polymers in all strains. Although amino groups were not detected on the surface of *Nocardia* bodies or *Thermoactinomyces* spores, they reacted to lysozyme like the other strains; all had surface carboxyl groups. Becker *et al.* (1965) and Yamaguchi (1965) studied walls of vegetative organisms of these genera, and DeJong & McCoy (1966) showed that the composition of spore walls was very similar to that of vegetative hyphae in *Streptomyces* species. All genera studied contained glucosamine, muramic acid, alanine and

glutamic acid in their wall hydrolysates, along with other amino acids and sugars, suggesting the hexosamine-peptide configuration of Gram-positive bacteria.

Past results on the effect of lysozyme on actinomycetes vary. Using optical density measurements to detect changes, Sohler, Romano & Nickerson (1958) obtained a positive response with *Streptomyces griseus* but none with *S. viridochromogenes* or *Nocardia rubra*; no effects on *S. viridochromogenes* spores were detected by DeJong & McCoy (1966) using optical density measurements and electron microscopy. Differences between these results and ours are probably due to differences in the sensitivity of the detection methods.

Many *Nocardia* species have a relatively high content of lipid, and the presence of lipid in cells of *Nocardia rubra* was detected by Adams & McClung (1962). The presence of lipid in *Thermoactinomyces* spores has not been previously reported. Several workers have detected lipid on the surface of other Gram-positive bacteria using electrophoretic techniques. Lipid in *Micrococcus aureus* was detected by changes in mobility after anionic detergent and lipase treatments (Dyar, 1948; Dyar & Ordal, 1946); SDS treatment detected lipid on *Streptococcus* cells (Hill *et al.* 1963*b*).

The genera studied here included two with relatively hydrophobic spores (*Streptomyces* and *Thermoactinomyces*) and two with readily wetted cells (*Micromonospora* and *Nocardia*). The hydrophobic properties of *Streptomyces* may be associated with the presence of lipid (Erikson, 1947) and Kalakoutski & Sokolov (1961) reported the presence of lipids in *Streptomyces violaceus* aerial mycelium. Our results did not indicate the presence of lipid on the surface of any of the *Streptomyces* spores. Of the genera which had surface lipid, one (*Thermoactinomyces*) had hydrophobic spores, while the other (*Nocardia*) was easily wetted. A similar lack of correlation between water-repellant properties and occurrence of surface lipid was noted in spores of fungi (Fisher & Richmond, 1969). It therefore seems that other explanations for the hydrophobic properties of *Streptomyces* spores must be sought.

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