Monoclonal antibodies for *Streptomyces lividans* and their use for immunomagnetic capture of spores from soil

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Monoclonal antibodies were produced to *Streptomyces lividans* spore surface antigens. One particular hybridoma cell line, 43H6, produced a monoclonal antibody that reacted exclusively with *Streptomyces* cluster group 21 in an enzyme-linked immunosorbent assay (ELISA). Antibody 43H6 was found to be of subclass IgG1, kappa light chain. Western blot (immunoblot) analysis revealed that 43H6 recognized a major outer spore polypeptide of about 37 000 Da. The epitope was stably maintained in *S. lividans* spores over at least seven sporulation cycles on laboratory medium and for at least 14 weeks in sterile soil systems. The species group specificity of antibody 43H6 was exploited in the development of an immunocapture technique for the isolation of streptomycetes from soil. Magnetic beads coated with antibody 43H6 were mixed with soil samples seeded with *S. lividans* spores. Spore–bead complexes were recovered using magnets. Treatment of beads with blocking agents and the inclusion of detergents in the recovery system lessened non-specific binding of spores to beads and improved recovery. In buffer solutions decreasing the spore concentration increased the recovery values for a fixed bead concentration. At a spore concentration of 5×10^7 ml⁻¹ the recovery was 4.3% whilst at 5×10^2 ml⁻¹ it was 76% for a fixed bead concentration of 0.6 mg ml⁻¹. Using a bead concentration of 2 mg per 10 g soil, approximately 30% of the target spore population of 10^6 c.f.u. was recovered from sterile soil and 4% from non-sterile soil. This method offers a rapid means of selectively recovering and concentrating *Streptomyces* spores from soil samples.

Keywords: immunocapture, magnetic beads, spore surface antigens, *Streptomyces lividans*, soil, streptomycetes

INTRODUCTION

The availability of selective detection and recovery methods is an important prerequisite for exploring the fate of micro-organisms released into the environment. Immunological techniques have long been employed for the detection and identification of micro-organisms in clinical situations and in turn in soil, water and other environmental samples (Strayer & Tiedje, 1978; Gates & Pham, 1979; Wright *et al.*, 1987; Levasseur *et al.*, 1992). The application of immunological methods to the detection of genetically manipulated micro-organisms in environmental samples is relatively recent (for example

Morgan *et al.*, 1989; Mason & Burns, 1990; Morgan *et al.*, 1991; Ramos-González *et al.*, 1992). An advantage of immunological techniques is that they can be used for capture of specific organisms using magnetic polystyrene beads coated with appropriate antibodies (Morgan *et al.*, 1989, 1991). In order to investigate the application of immunological methods to the detection and recovery of specific streptomycetes from the environment it is first necessary to isolate an appropriate antibody and to characterize and assess the suitability of its target antigen. Streptomycetes exist in soil mainly as spores or hyphal fragments (Mayfield *et al.*, 1972). However during periods of nutrient elevation the mycelium may be present in significant amounts (Cresswell *et al.*, 1992). Since survival in soil is most likely achieved as spores, antibodies directed against *Streptomyces* spore surface antigenic determinants are more applicable to immunological detection in the environment. It is now well recognized that the growth

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Abbreviation: mAb, monoclonal antibody.

Table 1. Actinomycete strains

Species	Strain	Cluster no.*	Source
<i>Streptomyces coelicolor</i>	ISP5233	1A	E. M. Wellington collection
<i>S. griseus</i>	ISP5236	1A	
<i>S. scabies</i>	ISP5086	3	
<i>S. violaceus</i>	ISP5082	6	
<i>S. spectabilis</i>	ISP5512	10	
<i>S. matensis</i>	ISP5188	12	
<i>S. aureofaciens</i>	ISP5127	14	
<i>S. albus</i>	ISP5313	16	
<i>S. cacaoi</i>	ISP5057	16	
<i>S. murinus</i>	ISP5091	17	
<i>S. azureus</i>	ISP5106	18	
<i>S. cyaneus</i>	ISP5108	18	
<i>S. lincolnensis</i>	ISP5355	19	
<i>S. vastus</i>	ISP5309	19	
<i>S. caesi</i>	ISP5419	21	
<i>S. coelestis</i>	ISP5421	21	
<i>S. coelicolor</i>	A3(2)	21	
<i>S. cyanocolor</i>	ISP5425	21	
<i>S. griseoruber</i>	ISP5275	21	
<i>S. humiferus</i>	E35	21	
<i>S. lividans</i>	1326	21	D. A. Hopwood†
<i>S. lividans</i>	TK24	21	D. A. Hopwood†
<i>S. lividans</i>	0783	21	E. M. Wellington collection
<i>S. tuir</i>	ISP5505	21	
<i>S. violaceolatus</i>	ISP5438	21	
<i>S. roseiscleroticus</i>	ISP5303	22	
<i>S. toyacaenensis</i>	ISP5030	29	G. P. Sharples‡
<i>S. hygroscopicus</i>	ICI14996	32	
<i>S. griseoflavus</i>	ISP5456	37	
<i>S. hirsutus</i>	ISP5095	37	
<i>S. bluensis</i>	ISP5564	39	E. M. Wellington collection
<i>S. violens</i>	ISP5597	40	
<i>S. albofaciens</i>	ISP5268	42	
<i>S. kanamyceticus</i>	ISP5500	42	
<i>S. rimosus</i>	ISP5260	42	
<i>S. tolypopharus</i>	N738	53	
<i>S. griseovercillatum</i>	ISP5507	58	
<i>S. columbiensis</i>	ISP5558	61	
<i>S. lavendulcolor</i>	ISP5216	61	
<i>S. lavendulae</i>	ISP5069	61	
<i>S. fradiae</i>	ISP506	68	

* Cluster group in accordance with Williams *et al.* (1983).

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environment of bacteria markedly influences phenotypic expression of surface characters (Smith *et al.*, 1991; Nelson *et al.*, 1991). Thus stability of the antigenic determinant is an important consideration in the development of an effective detection technique.

To date, studies on the immunology of streptomycetes have been very limited. Antisera have been raised against mycelium of *Streptomyces* spp. for use in taxonomic studies

of species groups (Cross & Spooner, 1963; Ridell & Williams, 1983; Ridell *et al.*, 1986). However, the specificity and continuous production of monoclonal antibodies (mAbs) make them more appropriate for use in the development of selective recovery and enrichment techniques.

This paper reports the production and characterization of mAbs to spore surface antigens of *Streptomyces lividans* and

Table 2. Microbial strains other than *Streptomyces*

Species	Strain	Source
<i>Bacillus cereus</i>	LPCC(B1)	LPCC*
<i>B. megaterium</i>	LPCC(B2)	LPCC
<i>B. subtilis</i>	LPCC(B3)	LPCC
<i>B. stearothermophilus</i>	LPCC(B3)	LPCC
<i>Escherichia coli</i>	HB101	LPCC
<i>E. coli</i>	ED8654	C. Winstanley†
<i>Trichoderma viride</i>	LPCC(T2)	LPCC
<i>Penicillium chrysogenum</i>	LPCC(P1)	LPCC
<i>Fusarium culmorum</i>	LPCC(F1)	LPCC

* LPCC, Liverpool John Moores University microbiology culture collection.

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Table 3. Bacterial plasmids

Plasmid	Characteristics*	Reference or source
pIJ486	<i>tsr</i>	Ward <i>et al.</i> (1986)
pIJ673	<i>tsr aph vpb</i>	T. Keiser (unpublished)
pNW4	<i>tsr λp_L xylE</i>	Wipat <i>et al.</i> (1991)

* *tsr*, *aph*, *vpb*: resistance to thiostrepton, neomycin, viomycin respectively; *λp_L*, lambda promoter leftward; *xylE*, gene for catechol 2,3-dioxygenase.

the use of such antibodies in the immunological capture of streptomycete spores from soil. The capture technique utilized magnetic polystyrene beads coated with mAb, to provide a large surface area for the attachment of *S. lividans* spores. Stable bead-spore complexes were attracted towards a strong magnet and subsequently recovered.

METHODS

Microbial strains and plasmids. The microbial strains are listed in Tables 1 and 2. Bacterial plasmids are described in Table 3. The plasmid pNW4 is a derivative of pIJ486 (Ward *et al.*, 1986) containing a cloned *xylE* gene expressed from the lambda *p_L* promoter. *Streptomyces* strains were routinely cultured on R2YE medium (Hopwood *et al.*, 1985) at 28 °C for 5 d or in YEME broth (Hopwood *et al.*, 1985) at 28 °C for 48 h. *Bacillus* spp. were cultured aerobically either on nutrient agar plates or in nutrient broth at 37 °C for 48 h. Fungal strains were cultured on Potato dextrose agar plates at 28 °C for 60 h.

Antigenic purification and mAb production. *S. lividans* 1326(pIJ673) spores were purified by density gradient centrifugation prior to use as antigen for mAb production. Crude spore suspensions, prepared as described by Hopwood *et al.* (1985), were resuspended in 40% (w/w) sucrose and layered on to 40%:50%:60% (w/w) sucrose step gradients. Gradients were centrifuged at 79700 *g* for 1 h at 20 °C using a Sorvall OTD65B centrifuge and a TST 41.14 swinging bucket rotor (6 × 12 ml). The band of spores forming at the 40%:50% interface was harvested, added to 20 ml sterile distilled water and centrifuged at 2800 *g* for 10 min at 4 °C. This process was

repeated twice in order to wash the spores. Spores were resuspended in phosphate-buffered saline, pH 7.0 [PBS; 8.5% (w/v) NaCl; 3.9% (w/v) NaH₂PO₄; 13.4% (w/v) Na₂HPO₄]. Female BALB/c mice (8 to 12 weeks old) were immunized intraperitoneally with a suspension of 200 µg (total protein) intact *S. lividans* 1326(pIJ673) spores in 150 µl sterile PBS and 150 µl Freund's incomplete adjuvant. Intraperitoneal immunization was repeated after 4 and 6 weeks using the same inoculum and the serum titre of each mouse to intact *S. lividans* 1326(pIJ673) spores was determined by enzyme-linked immunosorbent assay (ELISA). Three days before the intended fusion date mice that showed suitable serum titres were inoculated with 200 µg spores in sterile PBS alone. The mice were then killed and their spleens removed for fusion. Mouse spleen cells and NS1 myeloma cells were fused with 50% (w/v) polyethylene glycol (Boehringer Mannheim) by the method of Clark & Waldmann (1985). ELISA was used to screen the hybrid supernatants for the presence of specific antibodies. Positive hybridoma cell lines were cloned by limiting dilution using the method of Harlow & Lane (1988). mAb was purified from tissue culture supernatant on a protein A affinity column (Pharmacia).

SDS-PAGE and Western blot analysis. Spore polypeptides for analysis were prepared by alkaline sodium dodecyl sulphate (SDS) treatment and trichloroacetic acid precipitation as described by Guijarro *et al.* (1988). SDS-PAGE was performed on 10% (w/v) slab gels as described by Laemmli (1970). Western transfer of polypeptides from SDS-PAGE gels was carried out by the method of Towbin *et al.* (1979) using an electrophoretic transfer apparatus. Following transfer to nitrocellulose membrane, immunoblotting with mAb 43H6 was carried out using standard techniques and developed colorimetrically with 4-chloro-1-naphthol (Harlow & Lane, 1988).

Determination of epitope stability. Stability of the spore antigenic determinant of *S. lividans* 1326(pIJ673) was assessed on laboratory media and in sterile soil microcosms. For studies *in vitro*, *S. lividans* 1326(pIJ673) was continually subcultured on R2YE medium at 28 °C. Subculturing was performed in triplicate for seven sporulation cycles, each time using single colonies from the previous culture. Spore suspensions prepared from each generation were quantified by viable count and the reactivity of mAb 43H6 with the spores was assayed by ELISA. For studies in soil, spores from *S. lividans* 1326(pIJ673) were prepared as suspensions from R2YE plates as described by Hopwood *et al.* (1985). Soil was obtained from a wheat field site in Warwick, UK, as described previously (Wellington *et al.*, 1990). Soil microcosms were prepared by wetting 100 g aliquots of autoclaved unamended soil in glass, foil capped, 300 ml beakers to 20% (w/w) with sterile distilled water and seeded with 10⁷ *S. lividans* 1326(pIJ673) spores in 1 ml sterile distilled water. The microcosms were incubated at 28 °C for 14 weeks prior to spore isolation by soil homogenization and sucrose density gradient centrifugation. Soil (100 g) was homogenized with 100 ml sterile distilled water and the resulting mixture centrifuged at 2400 *g* for 5 min at 4 °C to pellet heavy material. The resulting pellet was re-homogenized with a further 100 ml sterile distilled water. The process was repeated and the supernatants from all three pooled and centrifuged at 2400 *g* for 10 min at 4 °C to give a pellet. This pellet was resuspended in 40% (w/w) sucrose and spores isolated by centrifugation in a 40% (w/w) and 60% (w/w) step sucrose gradient at 79700 *g* for 1 h at 20 °C. The spores banded at the 40%–60% interface and were harvested and washed in PBS, prior to examination by ELISA.

Immunomagnetic particle preparation. Immunomagnetic particles (Dynabeads M450 sheep anti-mouse 4.5 µm, Dynal

Fig. 1. Affinity of mAb 43H6 for spores from *S. lividans* 1326(pIJ673) and closely related species (from cluster 21). The affinity of mAb 43H6 for spores from *S. lividans* 1326(pIJ673) and closely related *Streptomyces* spp. (from cluster 21) was determined by ELISA. Negative control wells were coated with *S. lividans* 1326 spores as antigen and utilized PBS in place of mAb 43H6. The broken line near the base of the figure indicates the negative cut-off point [calculated as the mean plus three standard deviations of the readings from three negative control wells (Ngo & Lenhoff, 1985; Tjissen, 1985)]. Error bars indicate the standard error from three replicate samples. 1, *S. lividans* 1326(pIJ673); 2, *S. lividans* 1326; 3, *S. lividans* 0783; 4, *S. lividans* TK24; 5, *S. coelicolor* A3(2); 6, *S. coelestis*; 7, *S. humiferus*; 8, *S. violaceolatus*; 9, *S. caesi*; 10, *S. cyanocolor*; 11, *S. tuius*; 12, *S. griseoruber*.

Ltd, UK) were supplied coated with anti-mouse immunoglobulin G (IgG) sheep antibodies. Affinity coupling of mAb 43H6 was performed using the direct technique recommended by the manufacturers.

Recovery of spores using immunomagnetic particles. To examine the interaction of *S. lividans* 1326(pIJ673) spores and immunomagnetic beads, the beads were used either mAb 43H6 coated or with the sheep anti-mouse immunoglobulin coating as supplied, and were added to 1 ml spore suspension in PBS. The mixture was incubated for 10 min at 37 °C in a glass tube and a washing procedure was performed by placing the glass tube on a cobalt-samarium magnet. Beads and bead-spore complexes were drawn toward the inner wall of the tube close to the magnet for 2 min. Whilst in this position beads were washed with excess PBS three times and once with sterile distilled water. Beads and spores were then resuspended in 50 µl sterile distilled water. For examination by scanning electron microscopy drops of bead suspension were placed on glass coverslips that had been glued to scanning electron microscope studs using conductive glue. The stubs were allowed to dry overnight at room temperature, sputter coated with gold and examined under a Joel 840 scanning electron microscope operating at 25 kV.

To determine spore recovery from buffer suspensions a similar procedure was employed. However, the amount of beads used varied between experiments and after magnetic recovery, beads were resuspended in 100 µl sterile water, sonicated briefly in a sonicating water bath and quantified by plate counting on R2YE media. In some experiments the magnetic beads were treated with the blocking agents bovine serum albumin (2%, w/v), hydrolysed gelatin (2%, w/v) and skimmed milk (5%, w/v), prior to the recovery of *S. lividans* 1326 spores from PBS. In order to quantify the recovery of *S. lividans* 1326(pIJ673) spores from soil, tenfold serial dilutions of *S. lividans* spores [either *S. lividans* 1326(pIJ673) for use in sterile soil, or *S. lividans*

1326(pNW4) for use in non-sterile soil] were prepared in PBS. Soil samples (10 g) were placed in boiling tubes, autoclaved if required and wetted to 20% (w/w) with distilled water. The appropriate spore suspension was added in 100 µl distilled water and after mixing, the microcosms were incubated overnight at 4 °C.

To maximize the recovery of spores from soil using magnetic beads it was first necessary to produce a soil slurry to which beads could be added and then recovered by using a flow-through device. Soil samples of 10 g were resuspended in 75 ml sterile distilled water and treated with the repeated homogenization and centrifugation steps described above for determination of epitope stability up to the resuspension of the cell pellet in sucrose. The resulting pellet was instead resuspended in 6 ml sterile distilled water. Beads (2 mg), either mAb 43H6-coated or with sheep anti-mouse immunoglobulin coating as supplied, were added to each 6 ml of soil suspension, mixed and incubated for 30 min at 37 °C. Beads were then captured by passing the suspension through a disposable plastic syringe which had a strong magnet attached to the side and a reservoir mounted on top. The soil slurry was introduced into the reservoir and allowed to travel slowly past the magnet, the speed being controlled by the gauge of the hypodermic needle on the end of the syringe. Extracted slurry was passed through the apparatus twice to maximize capture of the immunomagnetic beads from the large sample volumes employed. In order to capture spores from non-sterile soil 0.05% (v/v) Nonidet P40 (BDH) was included in the capture buffer and mAb 43H6-coated beads were incubated in 5% (w/v) skimmed milk prior to use. After extraction, the magnetic beads were washed with 6 ml PBS whilst still captured, the syringe was removed and used together with its plunger to resuspend the captured particles in 1 ml PBS. Of the 1 ml, 0.5 ml was plated directly on to R2YE medium and the remaining 0.5 ml serially diluted before plating on to the same medium.

The improvements to the immunocapture system, by blocking of beads with skimmed milk and addition of Nonidet P40, were subsequently applied to enhance the recovery of *S. lividans* 1326(pNW4) spores from non-sterile soil. For spore isolation from non-sterile soil, the R2YE medium was replaced with actinomycete isolation agar (Difco) containing nystatin (50 µg ml⁻¹) and cycloheximide (50 µg ml⁻¹).

In experiments involving capture of spores from mixed suspensions in buffer, recovered spores were quantified by plate counts and were identified on the basis of spore and mycelium colouration or pigment production. *S. griseoruber* produced a grey/green spore mass, *S. violaceolatus* produced a purple pigment into the medium, *S. spectabilis* possessed a bright red/orange spore mass and *S. lividans* 1326(pNW4) (*p_{tc}*, *xyfE*) was identified on the basis of catechol 2,3-dioxygenase (C23O) activity (Wipat *et al.*, 1991).

Statistical analysis. Analysis of variance was performed on ELISA data using the Excel 4 software package (Microsoft).

RESULTS

Production of mAbs

mAbs were raised to spore surface antigens of *S. lividans* 1326(pIJ673). Of 194 primary hybridomas from one fusion, 34 were found to secrete antibodies to *S. lividans* spores as judged by ELISA. One particular cell line, 43H6, produced an antibody that reacted primarily with spores from the immunizing strain, only showing cross-reaction with a number of closely related species. mAb 43H6 reacted with all members of species cluster group

21, with the exception of *S. griseoruber* (Fig. 1). The affinity of the antibody was highest for spores from *S. lividans*, *S. coelicolor* and *S. coelestis*. There was no significant reactivity (negative cut-off point 0.203) with spores from other *Streptomyces* cluster groups tested and no reactivity with spores from selected spore-forming soil organisms (Table 4). mAb 43H6 was isotypized and found to be of

Fig. 2. SDS-PAGE of spore surface polypeptides from *S. lividans* 1326(pIJ673) and other cluster 21 strains. Polypeptides were eluted from the surface of spores of *S. lividans* 1326(pIJ673) and closely related strains from cluster 21 and subsequently separated and visualized by SDS-PAGE. Lanes: 1, molecular mass markers; 2, *S. griseoruber*; 3, *S. cyanocolor*; 4, *S. caesius*; 5, *S. violaceolatus*; 6, *S. humiferus*; 7, *S. coelestis*; 8, *S. coelicolor* A3(2); 9, *S. lividans* 1326(pIJ673).

Fig. 3. Western blot analysis of spore surface polypeptides from *S. lividans* 1326(pIJ673) and closely related strains from cluster 21. Polypeptides were eluted from the surface of spores of *S. lividans* 1326(pIJ673) and other strains from cluster 21, separated by SDS-PAGE and transferred to a nitrocellulose filter. ELISA was performed on the immobilized polypeptides to visualize immunoreactive bands. Lanes: 1, *S. griseoruber*; 2, *S. cyanocolor*; 3, *S. caesius*; 4, *S. violaceolatus*; 5, *S. humiferus*; 6, *S. coelestis*; 7, *S. coelicolor* A3(2); 8, *S. lividans* 1326(pIJ673).

Table 4. Affinity of mAb 43H6 for spores from a range of streptomycetes and other microbial strains as determined by ELISA

OD₄₉₀ data represent the means of at least three tests.

Strain	<i>Streptomyces</i> cluster*	OD ₄₉₀	SD
<i>S. griseus</i>	1A	0.088	0.022
<i>S. coelicolor</i>	1A	0.083	0.012
<i>S. scabies</i>	3	0.058	0.005
<i>S. violaceus</i>	6	0.175	0.034
<i>S. spectabilis</i>	10	0.061	0.009
<i>S. matensis</i>	12	0.060	0.007
<i>S. aureofaciens</i>	14	0.091	0.020
<i>S. albus</i>	16	0.079	0.018
<i>S. cacaoi</i>	16	0.061	0.007
<i>S. murinus</i>	17	0.163	0.040
<i>S. azureus</i>	18	0.091	0.015
<i>S. cyaneus</i>	18	0.073	0.013
<i>S. lincolnensis</i>	19	0.056	0.006
<i>S. vastus</i>	19	0.081	0.009
<i>S. lividans</i> 1326	21	> 2.000	—
<i>S. lividans</i> TK24	21	1.312	0.057
<i>S. roseiscleroticus</i>	22	0.118	0.060
<i>S. toyacensis</i>	29	0.081	0.075
<i>S. hygroscopicus</i>	32	0.130	0.054
<i>S. griseoflavus</i>	37	0.081	0.011
<i>S. hirsutus</i>	37	0.060	0.012
<i>S. bluensis</i>	39	0.075	0.008
<i>S. violens</i>	40	0.095	0.015
<i>S. albofaciens</i>	42	0.083	0.013
<i>S. kanamyceticus</i>	42	0.092	0.018
<i>S. rimosus</i>	42	0.125	0.016
<i>S. tolypopharus</i>	53	0.117	0.036
<i>S. griseovercillatus</i>	58	0.106	0.015
<i>S. columbiensis</i>	61	0.130	0.054
<i>S. lavendulcolor</i>	61	0.082	0.005
<i>S. lavendulae</i>	61	0.102	0.014
<i>S. fradiae</i>	68	0.208	0.089
<i>Bacillus cereus</i>		0.148	0.014
<i>Bacillus megaterium</i>		0.113	0.012
<i>Bacillus subtilis</i>		0.148	0.014
<i>Bacillus stearothermophilus</i>		0.079	0.011
<i>Trichoderma viride</i>		0.145	0.012
<i>Penicillium chrysogenum</i>		0.186	0.013
<i>Fusarium culmorum</i>		0.028	0.004
Negative control†		0.095	0.036
Negative cut-off point‡		0.203	

* Classification as Williams *et al.* (1983).

† Negative control wells contained *S. lividans* 1326 spores as antigen and received PBS-Tween in place of mAb 43H6.

‡ The negative cut-off point was defined as the mean of at least three negative controls plus three times the standard deviation (Ngo & Lenhoff, 1985; Tjissen, 1985).

IgG1 subclass, kappa type light chain. Western blot analysis and ELISA performed with mycelia cultured *in vitro* revealed that the antigenic polypeptide was also present on the surface of mycelia from cluster 21 streptomycetes (excluding *S. griseoruber*), but showed greatly reduced reactivity.

SDS-PAGE and Western blot analysis of *Streptomyces* spore polypeptides

SDS-PAGE of purified spore polypeptides from *S. lividans* and other members of cluster 21 revealed a high degree of similarity for all species in the group except *S. griseoruber*, which shared only a few common polypeptides (Fig. 2). The differences evident by visually examining the protein profiles were confirmed by numerical analysis of electrophoregrams. Western blot analysis using mAb 43H6 as probe revealed the presence of a single immunoreactive polypeptide of approximately 37 000 Da for each species of cluster 21 except *S. griseoruber* (Fig. 3). Spore polypeptide profiles for species from other cluster groups were heterogeneous and no band corresponding to the 37 000 Da antigenic polypeptide was detected by Western blot analysis (data not shown).

Epitope characterization and stability

ELISA dot-blot analysis of spore extracts indicated that mAb 43H6 recognized a polypeptide epitope, and time-based removal of outer spore polypeptides showed that this epitope is located on or near the spore surface (results not shown).

The reactivity of spores taken from each of seven sporulation cycles, with mAb 43H6, as tested by ELISA, remained fairly constant. No significant difference was indicated by analysis of variance performed on replicate data between sporulation cycles ($P < 0.01$). Following

Fig. 4. Scanning electron micrograph showing details of the attachment of *S. lividans* 1326(pIJ673) spores to mAb 43H6-coated beads. Bar, 1 µm.

incubation in sterile amended soil systems for 14 weeks the reactivity of spores with mAb 43H6 was compared with the original inoculum (data not shown). Analysis of variance was performed on ELISA data from three replicate samples and was found to indicate that reactivity was significantly lower with those spores derived from soil at the 95% confidence level ($P < 0.05$) but not significantly lower at the 99% confidence level ($P < 0.01$).

Capture of spores from soil using magnetic beads

Examination by scanning electron microscopy of magnetic beads that had been previously incubated in a spore suspension demonstrated the ability of mAb 43H6-coated beads to bind *S. lividans* 1326(pIJ673) spores (Fig. 4). Magnetic beads which had not received a mAb 43H6 coating did not show significant binding of spores (data not shown).

Table 5. Efficiency of *S. lividans* spore recovery from PBS by magnetic beads with increasing spore concentration

Magnetic beads (0.6 mg ml^{-1}) were added to spore suspensions in PBS for which the c.f.u. ml^{-1} had been determined by plate counts (giving a bead:spore ratio from 1:6 to 15 600:1). Spores were removed from bead-spore complexes by brief sonication and quantified by plate counts as described in Methods. Results are the means of at least two replicates.

Original concn of spores (c.f.u. ml^{-1})	Recovery using mAb 43H6-coated beads		Recovery using non-coated beads	
	Concn of spores recovered (c.f.u. ml^{-1})	Percentage recovery	Concn of spores recovered (c.f.u. ml^{-1})	Percentage recovery
5×10^7	2.18×10^6	4.3	4.20×10^5	0.84
5×10^6	2.66×10^5	5.32	1.90×10^5	3.20
5×10^5	4.20×10^4	8.40	1.50×10^4	3.00
5×10^4	7.10×10^3	28.40	2.20×10^3	4.40
5×10^3	1.60×10^3	32.00	2.60×10^2	5.20
5×10^2	3.80×10^2	76.00	3.00×10^1	6.00

Table 6. Effect of blocking agents applied to magnetic beads on the recovery of *S. lividans* 1326 spores from PBS

The original c.f.u. ml⁻¹ was 5.1×10^4 (as determined by plate counts); a total of 1.2 mg of beads was used per 2 ml reaction volume (bead:spore ratio 153:1). Results are the means of two replicates.

Blocking agent	Bead coating	Concn of spores recovered (c.f.u. ml ⁻¹)	Percentage recovery
None	mAb 43H6	1.97×10^4	38.62
None	No mAb	3.90×10^3	7.64
BSA (2%, w/v)	mAb 43H6	1.26×10^4	24.71
BSA (2%, w/v)	No mAb	4.26×10^3	8.35
Hydrolysed gelatin (2%, w/v)	mAb 43H6	2.70×10^2	0.53
Hydrolysed gelatin (2%, w/v)	No mAb	1.55×10^2	0.30
Skimmed milk (5%, w/v)	mAb 43H6	2.05×10^4	40.19
Skimmed milk (5%, w/v)	No mAb	9.55×10^2	1.87

The recovery of *S. lividans* 1326(pIJ673) spores, by mAb 43H6-coated beads, from spore suspensions in buffer solutions increased with decreasing spore concentration, reaching 76% for an initial spore concentration of 5×10^2 (Table 5), whilst spore recovery by beads which had not received a mAb 43H6 coating reached 6% using the same initial concentration. Spore recovery by mAb 43H6-coated beads was also increased by increasing the bead concentration (data not shown).

To facilitate magnetic bead recovery from soil it was necessary to employ a flow-through capture device (see

Table 8. Capture of *S. lividans* 1326(pNW4) spores from non-sterile soil using mAb 43H6-coated magnetic beads using skimmed milk blocking agent and Nonidet P40 detergent

S. lividans 1326(pNW4) spores were recovered from non-sterile soil samples which had been seeded with a range of spore concentrations and incubated overnight at 4 °C. Spore concentrations were quantified in terms of c.f.u. ml⁻¹ by plate counts prior to inoculation into soil. mAb 43H6-coated beads were used at a concentration of 0.33 mg (ml soil slurry)⁻¹ (bead:spore ratio varied from 84500:1 to 1:118). Results are the means of two replicates.

Concn of spores seeded [c.f.u. (g dry wt soil) ⁻¹]	Concn of spores recovered [c.f.u. (g dry wt soil) ⁻¹]	Percentage recovery
10	0	0
10 ²	1	1.00
10 ³	16	1.60
10 ⁴	1.23×10^2	1.20
10 ⁵	4.44×10^3	4.40
10 ⁶	3.42×10^4	3.40
10 ⁷	2.36×10^5	2.36
10 ⁸	8.78×10^4	0.09

Methods). Using such apparatus with 0.6 mg mAb 43H6-coated beads ml⁻¹ the recovery was 30.5% for spores seeded at a concentration of 10^5 (g sterile soil)⁻¹. However, using beads without mAb 43H6 resulted in a 16.5% recovery at the same initial spore concentration. In an effort to reduce such non-specific spore binding, various blocking agents were applied to beads (Table 6). Incubation of beads in skimmed milk (5%, w/v) prior to use had little effect on the specific binding of mAb 43H6

Table 7. Effect of washing and inclusion of non-ionic detergents in buffer solutions on non-specific binding of spores of *S. lividans* 1326

The initial concentration of spores (c.f.u. ml⁻¹) in the diluted suspension is shown and was determined by plate counts. A sample of 0.6 mg of beads was used per ml of spore suspension (bead:spore ratio 281:1). Spore recovery was performed in a total volume of 1 ml and was quantified by plate counts. Results are the means of two replicates.

Buffer	Bead coating	Initial concn of spores (c.f.u. ml ⁻¹)	Concn of spores recovered (c.f.u. ml ⁻¹)	Percentage recovery
PBS	mAb 43H6	2.78×10^4	6.8×10^3	24.4
PBS	No mAb	2.78×10^4	1.1×10^3	3.96
PBS/0.05% (v/v) Tween 20	mAb 43H6	2.78×10^4	5.14×10^3	18.48
PBS/0.05% (v/v) Tween 20	No mAb	2.78×10^4	1.27×10^3	4.59
PBS/0.05% (v/v) Nonidet P40	mAb 43H6	2.78×10^4	7.26×10^3	26.10
PBS/0.05% (v/v) Nonidet P40	No mAb	2.78×10^4	7.20×10^2	2.59

Table 9. Immunomagnetic recovery of *S. lividans* 1326(pNW4) spores from mixed spore suspensions in PBS buffer

S. lividans 1326(pNW4) spores were recovered by using mAb 43H6-coated magnetic beads from mixed spore suspensions in PBS (see Methods). The capture volume was 1 ml for each mixture and a total of 0.3 mg mAb 43H6-coated beads were used in each recovery (bead:spore ratio 4:1). Results are the means of at least two replicates.

	Initial spore concn (c.f.u. ml ⁻¹)	Concn of spores recovered (c.f.u. ml ⁻¹)	Percentage recovery
<i>S. lividans</i> 1326(pNW4)	10 ⁶	1.2 × 10 ⁵	12.5
<i>S. lividans</i> 1326(pNW4) +	10 ⁶	8.7 × 10 ⁴	8.7
<i>S. violaceolatus</i>	10 ⁶	2.5 × 10 ⁴	2.5
<i>S. lividans</i> 1326(pNW4) +	10 ⁶	1.3 × 10 ⁵	13.1
<i>S. griseoruber</i>	10 ⁶	3.2 × 10 ³	0.3
<i>S. lividans</i> 1326(pNW4) +	10 ⁶	6.8 × 10 ⁴	6.8
<i>S. violaceolatus</i> +	10 ⁶	1.2 × 10 ⁴	1.2
<i>S. griseoruber</i> +	10 ⁶	1.3 × 10 ³	0.1
<i>S. spectabilis</i>	10 ⁶	7.1 × 10 ²	0.07
<i>S. lividans</i> 1326(pNW4) +	10 ⁶	1.2 × 10 ⁵	12.5
<i>S. spectabilis</i>	10 ⁶	1.3 × 10 ³	0.13

beads, whilst reducing the level of spore binding shown by beads which had not received the mAb 43H6 coating. In addition the effect of adding detergents to buffer solutions was assessed (Table 7). Inclusion of Nonidet P40 (0.05%, v/v) in the magnetic capture buffer was similarly found to reduce the level of spore capture by beads with no mAb 43H6 coating.

Recovery of *S. lividans* 1326(pNW4) spores from non-sterile soil reached a maximum of 4.4% of a seeded concentration of 10⁵ g⁻¹ (Table 8), a value significantly lower than that from sterile soil. This was possibly due to spores from cross-reacting cluster 21 streptomycetes competing with those of the released strain in non-sterile soil. Indeed a number of XylE⁻ streptomycetes were evident on the plate cultures of spores which had been magnetically recovered (results not shown). In order to investigate the effect of cross-reactivity on the recovery of *S. lividans* 1326(pNW4), spores from mixed spore suspensions were examined. *S. lividans* spores were magnetically recovered from buffer suspensions in which they had been mixed with spores from related species from cluster 21 and with those from clusters outside this group (Table 9). Recovery of *S. lividans* 1326(pNW4) decreased significantly in the presence of cluster 21 strains, such as *S. violaceolatus*, but not *S. griseoruber*. Strains from other cluster groups, for example *S. spectabilis* (cluster 10)

had little effect on recovery. Recovery values correlated with the reactivities shown by mAb 43H6 against each spore type in ELISA.

DISCUSSION

An approach to developing immunological methods for the detection and recovery of *S. lividans* has been to raise mAbs to spore surface determinants. However, given the similarity of SDS-PAGE spore polypeptide profiles for most members of cluster group 21, mAbs have been obtained using intact spores of *S. lividans*, rather than a particular spore polypeptide, as immunogen. From all the mAb-producing cell lines screened, none produced an antibody that was unique to *S. lividans*, presumably due to the fact that there are few differences in spore surface polypeptides between members of the cluster group. However, species group specific mAbs were obtained, of which mAb 43H6 reacted with a polypeptide epitope of about 37 000 Da present in spores of all cluster 21 members except *S. griseoruber*. Interestingly this species, designated as the representative member of the group (Williams *et al.*, 1983), exhibited a markedly different spore polypeptide profile to other members. mAb 43H6 showed greatest affinity for the spores from the immunizing strain, *S. lividans* 1326(pIJ673), but also reacted strongly with spores from some other members.

Differences in the extent of ELISA reactivity of mAb 43H6 with spores from different members of the group may be attributable to the amount of the polypeptide antigen within the spore or to a variation in the configuration of the antigen within each strain. Reactivity of the mAb with mycelium suggests the presence of the antigenic determinant in the mycelial wall. Interestingly the ELISA results supported the grouping of species within cluster 21 as based on a numerical taxonomic study of phenotypic characteristics (Williams *et al.*, 1983). *S. caesius*, *S. humiferus* (*Actinopycnidium caeruleum*), *S. cyanocolor*, *S. lividans*, *S. coelestis* and *S. violaceolatus* formed a close group and reacted strongly with mAb 43H6. Data for *S. coelicolor* A3(2) were not available in the study of Williams *et al.* (1983).

An important consideration for mAbs that are intended for use in detecting specific micro-organisms in the environment is the stability of the target antigen. This is particularly applicable to streptomycetes, which exhibit a high degree of spontaneous mutation and genomic rearrangements (Altenbucher & Cullum, 1984; Betzler *et al.*, 1987; Dyson & Schrempf, 1987). Furthermore, modulation in nutrient status, as encountered in natural environments, has been shown to alter accessibility and expression of surface antigens in both Gram positive and Gram negative bacteria (McGroarty & Rivera, 1990; Nelson *et al.*, 1991; Smith *et al.*, 1991) in turn affecting antibody binding. The affinity of mAb 43H6 for spores isolated from sterile soil was lower than for spores from R2YE medium. It may be that the antigen concentration is lower in soil. Alternatively the spore isolation procedure may have affected spore surface structure. However, the stability of the antigen may also depend on the morphological state of the spore. Guijarro *et al.* (1983) have reported a degradation pattern of spore proteins which commences on spore germination and this may alter the antigen configuration.

Whilst reacting with spores from cluster 21, mAb 43H6 showed no significant affinity for spores from other cluster groups nor for spores from the other bacteria and soil-borne fungi tested. Such species group specificity was exploited to develop a selective capture procedure for *Streptomyces* spores from soil utilizing immunomagnetic beads. Anti-mouse IgG pre-coated magnetic beads were chosen, rather than uncoated beads, since they were more effective in spore recovery when subsequently coated with secondary antibody. However the pre-coated beads without mAb 43H6 appeared to recover some spores. This may be due to non-specific binding of spores to beads and/or trapping of spores between beads during magnetic capture. Non-specific binding of bacteria to beads has been reported by other workers (Lund *et al.*, 1988; Morgan *et al.*, 1991). Inclusion of the non-ionic detergent Nonidet P40 in the recovery buffer reduced the non-specific reaction of pre-coated beads alone and enhanced recovery of spores, when beads were subsequently coated with mAb 43H6, without affecting viability. This contrasts with the reduction in cell viability encountered when the detergent was used in immunocapture of bacterial cells (Morgan *et al.*, 1991). Treatment of beads

with blocking agents, such as skimmed milk, also reduced non-specific binding events. Such improvements in the recovery procedure permitted the capture of spores from both sterile and non-sterile soil. Recovery of *S. lividans* spores from non-sterile soil was significantly lower than that from sterile soil. This may be attributable to 'competition' from naturally-occurring cluster 21 members in the soil. Indeed when other cluster 21 strains were added to capture buffers there was a resultant decrease in the recovery of *S. lividans* spores. Whilst addition of strains from other cluster groups had little effect on recovery values.

Such an immunocapture technique offers a rapid and convenient means of partially recovering and concentrating target *Streptomyces* cluster 21 spores from crude soil samples for the subsequent application of specific detection techniques that may require cleaner samples. Moreover, if used in conjunction with an appropriate marker system, for example the *xyIE* system for *S. lividans* (Wipat *et al.*, 1991), specific marked strains could be identified directly.

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