Selective recovery of *Streptosporangium fragile* from soil by indirect immunomagnetic capture

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A polyclonal antibody raised to *Streptosporangium fragile* spores reacted strongly and specifically with the immunizing strain and to a number of related species of *Streptosporangium*, as determined by dot immunoblotting. An indirect immunomagnetic capture method was developed for the recovery of the target organism from sterile and non-sterile soil, using sheep anti-rabbit M-280 Dynabeads. The effects of different soil blocking agents, antibody labelling concentrations and spore/Dynabead capture times on the recovery of *S. fragile* spores were investigated. Pre-blocking of antibody binding sites within the soil, with either 2% partially hydrolysed gelatin or 10% skimmed milk, was essential prior to immunomagnetic capture. Increasing the capture time from 15 to 60 min did not affect spore recovery; however, a 10-fold decline in the magnetic bead concentration did result in a significantly lower recovery of spores from soil. *S. fragile* was selectively enriched (1:190-fold) when present as a mixed population with *Arthrobacter oxydans* in sterile soil. The indirect immunomagnetic capture method was used to selectively recover *S. fragile* spores seeded into non-sterile soil, although some background binding of non-target bacteria was noted. The target was successfully recovered from a sterile soil microcosm after 14 d incubation and the capture rate was increased by the inclusion of an initial soil dispersion and biomass concentration procedure, using the ion-exchange resin Chelex 100.

Keywords: immunocapture, *Streptosporangium*, soil, magnetic beads, selective isolation

INTRODUCTION

There is an increasing need to improve methods for the detection and isolation of bacteria from environmental samples, both for the recovery of genetically engineered strains and microbial inoculants (Ford & Olsen, 1988; Morgan *et al.*, 1989; Pickup, 1991; Trevors *et al.*, 1993) and for the isolation of rare and unusual bacteria for use in industrial screening programmes (Nolan & Cross, 1988; Shearer, 1987).

Immunomagnetic capture (IMC) represents a potentially important development within this field, allowing the specific recovery of target bacteria from a diverse range of environmental samples. The method relies upon the interaction between cell-surface antigens and antibodies which can be attached to magnetized polystyrene beads.

Separation of target cells from a suspension can be achieved using a magnetic field. IMC has been widely used in clinical studies for the separation or depletion of target cells from a mixed population (Gaudernack *et al.*, 1986; Gruhn *et al.*, 1991; Silvestri *et al.*, 1992). More recently it has been used to enrich for and enhance the detection of pathogenic bacteria present in contaminated foodstuffs (Johne *et al.*, 1989; Mansfield *et al.*, 1993; Parmer *et al.*, 1992; Skjerve *et al.*, 1990) and faeces (Luk & Lindberg, 1991; Tomoyasu, 1992). Work has also focused on the detection and isolation of bacteria by IMC from aquatic environments (Morgan *et al.*, 1991; Christensen *et al.*, 1992; Bifulco & Schaefer 1993); however, there has been only one reported study on the use of this method to recover bacteria from the soil environment (Wipat *et al.*, 1994).

Most previous studies have used a direct IMC approach, suitable when monoclonal antibodies are available. However, the value of the method for the isolation of bacteria may be extended by the use of polyclonal antibodies raised

Abbreviations: DIB, dot immunoblotting; IMC, immunomagnetic capture; MPC, magnetic particle concentrator; Non, Nonidet P-40.

to many different surface antigens. Such antibodies may avoid the potential problem of high specificity associated with monoclonal antibodies when the isolation of groups rather than individual strains is required. The IMC approach provides a useful alternative to the more traditional selective isolation procedures for the recovery of specific genera of actinomycetes from soil (Hayakawa & Nonomura, 1989; Wellington & Toth, 1994). However, there may be problems associated with this method: for example the non-specific binding of antibodies to soil particles, and the separation of cells from soil particles, will be greater than those previously encountered in aquatic and food systems. The study reported here investigated the use of an IMC method for the detection and recovery of bacteria from soil and used, as a model system, the sporogenous soil actinomycete *Streptosporangium fragile*. This genus was targeted because, unlike the more ubiquitous *Streptomyces* species, the group is difficult to isolate by traditional culturing methods due to very slow growth on isolation plates and competition from faster-growing actinomycetes.

METHODS

Bacterial strains. *Streptosporangium* species were kindly donated by Novo Nordisk. Other actinomycete and bacterial strains used to determine antibody specificity are listed in Table 1.

Isolation of spores. *S. fragile* was grown on Yeast Starch agar. Spores were harvested (Hopwood *et al.*, 1985), collected by centrifugation at 3000 g for 10 min and washed five times with sterile PBS. Spores (1×10^8 c.f.u. ml⁻¹) were either fixed in 4% (v/v) formalin, 0.15 M sodium chloride (Conway de Macario *et al.*, 1982) and subsequently washed with PBS prior to immunization, or known spore dilutions were prepared in coating buffer (0.15 M sodium carbonate, 0.35 M sodium hydrogen carbonate; pH 9.6) for dot immunoblotting. Spores were stored in 20% (v/v) glycerol at -16 °C.

Immunization schedule. Antibodies were raised against the outer spore coat of *S. fragile* in New Zealand White rabbits. Blood was taken at 8 weeks following two weekly booster injections and the IgG fraction purified by ammonium sulphate precipitation and DEAE Sephadex A-50 gel chromatography (Harboe & Ingild 1983); final protein concentration 5 mg ml⁻¹. Antibody was stored at 4 °C with 0.01% thimerosal (Sigma).

Dot immunoblotting (DIB) procedure. Spores and whole cells (10^7 – 10^8 c.f.u. ml⁻¹) were suspended in coating buffer. Sonicated mycelial wall was diluted in coating buffer at 1 mg ml⁻¹ following protein determinations (Bradford, 1976). For *S. fragile* spores, wall antigen stability after six generations was tested by DIB. Suspensions were blotted onto nitrocellulose membrane filters for DIB (Gordon & Billing, 1988).

Soil type. Warwick soil was used and prepared as previously described (Wellington *et al.*, 1990). For microcosm studies *S. fragile* spores were seeded into sterile soil amended with 1% (w/w) crab shell chitin (Sigma) and 1% (w/w) soluble starch, wetted to 30% (v/w) with distilled water; moisture-holding capacity 80%, matric potential -5 kPa.

Statistical methods. Means were calculated from duplicate or triplicate samples plated in triplicate. Percentage data were subjected to arcsine transformation (Sokal & Rohlf, 1969), prior to analysis of variance using the MINITAB statistical package (Minitab Statistical Systems). Mean significant difference (MSD)

values, at the 95% confidence level, were calculated from the analysis of variance (Petersen, 1985).

Isolation of target by IMC. Antibody-labelled spores were captured by sheep anti-rabbit (SAR) M-280 Dynabeads (Dyna, UK) and recovered with a magnetic particle concentrator (MPC), product numbers 12002 and 12004 (Dyna, UK). SAR Dynabeads ($6\text{--}7 \times 10^8$ beads ml⁻¹, 10 mg ml⁻¹), were washed five times in PBS/0.1% (w/v) bovine serum albumin (BSA) fraction V (Sigma) before use as recommended in the manufacturer's instructions. The indirect IMC method was used throughout this study to overcome the problem of non-specific antibodies present within the IgG fraction. Spores were initially labelled with the purified IgG fraction, and unbound and non-specific antibodies were subsequently removed by centrifugation.

Effect of IMC conditions on the recovery of *S. fragile* from PBS. Spores were added to 1 ml sterile PBS/0.1% BSA (4.0×10^4 c.f.u. ml⁻¹) in 1.5 ml microcentrifuge tubes and were incubated at 20 °C for 2 h on a Griffin shaker (speed 6) with 4 µl ml⁻¹ of either *S. fragile* antibody or normal rabbit serum. The antibody-labelled spores were then washed three times in fresh PBS/0.05% (v/v) Nonidet P-40 (Non) buffer and finally resuspended in 1 ml PBS/Non capture buffer. Tubes were gently agitated for 15 min at room temperature and then placed in the MPC for 3 min. The supernatant, containing uncaptured spores, was removed by pipette and 1 ml fresh PBS/Non was added. One set of tubes was removed from the MPC and the bead/spore complexes were resuspended by hand for 1 min to remove loosely bound spores. This was designated the first washing step (W1). The bead capture and washing steps were repeated a second (W2) and a third time (W3), when the bead/spore complexes were finally vortex-mixed for 10 s. A second set of tubes was treated as above, but the bead/spore complexes were not resuspended at the first and second washing steps, only at the third step (W3). Viable counts were made at each washing stage. Capture rates were expressed as the percentage recovery of spores remaining after each of the washing steps, relative to the initial spore concentration in the tubes before the addition of the Dynabeads. The effect of different capture buffers and capture times was investigated.

IMC conditions for the recovery of *S. fragile* from sterile soil. *S. fragile* spores were mixed into 0.1 g sterile soil in 1.5 ml microcentrifuge tubes. After 30 min, to allow binding of the spores to the soil, 0.9 ml sterile blocking buffer was added: either PBS/0.05% (v/v) Non, PBS/Non/10% (v/v) skimmed milk, or PBS/Non/2% (v/v) partially hydrolysed (Bohool & Schmidt, 1968, 1980) gelatin type B (Sigma). The tubes were shaken for 90 min on a Griffin shaker, before the addition of the specific spore antibody or normal rabbit serum. Tubes were re-shaken for 2 h and non-specific and unbound antibodies were removed by centrifugation at 3000 g for 10 min. Target spores were recovered using the previously described procedure and spore capture rates at the third washing step were determined. Capture rates were expressed as a percentage of the count obtained by traditional plating method. The effect of antibody concentration at 0.2, 2.0 and 40 µl (g soil)⁻¹ on recovery of *S. fragile* spores seeded into sterile soil was investigated.

Competitive IMC recovery of *S. fragile* and *Arthrobacter oxydans* from sterile soil. Microcentrifuge tubes, containing 0.1 g soil, were seeded with either *Arthrobacter oxydans* JCM 2521 or *S. fragile*, or were co-inoculated with *A. oxydans* and *S. fragile*. The indirect IMC procedure was used. *A. oxydans* was selected for competition to determine the extent of non-specific binding with an unrelated soil bacterium likely to occur under natural conditions.

IMC of *S. fragile* in non-sterile soil. *S. fragile* spores (4.0×10^5 c.f.u. g^{-1}) were inoculated and thoroughly mixed into 0.1 g non-sterile Warwick soil in 1.5 ml microcentrifuge tubes. After 30 min, 0.9 ml sterile PBS/Non/10% skimmed milk was added. The tubes were shaken for 2 h at 5 °C before the addition of 40 μl g^{-1} of spore antibody or normal rabbit serum. *S. fragile* spores, captured by the indirect IMC procedure, were enumerated before the addition of 30 μl Dynabeads ml^{-1} and after the final third washing step (W3). Appropriate dilutions were plated onto humic-vitamin (HV) medium (Nonomura, 1989) with nystatin (100 mg l^{-1} ; BDH), with or without gentamicin sulphate (5 mg l^{-1} ; Sigma). Putative *S. fragile* colonies were enumerated after 14 d incubation at 30 °C and their identity was confirmed phenotypically.

Indirect IMC of *S. fragile* from soil microcosms. The spore concentration method of Herron & Wellington (1990) was modified by substituting 0.05% (v/v) Non for sodium deoxycholate and polyethylene glycol. Warwick soil (30 g) was sterilized and inoculated with *S. fragile* spores (3.1×10^5 c.f.u. g^{-1}) and after 30 min a 5 g sample was suspended in 45 ml PBS/Non/2% hydrolysed gelatin and shaken for 2 h (Griffin shaker). Aliquots of 5 ml (0.5 g soil ml^{-1}) with 2 μl g^{-1} spore antibody or normal rabbit serum were used for indirect IMC with 20 or 200 μl Dynabeads (g soil) $^{-1}$. A second 5 g sample was suspended in 15 ml PBS/Non with 1 g Chelex, shaken for 2 h and the dispersed soil filtered through a diatomaceous earth filter to remove the resin. The larger soil particles were removed by centrifugation (1000 g, 30 s) and the pellet was re-extracted twice with 5 ml PBS; all supernatants were then pooled and centrifuged at 3000 g for 20 min. The final pellet was resuspended to 50 ml with PBS/Non/2% hydrolysed gelatin, stored overnight at 5 °C and then shaken for 10 min. Aliquots of 5 ml were mixed with spore antibody or rabbit serum [2 μl (g soil) $^{-1}$] and tubes were shaken for 2 h. SAR Dynabeads, 100 μl or 10 μl (200 or 20 μl g^{-1}), were added and a capture time of 15 min used to recover the target spores.

The two IMC recovery methods described above were repeated after 14 d incubation of the microcosm at 30 °C and cell capture rates were determined.

RESULTS

Antibody specificity

The antibody raised against *S. fragile* spores reacted strongly with spores and sonicated mycelium from the immunizing strain, and moderate cross-reactions to other *Streptosporangium* species, including *S. album*, *S. amethystogenes* and *S. violaceochromogenes*, were also detected (Table 1). There was no cross-reaction with other *Streptosporangium* species. Some very weak cross-reactions were noted to sonicated mycelium of *Streptomyces* species. The *S. fragile* spore antibody showed high levels of reactivity to the target spores even after six sporulation cycles.

Capture conditions for the recovery of target spores from PBS

There was a clear relationship between the number of washing steps and the proportion of the initial spore population recovered, with the greatest decline noted between the first and second washing steps (Fig. 1). This was anticipated, as the loosely and non-specifically bound

spores attached to the Dynabeads were removed. It was probable that the calculated capture rates underestimated the total recovery of target, as more than one spore may have been bound to a Dynabead, but only one colony would have been enumerated. An examination of bead/spore complexes at the third washing stage by electron microscopy indicated that two to three spores were bound to some beads.

A longer capture time did not significantly increase ($P > 0.05$) spore capture rates (Table 2). Spore recovery increased significantly when PBS/Non was used as the capture and washing buffer. Non-specific attachment of spores to the beads was low (0.1%) and did not vary significantly with the buffer used. A bead/spore capture time of 15 min followed by three washing steps, with resuspension between each step, was used throughout the rest of this study.

IMC of *S. fragile* from sterile soil

S. fragile spores were recovered from sterile soil using the indirect IMC method with various blocking agents (Table 3). Partially hydrolysed gelatin gave a significantly higher ($P < 0.05$) recovery of spores and was used as the blocking agent in the soil microcosm study. Preliminary studies indicated that 0.1 or 1% (w/v) BSA/PBS/Non and 1 or 10% (v/v) normal rabbit serum/PBS/Non did not provide sufficient blocking of the soil for use with the indirect IMC method.

The concentration of the labelling antibody significantly influenced the capture of *S. fragile* spores from soil only at the lowest antibody concentration of 0.2 μl g^{-1} (data not shown). Comparable results were obtained with hydrolysed gelatin as the blocking agent. *S. fragile* spores were successfully recovered by indirect IMC at the three seeding densities used, with significantly increased ($P < 0.05$) capture rates at 10^3 (40.3%) and 10^4 c.f.u. (g soil) $^{-1}$ (43.3%) in comparison to 10^5 c.f.u. g^{-1} (26.3%). Background capture rates, using the normal rabbit serum, were low ($< 0.05\%$) and not significantly ($P > 0.05$) different between treatments.

Selective recovery was achieved from mixtures of *S. fragile* and *Arthrobacter oxydans* in sterile soil (Table 4). *A. oxydans* was recovered at very low rates (0.1%), using either the spore antibody or normal rabbit serum as the labelling antibody (Table 4). This non-specific binding of cells to the Dynabeads was at a similar level to that observed for *S. fragile*. The blocking buffer and procedure used did not affect the viability of the *Arthrobacter* cells. Capture rates of the target were higher in the presence of *A. oxydans*; possibly *Arthrobacter* cells blocked antibody binding sites within the soil. The results demonstrated the efficiency of the method for the selective recovery of a target organism from a mixed population of cells present in soil.

IMC of *S. fragile* spores from non-sterile soil

Recovery of the *S. fragile* spore population from non-sterile soil (data not shown) was 6.1% (HV – gentamicin)

Table 1. Specificity of the *S. fragile* spore antibody determined by DIB

Immunoblots were 10 µl dots. Antibodies were raised to sonicated mycelium (sm) at 1 mg protein ml⁻¹ and to spores (sp) at 1 × 10⁷ c.f.u. ml⁻¹ and cells (cl) at 1 × 10⁸ c.f.u. ml⁻¹. Results are from two tests. —, Negative; ±, weak positive; +, positive; ++, strongly positive.

Strain	Material	Result
<i>Actinomadura pusilla</i> DSM 43357	sm	—
<i>Actinoplanes philippinensis</i> JCM 3001	sm	—
<i>Amycolatopsis mediterranei</i> NRRL B-3240	sm	—
<i>Arthrobacter oxydans</i> JCM 2521	cl	—
<i>Bacillus subtilis</i> NCIB 3610	cl	—
<i>Brevibacterium flavum</i> JCM 1308	cl	—
<i>Cellulomonas flavigena</i> JCM 1489	cl	—
<i>Kitasatoa purpurea</i> NRRL B-5403	sm	—
<i>Microbispora rosea</i> JCM 3006	sm	—
<i>Micrococcus luteus</i> ATCC 9341	cl	—
<i>Micromonospora echinoaurantiaca</i> NRRL B-16102	sm	—
<i>Planobispora rosea</i> JCM 3166	sm	—
<i>Planomonospora parontospora</i> ATCC 23864	sm	—
<i>Planomonospora venezuelensis</i> ATCC 23865	sp/sm	±
<i>Promicromonospora citrea</i> JCM 3051	cl	—
<i>Saccharothrix aerocolonigenes</i> NRRL B-3298	sm	—
<i>Streptomyces curacoi</i> ATCC 13385	sm	±
<i>Streptomyces hygroscopicus</i> ATCC 27438	sm	—
<i>Streptomyces lividans</i> TK 24	sm	—
<i>Streptomyces scabies</i> ATCC 23962	sp	—
<i>Streptomyces violaceus</i> ATCC 15888	sm	±
<i>Streptosporangium albidum</i> DSM 43870	sm	—
<i>Streptosporangium album</i> DSM 43023	sm	+
<i>Streptosporangium amethystogenes</i> DSM 43179	sm	++
<i>Streptosporangium corrugatum</i> DSM 43316	sm	—
<i>Streptosporangium fragile</i> DSM 43847	sm	++
<i>Streptosporangium fragile</i> DSM 43847	sp	++
<i>Streptosporangium fragile</i> DSM 43847 (six sporing cycles)	sp	++
<i>Streptosporangium longisporum</i> DSM 43180	sm	—
<i>Streptosporangium roseum</i> DSM 43021	sm	—
<i>Streptosporangium</i> sp. DSM 43137	sm	+
<i>Streptosporangium violaceochromogenes</i> DSM 43849	sm	+
<i>Streptosporangium viridogriseum</i> subsp. <i>viridogriseum</i> DSM 43850	sm	—
<i>Streptosporangium viridogriseum</i> subsp. <i>viridogriseum</i> DSM 43850	sp	—
<i>Streptosporangium vulgare</i> DSM 43802	sm	—
<i>Streptoverticillium abikoense</i> NRRL B-1516	sm	+

and 8.3% (HV + gentamicin); the difference between these media was not significant (MSD = 3.7, $P > 0.05$). Background spore capture rates were significantly lower ($P < 0.05$) using the negative control normal rabbit serum as the labelling antibody (0.2% HV — gentamicin, 0.1% HV + gentamicin). Spore recovery was slightly lower than that from sterile soil in comparable trials (Table 3), possibly due to the interference of other bacteria. A few

colonies of streptomycetes were also apparent on the isolation plates.

Soil microcosm studies

After 14 d incubation the total population of *S. fragile* increased significantly to day 14. No significant differences in spore capture rates were detected between the day 0 and

Fig. 1. Effect of washing conditions on the recovery of *S. fragile* spores by IMC from sterile PBS/0.1% BSA. Percentage recovery of initial spore number (4.0×10^4 c.f.u. ml⁻¹) after first (W1), second (W2) and third (W3) washing steps, with or without resuspension (nr) of bead/spore complexes, is shown. Antibody/spore labelling time was 120 min, concentration of spore antibody (■) or normal rabbit serum (■) 4 µl ml⁻¹, capture time 15 min, Dynabeads 10 µl ml⁻¹. Results are means of duplicate samples plated in triplicate. The error bar indicates MSD ($P < 0.05$).

Table 2. Effect of capture buffer and IMC time on the recovery of *S. fragile* spores

IMC time was 15 or 60 min, with 10 µl SAR Dynabeads ml⁻¹. Antibody concentration was 4 µl ml⁻¹ with spore labelling time 120 min in sterile PBS/0.1% BSA. Results are given as percentage recovery of initial *S. fragile* spore number (trial 1, 2.7×10^4 ; trial 2, 1.8×10^4 c.f.u. ml⁻¹) after the third washing step, using three capture and washing buffers. Results are means of duplicate samples plated in triplicate. MSD ($P < 0.05$): trial 1, 3.0; trial 2, 5.7.

Capture buffer	Trial no.	Capture time (min)	Percentage recovery	
			Rabbit serum	Spore antibody
PBS	1	15	0.1	4.8
PBS/0.1% BSA	1	15	< 0.1	2.4
PBS/0.05% Non	1	15	0.1	8.3
PBS/0.05% Non	2	15	0.1	9.4
PBS/0.05% Non	2	60	< 0.1	7.4

day 14 microcosms using the IMC method without an initial Chelex dispersion step (Fig. 2). The modified Chelex method gave reduced recovery rates in comparison to the standard IMC method at day 0; however, at day 14 the Chelex dispersion step significantly improved ($P < 0.05$) the capture of *in situ*-grown *S. fragile*: 83% in comparison to 61% using the standard IMC method. This may be due to the increased dispersion of the soil particles and target cells, allowing greater access for the labelling antibody and reducing binding of cells to soil particles. A

Table 3. Comparison of blocking agents for IMC of *S. fragile* spores from sterile soil

Pre-blocking of soil was done for 90 min with partially hydrolysed gelatin or skimmed milk. Rabbit serum (RS) or spore antibody (SA) at 40 µl (g soil)⁻¹ was used for spore/antibody labelling over 120 min. IMC time was 15 min with SAR Dynabeads (100 µl g⁻¹). The results are percentage capture of initial spore number [5.8×10^5 c.f.u. (g soil)⁻¹] after the third washing step; means of two replicates plated in triplicate are shown. MSD = 3.3 ($P < 0.05$).

Blocking agent	Antibody	Percentage capture
PBS/Non	RS	< 0.1
PBS/Non	SA	0.1
PBS/Non/10% skim milk	RS	< 0.1
PBS/Non/10% skim milk	SA	10.3
PBS/Non/2% gelatin	RS	< 0.1
PBS/Non/2% gelatin	SA	15.4

Table 4. Competitive recovery of *S. fragile* from sterile soil co-inoculated with *Arthrobacter oxydans*

Normal rabbit serum (RS) and spore antibody (SA), each at 2 µl (g soil)⁻¹, were used with a labelling time of 120 min in PBS/Non/10% skimmed milk, after 90 min pre-blocking. IMC time was 15 min with SAR Dynabeads at 100 µl (g soil)⁻¹. Results are percentage recovery of initial spore or cell number [2.0×10^5 c.f.u. (g soil)⁻¹] after the third washing step. Means of duplicate samples plated in triplicate are shown. MSD ($P < 0.05$): *A. oxydans*, 0.6; *S. fragile*, 15.0.

Species	Labelling antibody	Percentage capture
<i>A. oxydans</i>	RS	0.1
<i>A. oxydans</i>	SA	0.1
<i>S. fragile</i>	RS	< 0.1
<i>S. fragile</i>	SA	23.5
<i>A. oxydans</i> *	SA	0.3
<i>S. fragile</i> *	SA	55.8

* Present as mixed population in soil.

spore/Dynabead ratio of at least 1:100 ensured optimal recovery of target cells.

DISCUSSION

Initial studies indicated that spore capture rates using a direct IMC procedure were poor (less than 1%), although this method has previously proved suitable for use with monoclonal antibodies (Skjerve *et al.*, 1990). This may be due to the low concentration of specific antibodies to the outer spore wall present within a purified IgG fraction, typically between 3 and 30% (Harboe & Ingild, 1983). Using an indirect IMC method this problem was overcome by initially labelling the target spores with the IgG

Fig. 2. Effect of Dynabead concentration and a preliminary Chelex (Cx) dispersion and spore concentration procedure, on the IMC of *S. fragile* from a sterile soil microcosm at day 0 and day 14. Percentage recovery of initial spore number on day 0 (3.1×10^5 c.f.u. ml⁻¹) and day 14 (1.2×10^6 c.f.u. ml⁻¹), after the third washing step, is shown. Soil was pre-blocked for 90 min with 2% partially hydrolysed gelatin; spore antibody or rabbit serum labelling time was 120 min, concentration $2 \mu\text{l (g soil)}^{-1}$, capture time 15 min, Dynabeads, 200 or $20 \mu\text{l (g soil)}^{-1}$. Results are means of triplicate samples, plated in triplicate. The error bar indicates MSD ($P < 0.05$). □, Rabbit serum, 200 $\mu\text{l Dynabeads (g soil)}^{-1}$; ▨, spore antibody, 200 $\mu\text{l Dynabeads (g soil)}^{-1}$; ■, spore antibody, 20 $\mu\text{l Dynabeads (g soil)}^{-1}$.

fraction and then removing non-specific and unbound antibodies during subsequent centrifugation steps. This indirect approach was successfully developed for the recovery of *S. fragile* spores from buffer solutions and from sterile and non-sterile soil.

IMC recovery rates for *S. fragile* spores from soil varied between 5 and 80%, but were usually in the range of 10–20% of the count obtained by traditional plating. Whilst selective plating allowed more efficient recovery, the capture method produced cleaner isolation plates, which in many soils would greatly increase the successful isolation of slow-growing genera. Recovery of *S. fragile* spores was significantly higher at low population densities, indicating that the method would be suitable for the isolation of target bacteria present at low population densities in soil. Comparable recovery rates were reported for the IMC of bacterial cells both from aquatic environments (Bifulco & Schaefer, 1993; Morgan *et al.*, 1991) and from a variety of dairy and meat products (Fratamico *et al.*, 1992; Lund *et al.*, 1988; Skjerve *et al.*, 1990; Vermunt *et al.*, 1992). However, Skjerve & Olsvik (1991) noted that the IMC method was not suitable for use with certain foodstuffs, including yoghurt, due to the presence of high levels of indigenous microflora.

Increasing the spore/bead capture time did not improve the recovery rate of spores, supporting the observations of Blackburn *et al.* (1991), who found that the greatest immunobinding of *Salmonella* to Dynabeads occurred within the first few minutes, with near maximal levels after 15 min. Increased capture time was always associated

with greater non-specific binding of bacterial cells to the magnetic beads (Fratamico *et al.*, 1992; Morgan *et al.*, 1991). This increase in the level of non-specific binding was not observed during this study.

The ratio of magnetic beads to target spores significantly influenced the capture of *S. fragile* spores. Morgan *et al.* (1991) found that an increase in the cell/bead ratio from 10:1 to 10000:1 did not markedly affect the recovery of *Pseudomonas* cells from lake water. A high cell/bead ratio is suitable for studies with soil due to binding of cells to soil particles and reduced mixing. In this study, non-specific binding of antibody to soil particles was reduced by use of gelatin as a blocking agent; this technique has been used for immunofluorescence studies of soil for *Rhizobium* (Bohool & Schmidt, 1980; Postama *et al.*, 1988; Schmidt, 1974) and *Salmonella* (Turpin *et al.*, 1993a). The use of the gelatin block also reduced the compaction of the soil after centrifugation, allowing greater dispersion of the soil particles during the resuspension steps required in the indirect IMC method. The nature of the washing conditions used significantly affected the proportion of target cells recovered, confirming the findings of others (Blackburn *et al.*, 1991; Lund *et al.*, 1988; Morgan *et al.*, 1991).

The enrichment ratio of 1:190 obtained for target spores from mixed cultures in soil was similar to that reported by Lund *et al.* (1988) for *Escherichia coli* (> 190-fold) and Blackburn *et al.* (1991) for the enrichment of *Salmonella* from four different bacteria (3- to > 2700-fold). The inclusion of a detergent in the washing and capture buffer improved the dispersion of target cells from the soil particles, facilitating capture of the bead/spore complexes. No detrimental effects of exposure to detergent were noted, in contrast to other studies (Morgan *et al.*, 1991). Further chemical disruption of the soil using the ion-exchange resin Chelex 100, in a preliminary spore dispersion and concentration step (Herron & Wellington 1990; Turpin *et al.*, 1993b), resulted in an increased recovery of *in situ*-grown cells from the day 14 soil microcosm. No effect was seen at day 0 as the spores seeded into the soil were not tightly bound to, or entrapped within, the soil matrix.

No decline in antibody reactivity to the spores was detected. Previous studies have reported that epitope stability in soil is maintained at least over several cell cycles, or for a few months (Mason & Burns, 1990; Kinkle & Schmidt, 1992). However, Wipat *et al.* (1992) noted a slight decline in the reactivity of *Streptomyces lividans* spores after 14 weeks incubation in soil, probably as a result of lower levels of antigen expression or accessibility. Variations in the expression of surface antigens, in response to growth conditions, have been reported in Gram-negative bacteria (Nelson *et al.*, 1991).

In conclusion, an indirect IMC method was developed for the recovery of a high proportion of the *S. fragile* spores present in both sterile and non-sterile soil. This method was shown to be suitable for the recovery of *in situ*-grown cells and, when used in conjunction with an initial Chelex dispersion step, resulted in the capture of up to 83% of

the spore population present in the soil, within a concentrated bead/spore pellet that was substantially free from contaminating soil particles. Spores produced in the soil were equally reactive but more tightly bound to soil particles. Further studies will investigate the distribution of spores in soil and determine their physiological status.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of Novo Nordisk A/S. We wish to thank Annette Petersen for raising and purifying the antibody, and Paul Baker for technical assistance.

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Received 10 March 1995; revised 23 May 1995; accepted 25 May 1995.