Journal of Dairy Research (2006) **73** 235–243. © *Proprietors of Journal of Dairy Research 2006*
doi:10.1017/S0022029906001749 *Printed in the United Kingdom*

Survival of thermophilic spore-forming bacteria in a 90^{+} year old milk powder from Ernest Shackelton's Cape Royds Hut in Antarctica

Ron S Ronimus*, Andreas Rueckert and Hugh W Morgan

Thermophile Research Unit, Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton New Zealand

Received 10 May 2005 and accepted for publication 22 September 2005

Milk powder taken to Antarctica on Shackelton's British Antarctic Expedition in 1907 was produced in New Zealand by a roller drying process in the first factory in the world dedicated to this process. Thermophilic bacilli are the dominant contaminants of modern spray-dried milk powders and the 1907 milk powder allows a comparison to be made of contaminating strains in roller-dried and spray-dried powders. Samples of milk powder obtained from Shackelton's Hut at Cape Royds had low levels of thermophilic contamination (<500 cfu ml^{-1}) but the two dominant strains (*Bacillus licheniformis* strain F and *Bacillus subtilis*) were typical of those found in spray-dried powders. Soil samples from the floor of the hut also contained these strains, whereas soils distant from the hut did not. Differences in the RAPD profiles of isolates from the milk powder and the soils suggest that contamination of the milk from the soil was unlikely. It is significant that the most commonly encountered contaminant strain in modern spray-dried milk (*Anoxybacillus flavithermus* strain C) was not detected in the 1907 sample.

Keywords: *Bacillus licheniformis*, *Bacillus subtilis*, RAPD-PCR, thermophilic, *Anoxybacillus flavithermus*, *Geobacillus stearothermophilus*, Milk powder, Antarctica.

Due to the vital role in human nutrition that milk plays, milk products are routinely analysed for a number of potential microbial contaminants. Thermophilic spore-forming bacterial contamination is routinely monitored despite there being no evidence of their involvement in human disease; but they are a useful indicator organism of good hygiene practices within milk powder producing factories and for downstream food processing requirements (Stadhouders et al. 1982; Kwee et al. 1986; Murphy et al. 1999). The milk industry is usually required to store samples of powder products for a number of years (commonly 2 to 3), after which they are usually destroyed, thus precluding the analysis of the survival of contaminants in powder stored for a long period. To our knowledge, in no published cases have the number of surviving thermophilic bacilli been assessed in very old milk powders (even over a few years old), although several studies have shown that bacterial spores can survive for extensive time-periods (Wilson & Shipp, 1938; Sneath, 1962; Dombrowski, 1963; Bartholomew & Paik, 1966; Setlow, 1994; Cano & Borucki, 1995).

*For correspondence; e-mail: ron.ronimus@agresearch.co.nz

Studies on the effect of storage conditions on the longevity of microorganisms in milk powder, or their spores in the case of bacilli, have shown that the storage temperature, total solids content and the relative humidity are important factors (McDonough & Hargrove, 1968; Higginbottom, 1969; Reddy et al. 1975; Mercurio & Tadjalli, 1979). With regard to possible freeze-thaw cycles affecting the long-term survival, *Bacillus* endospores are known to possess both extreme resistance to long-term desiccation and to multiple cycles of freezing and thawing (Nicholson et al. 2000).

Recently, two large-scale molecular-based surveys using randomly amplified polymorphic DNA-PCR (RAPD-PCR) were undertaken in which nearly 2400 isolates of thermophilic bacterial contaminants of milk powders were analysed, one using powders solely from New Zealand, and the other from a wide-geographic spread of 18 countries (Ronimus et al. 2003; Rueckert et al. 2004). The results show that seven strain groups from four species of bacilli are the major cause of contamination. These are *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) strain A, *Anoxybacillus flavithermus* (strains B, C and D), *Bacillus licheniformis* (strains F and G) and *Bacillus subtilis*. The presence of thermophilic

Fig. 1. Digital image of the original container of milk powder at Shackelton's Cape Royds hut used for sampling. Note finger on left for size perspective.

strains of *G. stearothermophilus*, *B. licheniformis* and *B. subtilis* has been confirmed in both raw milk and milk powders in numerous instances (Reddy et al. 1975; Stadhouders et al. 1982; Chopra & Mathur, 1984; Kwee et al. 1986; Phillips & Griffiths, 1986; Phillips & Griffiths, 1990; Crielly et al. 1994; Murphy et al. 1999). Significantly, strains of *A. flavithermus*, which were originally isolated from a hot spring in New Zealand (Heinen et al. 1982) and subsequently from hot springs in Yellowstone National Park, USA (Nold et al. 1996) and Turkey (Beldüz et al. 2000), have recently been conclusively identified in milk powders (Flint et al. 2001; Ronimus et al. 2003; Rueckert et al. 2004). The *A. flavithermus* strains (groups B, C and D) have no doubt over the years been routinely classified by the dairy industry as *G. stearothermophilus*.

Prior to spray drying, milk powder was produced by roller drying and although thermophilic bacilli were recognized as major contaminants it is unclear whether the strains that predominate in modern spray drying were also those found in roller-dried powder. Recently, we were able to obtain a milk powder sample from one of the earliest roller-dried powders produced, and this allowed analysis of the thermophilic contaminants.

Ernest Shackelton's ship *Nimrod* of the British Antarctic Expedition left New Zealand on January 1, 1908, from Lyttelton Harbour, Christchurch, bound for Antarctica. Of particular relevance to this communication were supplies of dried whole milk powder obtained from the LD Nathan Company packaged under the local name Defiance Brand, but registered in 1906 in the UK under the name Glaxo (Shackelton, 1910), for the Imperial Dry Milk Company, Ltd. of the UK (28 Gracechurch Street, London; see Fig. 1). The date of manufacture of the milk powder is likely to be 1907, since The Imperial Dry Milk Company was registered in 1905 (Millen, 1991), and subsequently was registered as Glaxo in the UK on October 27, 1906 (Jephcott, 1969). A dedicated milk powder factory at Bunnythorpe, New Zealand, was completed in late 1905 under the Defiance name with a Black Rooster logo (Jephcott, 1969). Furthermore, on the side of the milk powder tin was the promotional statement 'The only milk supplied to the Royal Navy and Military Tournament, Islington, 1905'. Finally, the manifest for the *Nimrod* includes the purchase and storage onboard of 68 cases of Glaxo non-fat dried milk powder from LD Nathan (Antarctic Heritage Trust report; Shackelton, 1910).

The Defiance Brand milk powder was produced in the first dedicated milk powder factory in the world using atmospheric-double-drum driers (no assistance of vacuum) with super-heated steam as the drum heat source – also termed hot-roller process dried milk (Eckles et al. 1943; Jephcott, 1969; McGillivray, 1978). The milk

https://doi.org/10.1017/S0022029906001749 Published online by Cambridge University Press

pre-treatment included pasteurization followed by chilling to 10 °C before drying (Millen, 1991). Roller, or drum drying, generally produces powders with larger particle sizes than spray-dried powders, 60% to 70% reduced solubilities (Eckles et al. 1943) and a relatively high degree of scorching, somewhat akin to high heat-treatment spray-dried powders (Lovell, 1990). Shackelton's Hut at Cape Royds still has containers of this milk powder and we were able to obtain samples of powder from a previously opened canister (presumably by members of Shackelton's team in 1908). The prospect of being able to analyse a milk powder produced at the start of the 1900s in New Zealand, by roller drying and stored in very favourable conditions (cold and dry), represented a remarkable opportunity. Results could aid in our understanding of the source of present day factory-derived contamination in the New Zealand milk powder industry. Additionally, we were able to obtain a sample of a spray-dried powder produced by the NZ Dairy Company in 1966, and stored at ambient temperature since that time which was included in this survey.

Materials and Methods

Milk powders

A sample of whole milk powder from Shackelton's Hut at Cape Royds was obtained in the Austral summer of 2002 and stored at 4 °C until analysed. The storage tin had been opened leaving the possibility of some contamination, although the vast bulk of the milk powder was still in place. The compositional analysis on the 10 kg metal storage box was as follows: 4·9% moisture, 5·6% mineral matter, 26·2% proteins, 27% fat and 36·3% milk sugar. The powder had a flaky appearance and was slow to re-constitute after incubation for 15 minutes in 50 °C warm water and vigorous vortexing. The 1966 whole milk powder was produced by Anchor Products, New Zealand, for the New Zealand Co-operative Dairy Co., Ltd., Hamilton, New Zealand and had an expiry date of January, 1967. The compositional analysis was: 26·5% butterfat, 28% protein, 6·0% mineral salts, 35·5% lactose, 3·0% moisture and 1·0% minor constituents (100 calories per 100 g). Thus, the two whole milk powders were essentially identical with respect to their compositions. The 1966 whole milk powder had a fine powder appearance and it was easily reconstituted after only 15 min incubation in 50 °C warm water and vigorous vortexing.

Soil samples

Nine soil samples were derived from different geographical locations during the Antarctic summer 2002. The three samples 'Mainway', 'Hallway' and 'Entrance' were collected within the hut domain while four soil samples were derived from the close hut surrounding with local distances of approximately 0, 10, 50 and 2500 m from the hut, respectively while two soils were derived from the Antarctic continent approximately 120 and 400 km away from the hut. Soil samples were collected in sterile 50 ml Falcon tubes using sterilized spatulas. The samples were stored at −20 °C until culturing was performed.

Culturing techniques

The powder samples were reconstituted and diluted in sterile deionised water. For total counts of bacilli, aliquots were pour plated with tryptic-soy agar (TSA) medium supplemented with 0·2% (w/v) soluble potato starch (Sigma; S2004) followed by incubation at 55 °C for 16 to 24 hours. The number of spores was determined after heat-treatment of the reconstituted milk at 80 °C for 20 min, followed by incubation at 55 °C in TSA-starch. Individual colonies from the dilution plates were then re-streaked to TSA-starch plates and re-incubated at either 35 or 55 °C (dependent on the colonies previous isolation temperature) for 16 h to obtain sufficient bio-mass for DNA preparation. The cells were harvested with sterile disposable spreaders and DNA isolated as described by Ronimus et al. (1997).

The thermophilic load of the soil samples was determined by resuspending 4 gram of soil into 10 ml sterile water and plating as described for the milk powder samples.

RAPD analyses

RAPD analyses employed Operon Technologies 10-mer primer OPR13 (GGACGACAAG) in a 35 cycle PCR reaction with an annealing temperature of 36 °C, again as described by Ronimus et al. (1997). PCR products were analysed by agarose gel electrophoresis using 1·5% LE SeaPlaque agarose gels (FMC Corp., San Diego, USA) and stained with ethidium bromide followed by image capture using an Eagle Eye System (Stratagene Corp., USA).

Results

A total count and spore count, together with the composition of strains or species identified are shown in Table 1. Total plate counts for thermophilic bacilli were all low, with the New Zealand milk samples from 1966 having a maximum of 360 per gram. For the Shackelton Hut samples the highest total count was found in the milk powder itself, soil samples from within the Hut had only slightly lower total counts but the ramp outside the Hut and more distant soil samples recovered no thermophilic bacilli. This result would be consistent with the soil in the Hut having been contaminated with organisms from the milk powder. Spore counts do not follow this trend, with the exception that spore numbers on the ramp sample were barely countable and the more distant soils contained no thermophilic spores. However, spore numbers in all soil samples in the Hut exceeded numbers of spores in

https://doi.org/10.1017/S0022029906001749 Published online by Cambridge University Press

Table 1. Numbers of thermophilic cells and spores in milk powders and soils and distribution of RAPD profiles in each sample

Sample	Sample type	Total count [cfu g^{-1}]	Spore count[a] [cfu g^{-1}]	Number of isolates[b]	Thermophiles [cfu g^{-1}]				
					A	F	G	*B. subtilis*	Other
Shackelton Hut	Milk powder	170	—	36	—	14	—	156	—
Shackelton Hut	Milk powder	—	17	30	0·5	14	2·5	—	—
Mainway	soil	71	—	18	—	24	3	44	—
Mainway	soil	—	320	14	—	68	—	252	—
Hallway	soil	140	—	18	—	78	—	54	8[c]
Hallway	soil	—	44	18	—	42	—	—	2[d]
Entrance	soil	120	—	18	—	73	—	47	—
Entrance	soil	—	160	17	—	66	—	94	—
Ramp	soil	0	—	0	—	—	—	—	—
Ramp	soil	—	4	10	—	3·6	—	0·4	—
Antarctic soils	soil	0	0	0	—	—	—	—	—
NZ 1966	Milk powder	360	—	53	34	306	13	7	—
NZ 1966	Milk powder	—	330	28	12	236	—	12	70[e]

A = *G. stearothermophilus*; F and G = *B. licheniformis*
[a] Activation by heat treatment at 80 °C for 20 min and incubation at 55 °C for >16 h
[b] Numbers of colonies isolated from each sample and subjected to RAPD-PCR
[c] Unidentified profile; [d] *Ureibacillus thermosphaericus*; [e] *B. coagulans* (58) unidentified (12)

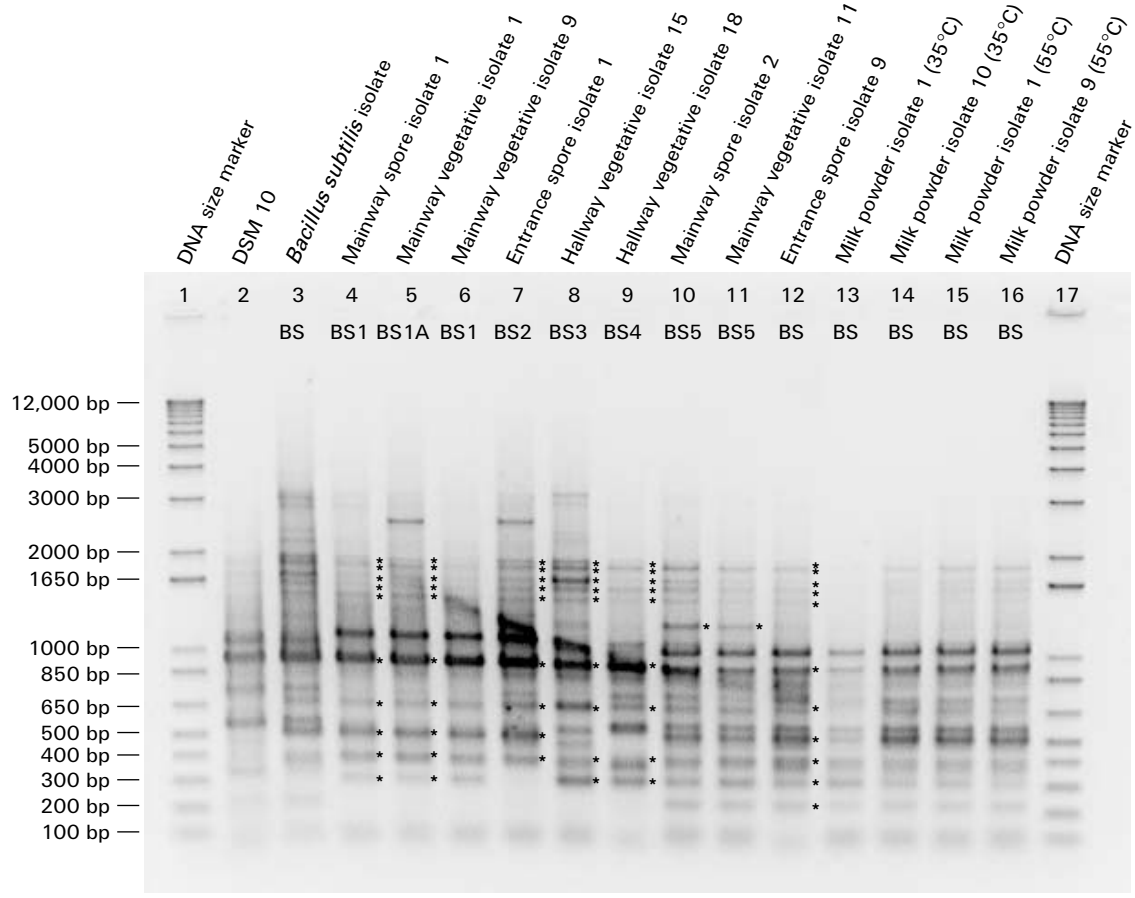

Fig. 2. RAPD-fingerprint profiles of selected *Bacillus subtilis* soil (tracks 4 to 12) and Shackelton Hut milk powder isolates (tracks 13 to 16). Track 2 shows the fingerprint of the reference strain DSM 10 and track 3 the common *B. subtilis* isolate found in New Zealand factory powders (Ronimus et al. 2003). Asterisks identify inter-sample distinguishing bands between milk-derived and soil-derived RAPD profile patterns.

https://doi.org/10.1017/S0022029906001749 Published online by Cambridge University Press

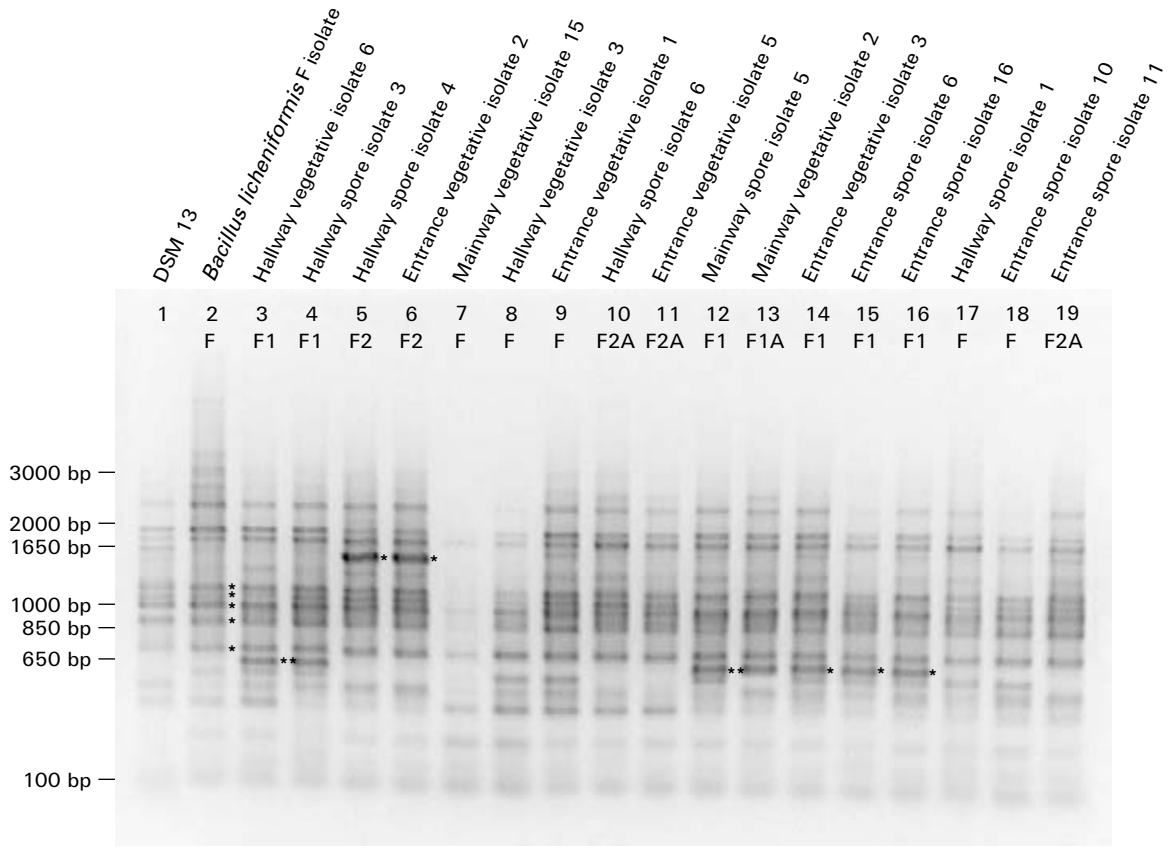

Fig. 3. RAPD-fingerprint profiles of thermophilic *B. licheniformis* strain F isolates from Antarctic soil samples. Asterisks identify inter-sample distinguishing bands between milk-derived and soil-derived RAPD profiles pattern.

the milk powder, sometimes by over an order of magnitude and it is difficult to explain this result as contamination of these samples directly by milk powder (where the ratio of spores to total count would have been expected to be relatively constant). A total of 260 isolates from the various samples were subjected to RAPD profiling, 66 from the roller-dried Shackelton Hut powder, 113 soil-derived isolates from the floor and the adjacent surroundings of Hut and 81 from the 1966 spray-dried powder. Two species dominated all these samples viz *B. licheniformis* followed by *B. subtilis*. The cell density of each strain present in any sample can be derived from the proportion of RAPD fingerprints obtained from the isolates subjected to RAPD analysis for that sample. The distribution of these RAPD fingerprints differed between the Shackelton milk sample and the Hut soil samples. For example, in the former, the total count was clearly dominated by *B. subtilis*, whereas the spore count was largely constituted of *B. licheniformis* strain F (Table 1). This distribution was quite different in the soil samples where generally both strains were present in more equal proportions.

A more detailed analysis of the RAPD patterns of isolates from milk powder and Hut soil sources is shown in Figs 2 to 5. When the RAPD fingerprints for *B. subtilis* were examined for variations between the milk and soil samples (Fig. 2), the patterns obtained for all milk powder isolates were identical (tracks 13 to 16) and consistent with the RAPD pattern of both the DSM type strain for *B. subtilis* (track 2) and our own reference strain of *B. subtilis*, which was isolated from a spray-dried powder (track 3). Some of the patterns obtained from soil isolates also had identical RAPD profiles to that of the milk isolates, e.g. track 12, while others were very similar, e.g. tracks 10 and 11. However, the majority of soil profiles were clearly different from those of the milk isolates. In particular, they contained a prominent distinguishing band at 1165 bp, (shown by an asterisk in Fig. 2), and other less prominent bands in some of the isolates reinforce this difference.

Isolates identified as *B. licheniformis* strain F all have common bands at 680, 850, 1000, 1040 and 1150 bp as indicated with asterisks in Fig. 3 track 2, but these isolates display a greater degree of genetic diversity than shown with *B. subtilis* (Figs 3 and 4). While all profiles are clearly of the *B. licheniformis* type (tracks 1 and 2 in each Fig. are of the DSM type strain and our reference strain isolated from spray-dried milk powder, respectively), consistent sub-strain variations are observed. Profiles, which are characteristic of the type strain of *B. licheniformis* F are found in milk isolates (Fig. 4, tracks 6 and 7) and soils from

https://doi.org/10.1017/S0022029906001749 Published online by Cambridge University Press

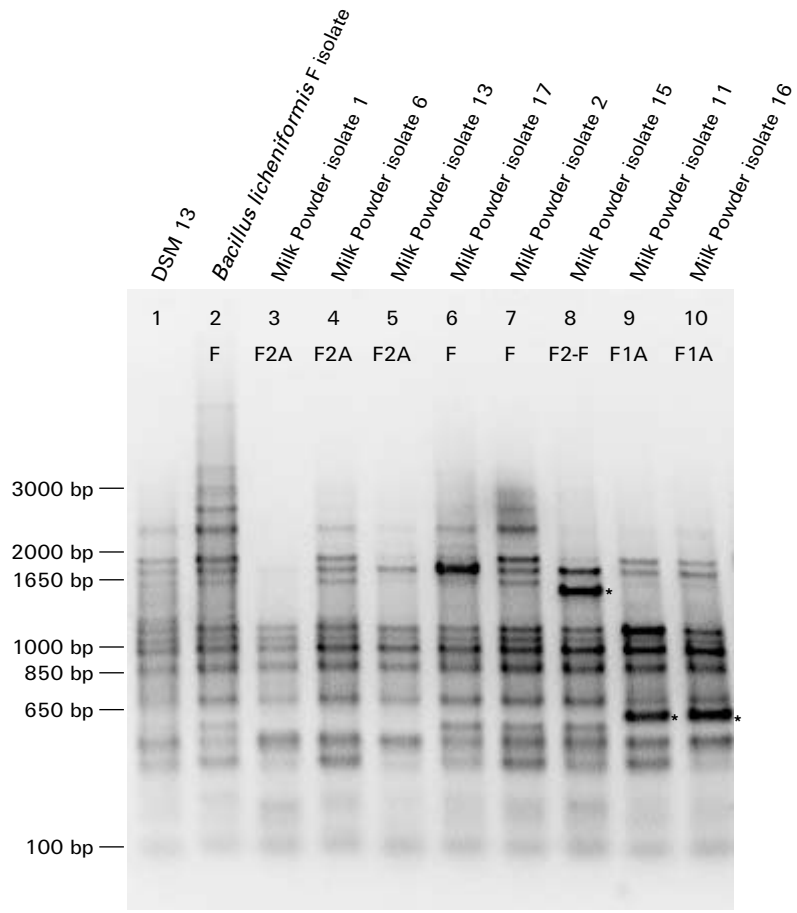

Fig. 4. RAPD-fingerprint of thermophilic *B. licheniformis* strain F spore isolates from the Shackelton Hut milk powder. Asterisks identify inter-sample distinguishing bands between milk-derived and soil-derived RAPD profile patterns.

the Hut (Fig. 3 tracks 7 to 9, 17 and 18). A variant of this profile (which we term F1) is characterised by a prominent band at approximately 640 bp and was present in isolates from milk (Fig. 4 tracks 9 and 10) and soils (Fig. 3 tracks 3, 4 and 12 to 16). Variant profile F2 was again found in soil and milk isolates (Fig. 3 tracks 5, 6, 10, 11 and 19; Fig. 4 tracks 3 to 5 and 8).

Of the 179 RAPD profiles that were run for the milk and soil samples from the Hut 171 were easily identified as *B. subtilis* or *B. licheniformis* strain F. Of the remaining 8 profiles, four were identified as *B. licheniformis* strain G, one as *Ureibacillus thermosphaericus*, one was unidentified and one as *G. stearothermophilus* strain A. The latter was only isolated as a spore from the milk powder and is significant since this profile is common in surveys of present day spray-dried milk powders (Ronimus et al. 2003; Rueckert et al. 2004). We have previously reported the occurrence of *U. thermosphaericus* in spray-dried milk, but only at very low levels, where it is considered to indicate contamination present in the raw milk from soil.

Eighty-one isolates from the 1966 spray-dried powder were RAPD profiled and contamination by *B. licheniformis* strain F clearly dominated this powder. Typical results are shown in Fig. 5 illustrating profile F (tracks 2, 3, 4, 6 and 8) and profile F2 (track 11). In addition, profiles identified as *G. stearothermophilus* strain A (track 5) *Bacillus coagulans* (tracks 7, 9 and 10) and *B. subtilis* (not shown) were less frequently encountered.

Discussion

The three critical storage parameters enhancing the survival of microorganisms following milk powder production and long-term storage are the water activity, relative humidity and the temperature (Higginbottom, 1969; Thompson et al. 1978; Stapelfeldt et al. 1997). Extremely dry conditions can lead to morphological changes in cells and generally, a slow reduction of the microbial population (Troller, 1991). Additional contributing factors include the preheat-treatment of the powder (Findlay et al. 1946), the milk powder structure, storage conditions (particularly the lack of oxygen), the type of organisms present (Thompson et al. 1978; Kieseker & Aitken, 1993; Celestino et al. 1997) and type and concentration of milk solids, i.e. skim or whole milk (Mazas et al. 1999).

https://doi.org/10.1017/S0022029906001749 Published online by Cambridge University Press

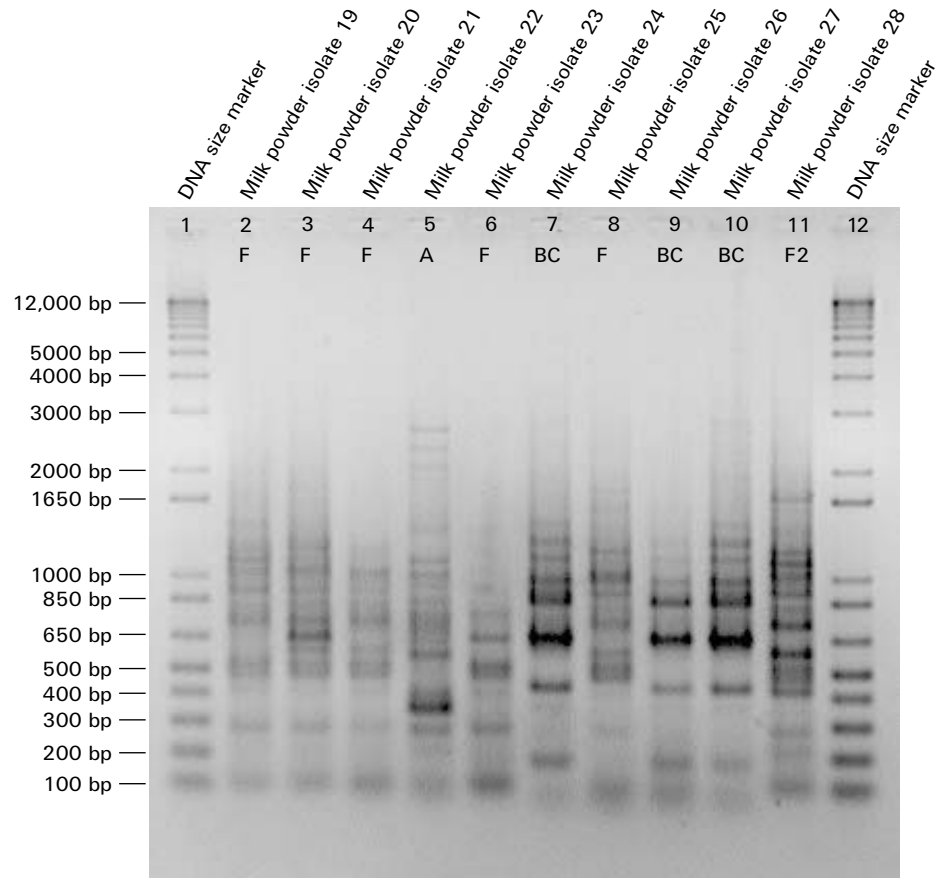

Fig. 5. RAPD-fingerprint profiles of thermophilic spore isolates of bacilli isolated from the New Zealand 1966 milk powder (F and F2 = *B. licheniformis*; A = *G. stearothermophilus*; BC = *B. coagulans*).

Recovery of growth after storage will be affected by the aeration (Long & Williams, 1959), incubation temperature, growth medium, pH and any spore activation procedures (Daemen, 1981; McGuiggan et al. 1994), although many bacilli do not necessarily require an activation step for germination to proceed (Sneath, 1962). Spores can be activated by the factory heat treatments (Lane, 1988), and at the same time can also be protected by milk solids themselves from heat (Raju & Kumar, 1989). The modern day shelf-life of whole milk powder is considered to be about 6 months at room temperature (Celestino et al. 1997), but when stored under vacuum or with an inert gas then it is 12 months (Kieseker & Aitken, 1993). Without protection from oxygen, auto-oxidation products might be expected to have a deleterious effect on the longevity of microorganisms (Celestino et al. 1997). Overall, a decrease in the total microbial content occurs after prolonged storage of dried milk powders, with endospore-forming bacilli being able to survive both the heat-treatments during powder production and the resulting desiccation, and are thus, the most likely to be isolated after prolonged storage (Keogh, 1966). Indeed, in a study using pure cultures of *B. subtilis* spores added to milk before drying no change in the viable count was observed up to 110 weeks at relative humidities between 5 and 50% (Higginbottom, 1969). Long-term studies dealing with the numbers of thermophiles with time of storage are relatively few in number. In a study using mesophilic recovery of *B. subtilis* at 32 °C by Thompson et al. (1978), the authors studied the effect of varying temperatures (71·1 to 93·3 °C) during spray drying on the survival of cultures spiked into non-fat milk powder and found the survival rate ranged between 32·2 and 40·4% after 36 weeks. Of those species that did survive *B. subtilis* was the most resistant to drying and recovery after storage (Thompson et al. 1978). In another investigation performed by Celestino et al. (1997) a medium-heat whole-milk powder (which was neither stored under an inert gas nor vacuum) showed no significant difference between the total plate counts of aerobic thermophiles and anaerobic thermophiles over an eight month period. In contrast, in a study looking at the survival of *Salmonella* in skim milk powders stored at ambient temperature with 4·4% moisture content there was an approximate 90% die off after 15 weeks (McDonough & Hargrove, 1968). The total mesophilic plate count decreased significantly, by approximately 80%, attributed to low water activity. In a New Zealand-based study no significant decrease in thermophile count was observed

https://doi.org/10.1017/S0022029906001749 Published online by Cambridge University Press

after 5 months storage at ambient temperature (Lane, 1988). As most of these studies are 'short-term' with respect to the powders reported here it is difficult to extrapolate the findings to that of a powder nearly 100 years old.

We must assume that the container of milk powder in Shackelton's Hut had been opened by members of Shackelton's party, and thereafter remained covered. It is also possible that Hut visitors since the late 1950's may have opened and disturbed some of the contents, and therefore contamination of the powder from the Hut surroundings cannot be excluded. Given the prevailing low temperature in the Hut throughout the year, it is likely that the temperature inside the Hut never exceeded 20 °C throughout the nearly 100 years, and it is reasonable to assume that allied with the low humidity and water content of the powder that no growth of vegetative cells of thermophilic bacilli or germination of their spores would likely occur. Similar assumptions can be made about the soil environment within the Hut. Therefore the occurrence of thermophilic cells and spores reflects their original distribution and not growth.

Our analysis of many spray-dried powders from NZ factories shows that the most common RAPD profiles encountered are *B. licheniformis* strain F, *G. stearothermophilus* strain A and *A. flavothermus* strain C (Ronimus et al. 2003; Rueckert et al. 2004). The latter is completely absent from the Antarctic powder, but was also not detected in the 1966 spray-dried powder so possibly this strain either does not survive long-term storage or was never present in either powder originally. It is of interest that of all the samples examined from Antarctica only the milk powder contained a representative of *G. stearothermophilus* strain A, albeit only a single spore from the 94 isolates profiled. In the 1966 spray dried powder 6 of the 81 isolates examined were of this strain. Thus, the two most typical organisms of modern day powders were either absent (*A. flavitherms* C), nearly absent (*G. stearothermophilus*) or did not survived long-term storage.

Based on the proportions of the profiles of the two most common strains present in the Antarctic samples it would appear unlikely that the milk powder has been contaminated by soil organisms. Firstly, the total count (consisting largely of vegetative cells) of the milk powder sample is dominated by *B. subtilis*, with spores only of *B. licheniformis* strain F. In the soil samples, apart from the Hallway, soils contain spores of *B. subtilis*, sometimes as the dominant form, e.g. the Mainway (Table 1). Additionally, all profiles of isolates of *B. subtilis* from the milk were identical, whereas those from the soil were varied and different (Fig. 2). Secondly, the total counts of soil samples contained a far greater proportion of *B. licheniformis* strain F cells as a percentage of the total compared with the milk powder. Since growth or spore germination is unlikely in these habitats, these differences can only arise from different sources of contamination. It would appear unlikely that the milk had been contaminated with thermophilic bacilli from the soil or other habitats within the Hut; if so, a greater proportion of *B. subtilis* spores would have been predicted in the powder sample, and relatively more *B. licheniformis* strain F cells contributing to the total count. In summary the results obtained are consistent with the milk powder containing the remnants of a population of thermophilic bacilli present in the powder at the time of formation, whereas the soil samples have derived their thermophilic bacilli from a different source. While it is not possible to discount the milk powder as the source of some of these contaminants in the soil, other strains are more likely to have derived from other sources. The hay taken as animal fodder, the animals themselves and human activities resulting from Hut occupancy are all possible sources of these organisms.

Conclusive support for this hypothesis can most readily come from analysis of milk powders in canisters with their seals intact. These are still present in the Hut, but access to such samples is restricted and unlikely. Meanwhile, these results form a base which can be compared over time with further samples.

The authors appreciate and thank the efforts taken by Shona Duncan, Ryuji Minasaki and Professor Roberta Farrell at the University of Waikato for obtaining the Antarctic milk sample from Cape Royds. The offer of samples of the 1966 powder by Mike Weeks of Anchor Products, Hamilton, for our study is appreciated. We also acknowledge the historical investigative work by Robert Norris at the New Zealand Dairy Research Institute.

References

Atwal JS, Shroff DN, Chand R & Srinivasan RA 1974 Studies on aerobic spore forming bacteria in milk and milk products. *Indian Journal of Dairy Science* **27** 22–28

Bartholomew JW & Paik G 1966 Isolation and identification of obligate thermophilic spore-forming bacilli from ocean basin cores. *Journal of Bacteriology* **92** 635–638

Beldüz AO, Dülger S, Demirbag Z & Ertürk Ö 2000 Isolation and characterisation of *Bacillus flavothermus* strains from a hot spring in North-East Turkey. *Biologia, Bratislava* **55** 249–258

Cano RJ & Borucki MK 1995 Revival and identification of bacterial spores in 25- to 40-million year-old Dominican amber. *Science* **268** 1060–1064

Celestino EL, Mani I & Roginski H 1997 The effects of refrigerated storage of raw milk on the quality of whole milk powder stored for different periods. *International Dairy Journal* **7** 119–127

Chopra AK & Mathur DK 1984 Isolation, screening and characterisation of thermophilic *Bacillus* species isolated from dairy products. *Journal of Applied Bacteriology* **57** 263–271

Crielly EM, Logan NA & Anderton A 1994 Studies on the *Bacillus* flora of milk and milk products. *Journal of Applied Bacteriology* **77** 256–263

Daemen ALH 1981 The destruction of enzymes and bacteria during the spray-drying of milk and whey, 1. The thermoresistance of enzymes and bacteria in milk and whey with various total solids contents. *Netherlands Milk and Dairy Journal* **35** 133–144

Dombrowski HJ 1963 Bacteria from palaeozoic salt deposits. *Annals of the New York Academy of Sciences* **108** 453–460

Eckles CH, Combs WB & Macy H 1943 Milk and Milk products, Third Edition. New York and London: McGraw-Hill Book Company

Findlay JD, Higginbottom C & Smith JAB 1946 The effect of the pre-heating temperature on the bacterial count and storage life of whole

milk powder spray-dried by the Krause process. *Journal of Dairy Research* **14** 378–399

Flint SH, Ward LJH & Walker KMR 2001 Functional grouping of thermophilic *Bacillus* strains using amplification profiles of the 16S-23S internal spacer region. *Systematic and Applied Microbiology* **24** 539–548

Heinen W, Lauwers M & Mulders JWM 1982 *Bacillus flavothermus*, a newly isolated facultative thermophile. *Antonie von Leeuwenhoek* **48** 265–272

Higginbottom C 1969 The effect of storage at different relative humidities on the survival of micro-organisms in milk powder and in pure cultures dried in milk. *Journal of Dairy Research* **20** 65–75

Jephcott H 1969 The first fifty years, pp. 68. Butter Market, Ipswich, England: Cowell WS, Ltd

Kieseker FG & Aitken B 1993 Recombined full-cream milk powder. *Australian Journal of Dairy Technology* **37** 132–135

Keogh BP 1966 Bacteriological problems in the condensing and drying of milk. *Food Technology Australia* **18** 126–133

Kwee WS, Dommett TW, Giles JE, Roberts R & Smith RAD 1986 Microbiological parameters during powdered milk manufacture. 1. Variation between processes and stages. *The Australian Journal of Dairy Technology* March 3–6

Lane A 1988 Microbiological effects in evaporation and spray drying. In *Evaporation and spray drying in the New Zealand Dairy Industry* (Ed. RS Jebson). Palmerston North, New Zealand: Food Technology Department, Massey University

Long SK & Williams OB 1959 Factors affecting the growth and spore formation of *Bacillus stearothermophilus*. *Journal of Bacteriology* **79** 625–628

Lovell HR 1990 The microbiology of dried milk powders. In *The Microbiology of milk*, 2nd Edition, pp. 245–269 (Ed. RK Robinson). NY: Elsevier Press

Mazas M, López M, Martínez S, Bernardo A & Martin R 1999 Heat resistance of *Bacillus cereus* spores: effects of milk constituents and stabilizing additives. *Journal of Food Protection* **62** 410–413

McDonough FE & Hargrove RE 1968 Heat resistance of *Salmonella* in Dried milk. *Journal of Dairy Science* **51** 1587–1591

McDowall FH 1953 The buttermaker's manual. Volume II. Wellington, New Zealand: New Zealand University Press

McGillivray WA 1978 New Zealand Dairy Research Institute: the first fifty years. Palmerston North, New Zealand: New Zealand Dairy Research Institute

McGuiggan JTM, Gilmour A & Lawrence LM 1994 Factors influencing the recovery of psychrotrophic, mesophilic and thermophilic *Bacillus* spp. from bulk raw milk. *Journal of the Society of Dairy Technology* **47** 111–116

Mercurio K & Tadjalli VA 1979 Nutritive and sensory evaluation of 20-year old non-fat dry milk. *Journal of Dairy Science* **62** 633–636

Millen J 1991 From Bonnie Babies to Better Medicines, The people who made Glaxo (Ed. MD Somma). Palmerston North: Glaxo New Zealand Ltd

Murphy P, Lynch D & Kelly P 1999 Growth of thermophilic spore forming bacilli in milk during the manufacture of low heat powders. *International Journal of Dairy Technology* **52** 45–50

Nicholson WL, Munakata N, Horneck G, Melosh HJ & Setlow P 2000 Resistance of *Bacillus* endospores to extreme terrestrial and extra-terrestrial environments. *Microbiology and Molecular Biology Reviews* **64** 548–572

Nold SC, Kopczynski ED & Ward DM 1996 Cultivation of aerobic chemoorganotrophic proteobacteria and gram-positive bacteria from a hot spring microbial mat. *Applied and Environmental Microbiology* **62** 3917–3921

Phillips JD & Griffiths MW 1986 Factors contributing to the seasonal variation of *Bacillus* spp. in pasteurized dairy products. *Journal of Applied Bacteriology* **61** 275–285

Phillips JD & Griffiths MW 1990 Pasteurised dairy products: constraints imposed by environmental contamination. In *Food contamination from environmental sources*, pp. 387–456 (Eds JO Nriagu & MS Simmons). USA: John Wiley and Sons

Raju VVR & Kumar MK 1989 Heat resistance of aerobic spore forming bacteria in milk. *Indian Journal of Dairy Science* **42** 71–74

Reddy PC, Atwal JS & Srinivasan RA 1975 Studies on the survival of thermophilic bacteria during manufacture and storage in dried milks. *Indian Journal of Dairy Science* **28** 289–295

Ronimus RS, Parker LE & Morgan H 1997 The utilisation of RAPD-PCR for identifying thermophilic and mesophilic *Bacillus* species. *FEMS Microbiology Letters* **147** 75–79

Ronimus RS, Parker LE, Turner N, Poudel S, Rueckert A & Morgan HW 2003 A RAPD-based comparison of thermophilic bacilli from milk powders. *International Journal of Food Microbiology* **85** 45–61

Rueckert A, Ronimus RS & Morgan HW 2004 A RAPD-based survey of thermophilic bacilli in milk powders from different countries. *International Journal of Food Microbiology* **96** 263–272

Setlow P 1994 Mechanisms which contribute to the long-term survival of spores on *Bacillus* species. *Applied Bacteriology Symposium Supplement* **76** 49S–60S

Shackelton EH 1910 The heart of the Antarctic: being the story of the British Antarctic Expedition 1907–1909. London: W. Heinemann

Sneath PH 1962 Longevity of micro-organisms. *Nature* **18** 643–646

Stadhouders J, Hup G & Hassing F 1982 The conceptions index and indicator organisms discussed on the basis of the bacteriology of spray-dried milk powder. *Netherlands Milk and Dairy Journal* **36** 231–260

Stapelfeldt H, Nielsen BR & Skibsted LH 1997 Effect of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder. *International Dairy Journal* **7** 331–339

Thompson SS, Harmon LG & Stine CM 1978 Survival of selected organisms during spray drying and storage of non-fat dry milk. *Journal of Food Protection* **41** 16–19

Troller JA 1991 Trends in research related to the influence of ''water activity'' on microorganisms in food. *Exp Med Biol* **302** 305–313

Wilson GS & Shipp HL 1938 Part IV – bacteriological investigations. *Journal of the Society Chemical Indiana* **36** 834–836