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Genomic and phenotypic comparison of *Bacillus fumarioli* isolates from geothermal Antarctic soil and gelatine

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Abstract

Bacillus fumarioli was originally isolated from geothermal soils in continental and maritime Antarctica, and recently, it has been shown to be a frequent contaminant of gelatine extracts obtained from European and American production plants. These habitats are geographically widely separated, share similar temperature and pH conditions, but have substantially different organic loads. Because of the prevalence in gelatine extracts and the dissimilarity of this habitat to geothermal soil, a comparative study was performed to assess the diversity among *B. fumarioli* strains and reveal possible intraspecies differences that might correspond to their niches of origin. Genomic (rep-PCR, 16S rDNA sequencing, DNA–DNA hybridisations) and phenotypic techniques (analysis of fatty acid content, total cellular proteins, metabolic and morphological traits) illustrate the very close relationship between isolates from the two niches. An abundant protein band was demonstrated for gelatine isolates only. This band was shown to result from a protein with high similarity to a stress response protein. Furthermore, subtractive hybridisation revealed genomic differences between Antarctic and gelatine isolates that may indicate adaptive evolution to a specific environment.

Keywords: *Bacillus fumarioli*; Diversity; Gelatine; Antarctica

1. Introduction

Bacillus fumarioli was originally isolated from geothermal soils in continental and maritime Antarctica [18], and it has been shown to be a frequent contaminant of gelatine extracts [8,9]. Gelatine is an animal protein hydrolysate that is of great value because of its gelling and stabilising properties in food and other industries. *B. fumarioli* was isolated from crude gelatine extracts during production [8] and has also been found in UHT-treated, semi-final gelatine batches from several European and American production plants [9]. Since isolations were performed in different gelatine production plants, the incidence of *B. fumarioli* as an occasional contaminant in gelatine may be discounted, especially as there have been no further reports on the isolation of this organism in other niches except the Antarctic environment.

Isolates of *B. fumarioli* from Antarctic geothermal environments, the only other habitat known for this organism, are of special biological interest as Antarctica is largely an ice-bound continent, supporting only sparse growth of terrestrial life, while these geothermal sites exhibit unique selective pressures. *B. fumarioli* is described as a moderately thermophilic and acidophilic, aerobic endospore-former, growing optimally at pH 5.5 and 50 °C. Its isolation from geothermal soils on the one hand, and from gelatine on the other is remarkable. Although both kinds of ecosystems have similarly low pH values and moderately high temper-

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atures, their geographical separations are considerable, and the intrinsic nutrient-status differences between the niches are striking. The organic load in Antarctic soils is very low compared with that of gelatine.

Comparson of two subsets of isolates attributed to a single species, but with widely divergent biological origins, can provide valuable ecological information and allow insights into the special traits needed by the organisms for survival in their different habitats. Because of the prevalence of *B. fumarioli* in gelatine extracts and the dissimilarity of this habitat with geothermal soil, the present study aimed to assess the diversity between the strains and reveal possible intraspecies differences that might correspond with their niches of origin. Therefore, we performed a polyphasic study on *B. fumarioli* isolates from gelatine and geothermal soils by means of $(GTG)_5$ primed rep-PCR (repetitive element primed-PCR) fingerprinting, 16S rDNA sequencing, DNA–DNA hybridisations, SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) of whole cell proteins, fatty acid analysis and phenotypic analysis (API 50CHB, cultural and morphological characters). In addition, genome differences between Antarctic and gelatine isolates were studied by suppression subtractive hybridisation. This technique has been shown to be efficient for the detection and characterisation of genome differences among strains of a single species [2,3] or closely related species [14,24].

2. Materials and methods

2.1. Bacterial strains and growth conditions

Details of the origin and the affiliation of the strains tested are shown in Table 1. *B. fumarioli* strains were grown aerobically on trypticase soy agar (TSA, BBL), pH 5.5 at

Table 1
List of strains studied

Species	Strain No.	Other designation	Source (if known)
B. fumarioli	LMG 17487	Logan Rcp L1	Soil from active fumarole, Antarctica
	LMG 17491	Logan Rcu Ir1	Soil from active fumarole, Antarctica
	LMG 17494	Logan Rfu S10	Soil from active fumarole, Antarctica
	LMG 18417	Logan Rfu S9	Soil from active fumarole, Antarctica
	LMG 18430	Logan Rcu Sm3	Soil from active fumarole, Antarctica
	LMG 18432	Logan Rcu S5	Soil from active fumarole, Antarctica
	LMG 18469	Logan SS1096	Soil from dead fumarole, Antarctica
	LMG 18470	Logan SS1097	Soil from dead fumarole, Antarctica
	LMG 18472	Logan SS1010	Soil from dead fumarole, Antarctica
	LMG19448^T	LMG 17489, Logan Rcp Sm1	Soil from active fumarole, Antarctica
	R-10919		Gelatine, production chain, Belgium
	R-13595		Gelatine, semi-final product, Belgium
	R-13623		Gelatine, semi-final product, Belgium
	R-13624		Gelatine, semi-final product, Belgium
	R-13860		Gelatine, semi-final product, Belgium
	R-13992		Gelatine, semi-final product, Belgium
	R-14704		Gelatine, semi-final product, Belgium
	R-14705		Gelatine, semi-final product, Belgium
	R-14711		Gelatine, semi-final product, Belgium
	R-16112		Gelatine, semi-final product, France
	R-16404		Gelatine, semi-final product, France
	R-19905		Gelatine, semi-final product, France
	R-19906		Gelatine, semi-final product, France
	R-19910		Gelatine, semi-final product, France
	R-20081		Gelatine, semi-final product, France
	R-20285		Gelatine, semi-final product, France
	R-20287		Gelatine, semi-final product, France
	R-20342		Gelatine, semi-final product, US
	R-20444		Gelatine, semi-final product, US
B. cereus	LMG 6910	ATCC 7004	Pasteurised milk
	R-13611		Gelatine, semi-final product, Belgium
B. coagulans	LMG 12401	Logan B0607	
	R-16392		Gelatine, semi-final product, France
B. licheniformis	LMG 17334	DVL 8400227	Aborted bovine, placenta
	R-13938		Gelatine, semi-final product, Belgium
'Paenibacillus cookii'	LMG18419^T		Soil from active fumarole, Antarctica
	R-11600		Gelatine, production chain, Belgium

Abbreviations: ATCC, American Type Culture Collection, Rockville, Maryland, US; DVL, Danish Veterinary Laboratory, Department of Pathology and Epidemiology, Copenhagen, Denmark; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie Gent, Ghent University, Belgium.

45 °C, while strains of other species were grown under the same conditions, but at neutral pH.

2.2. Repetitive element primed genomic fingerprinting (Rep-PCR)

Template DNA was prepared using a slight modification of the method of Pitcher et al. [22] as previously described [15]. The $(GTG)_5$ primer (5′-GTGGTGGTGGTGGTG-3′) was used as previously described [27]. PCR amplifications were performed as described before [27] using a DNA thermocycler (Perkin–Elmer 9600) and Goldstar DNA polymerase (Eurogentec, Belgium). The PCR products were subjected to electrophoresis in a 1.5% agarose gel (15 × 20 cm) for 16 h at 1.9 V cm^{-1} in 1 × TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 4 °C. The rep-PCR profiles were visualised after staining with ethidium bromide under ultraviolet light, followed by digital image capturing using a CCD camera. The resulting fingerprints were analysed by the BioNumerics V3.0 software package (Applied Maths, Belgium). Similarities of digitised profiles were calculated using the Pearson correlation method and an average linkage (UPGMA) dendrogram was obtained.

2.3. 16S rDNA sequencing

A fragment of the 16S rDNA gene was sequenced using an ABI 3100 automated DNA sequencer, as previously described [16]. The sequencing primers used were 5′-CTCCTACGGGAGGCAGCACT-3′ (forward primer, corresponding to positions 339–358 in *E. coli* numbering), 5′-AACTCAAAGGAATTGACGG-3′ (forward, 908–926), 5′-AGTCCCGCAACGAGCGCAAC-3′ (forward, 1093–1112), 5′-ACTGCTGCCTCCCGTAGGAG-3′ (reverse, 358–339), 5′-GTATTACCGCGGCTGCTG-3′ (reverse, 536–519) and 5′-GTTGCGCTCGTTGCGGGACT-3′ (reverse, 1112–1093). The FASTA programme [21] was applied to find the most closely related sequences from the EMBL database.

2.4. DNA–DNA hybridisations and determination of DNA base composition

Large-scale DNA extraction for determination of the G + C content and DNA–DNA hybridisations was performed as described before [10]. Determination of the G + C content was performed as described previously [18]. DNA–DNA hybridisations were performed using a modification of the microplate method described by Ezaki et al. [12], as described before [29]. A hybridisation temperature of 37 °C was used.

2.5. Fatty acid methyl ester analysis (FAME)

After an incubation period of 24 h, fatty acid methyl esters were prepared and separated as described before [30].

2.6. Analysis of whole-cell proteins and identification of selected proteins

After an incubation period of 24 h, preparations of whole-cell proteins and SDS-PAGE were performed as described before [23]. Based on these results, bands were selected for N-terminal amino acid sequence determination and mass spectrometric identification. Therefore, proteins were transferred onto a ProBlott membrane (Applied Biosystems) using standard protocols. The membrane was stained with Coomassie blue, and the corresponding protein band was subjected to N-terminal sequence determination using a 477A pulsed liquid sequenator (Applied Biosystems) following the manufacturer's instructions.

For mass spectrometric identification, the band of interest was excised from the SDS-PAGE gel, and slices of 4 mm^3 were washed twice with 150 µl of 200 mM NH_4HCO_3 in 50% ACN for 30 min at 30 °C, and air-dried. The proteins were reduced and alkylated as described before [17]. After a final drying step, in-gel digestion and extraction of the peptides were done as previously described [11]. From MALDI-MS spectra, generated using a 4700 Proteomics Analyzer (Applied Biosystems) with TOF/TOF optics, precursor ions were selected and fragmented in MS/MS experiments. Fragmentation spectra were manually interpreted with the assistance of the 'Data Explorer' data analysis software (Applied Biosystems). For identification, 'de novo' sequence information was combined with N-terminal sequence information. The FASTS programme [20] was used to search against the NCBI nonredundant database using default settings.

2.7. Phenotypic characterisation and numerical analysis

All strains of *B. fumarioli* were phenotypically characterised as described before [18], with incubation at 50 °C and reading at 24 and 48 h. With the exception of growth temperature ranges, all other tests were incubated at 45 °C. Numerical analysis of data followed the approach described before [18].

2.8. Analysis of genome differences

Genome comparison by suppression subtractive hybridisation (SSH) was performed as previously described [3] using *Sau*3A-digested DNA fragments. SSH identifies DNA sequences that are specific to one genome (tester) and absent from the other genome (driver). The gelatine isolate R-13624 was chosen as tester and the Antarctic isolate LMG 19448^T as driver.

Resulting products were inserted in the pMos*Blue* vector and transformed into MOS*Blue* competent *Escherichia coli* cells using the pMos*Blue* blunt ended cloning kit (Amersham Biosciences) following the guidelines of the manufacturer. Screening of recombinants was performed by direct colony PCR screening, applying PCR conditions as pro-

posed by the manufacturer and primers used in the subtractive hybridisation protocol [3].

Specificity of the final products was verified by Southern hybridisation. Transfer of PCR amplified difference products to a Hybond-N^+ membrane (Amersham Biosciences) after separation on an agarose gel was performed by standard Southern blot protocols [25]. Southern blots were hybridised with genomic, *Sau*3A-digested driver DNA on the one hand and tester DNA on the other, using the 'ECL direct nucleic acid labelling and detection system' (Amersham Biosciences) according to the manufacturer's recommendations.

Hybridisation of difference products with genomic DNA of a selection of *B. fumarioli* strains with different origins and strains attributed to other relevant species was verified. Therefore, dot blotting of genomic DNA of these strains was performed using a Bio-Dot® apparatus according to the instruction manual (BIO-RAD) and resulting dot blots were hybridised with difference products using the 'ECL direct nucleic acid labelling and detection system' (Amersham Biosciences) following the manufacturer's recommendations. In each hybridisation reaction, the tester strain and driver strain were included as a positive and negative control, respectively.

Difference products specific for gelatine isolates were sequenced. Sequence analysis was performed on PCR amplified fragments (as described above) in both directions with an ABI 3100 automated DNA sequencer using the original primers [3] and PCR conditions as described before [16]. The BLASTX search tool [13] was applied to find the most closely related sequences from publicly available databases.

3. Results

Rep-PCR analysis (Fig. 1) revealed comparable patterns for *B. fumarioli* strains, with minor inter-strain variations. No clear distinction was observed between the banding patterns of the Antarctic isolates and the gelatine isolates.

Almost the complete 16S rDNA sequences of gelatine isolates R-10919 (accession No. AJ438295), R-13595 (AJ581124), R-13624 (AJ581125) and R-14705 (AJ581126) were determined and FASTA analysis [21] revealed more than 99.9% sequence similarity of each of these isolates with the Antarctic isolate and type strain of *B. fumarioli*, LMG 19448^T, illustrating a close phylogenetic relationship.

Gelatine isolates R-13624, R-14705 and R-16404 showed more than 98% DNA–DNA reassociation amongst themselves and 87, 88 and 94% DNA–DNA reassociation respectively with the type strain and Antarctic isolate LMG 19448^T. According to the recommendations for species delineation [26] these findings clearly assign these strains to one single species, *B. fumarioli*. The G + C content for these four strains has a limited range, between 40 and 42%.

The fatty acid patterns of all gelatine isolates (not shown) fit within the mean profile set up for *B. fumarioli* based on

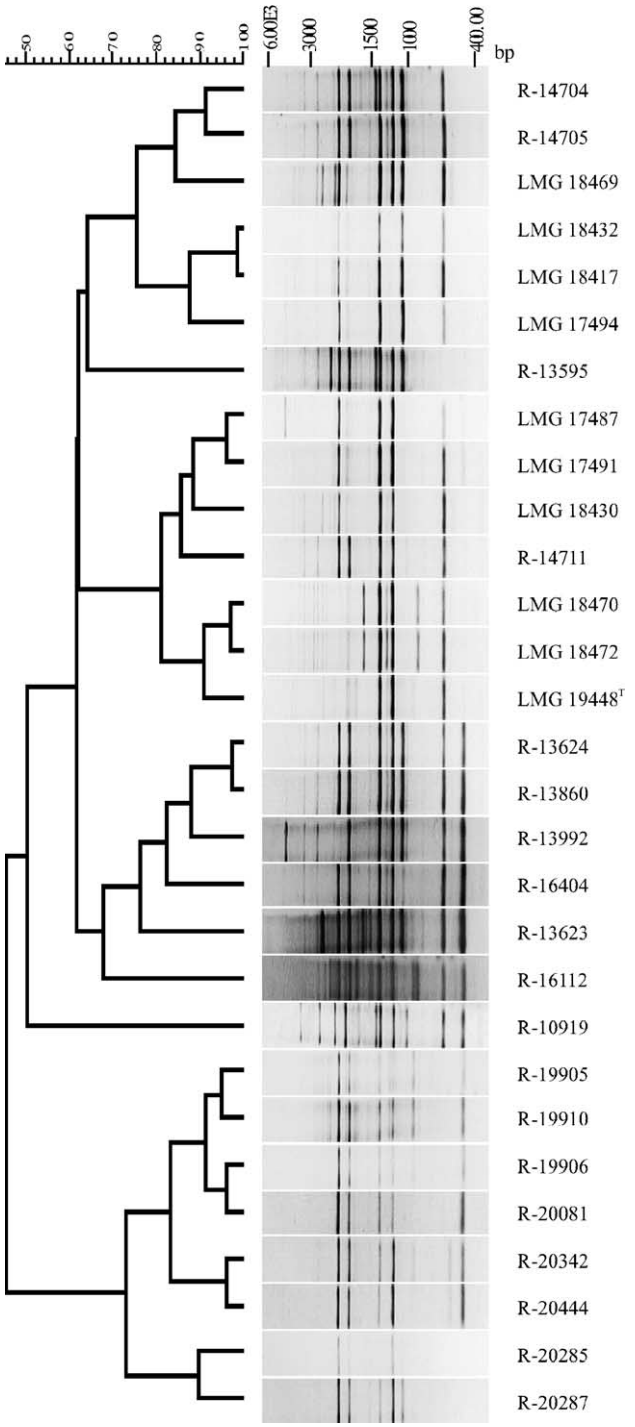

Fig. 1. Cluster analysis of digitised banding patterns, generated by rep-PCR using the $(GTG)_5$ primer, of *B. fumarioli* isolates among other *Bacillus* species. Gelatine isolates are indicated with an R-number, while Antarctic isolates are indicated with an LMG number. The dendrogram was constructed using the unweighted pair-group method using arithmetic averages with correlation levels expressed as percentage values of the Pearson correlation coefficient.

profiles of Antarctic isolates [18]. There was no clear-cut distinction between profiles of isolates from the two habitats.

Biochemical and morphological characterisation did not reveal any characters that could consistently and con-

vincingly distinguish between the group of Antarctic isolates and the set of gelatine isolates (data not shown). All strains possessed gelatinase activity. The description of this species [18] stated that acid without gas was produced from D-fructose, D-glucose, mannitol, D-mannose, *N*-acetylglucosamine (weak), sucrose and trehalose (weak), and indicated that appreciable interstrain variability was observed in the production of acid from galactose, glycerol, lactose, maltose, D-melibiose, D-melezitose, D-methyl-D-glucoside, D-raffinose, and D-turanose. A similar pattern was seen with the gelatine isolates. In both sets of strains this interstrain variability was seen mainly as wide differences in strengths of reaction, which ranged from too weak to be scored as positive in some strains, to sufficiently strong to be scored as positive reactions in others. One character, acid production from ribose, varied from negative to moderately positive in Antarctic isolates, but it was never positive for gelatine isolates, while acid production from sorbitol and D-xylose ranged from weak to positive in the gelatine isolates, but was never strong enough to count as positive in the Antarctic strains. Overall, however, the metabolic profile of the gelatine isolates fits within the profile described for Antarctic isolates [18].

SDS-PAGE of whole cell proteins showed a similar, species-specific pattern for all isolates (Fig. 2). Remarkably, profiles of gelatine isolates from semi-final batches showed a thick band, absent in profiles of Antarctic isolates and the strain isolated from a crude gelatine extract during production (R-10919). This band points to an abundant protein of approximately 26 kDa that could be specific for isolates from semi-final gelatine batches or is overexpressed in these strains. Surprisingly, this abundant protein band was less pronounced or even absent when organisms had been cultivated in trypticase soy broth at 45 °C for 6 h. Further attempts have been made to identify this protein. N-terminal sequence analysis resulted in a stretch of 40 amino acids (MANLLRLKNVIMADLHEALEQRENQTPQAMLNEYLRQDXH). Comparison with nonredundant protein databases revealed significant homology (77%) with the 26 kDa phage shock protein A (pspA) of *Bacillus cereus*. Peptide mass fingerprinting and MS/MS confirmed the 40 N-terminal residues, and additionally obtained sequence tags supporting the differentially expressed protein as a pspA family member.

From the SSH procedure, 20 difference products were retrieved that showed Southern and dot blot hybridisation signals with tester DNA (R-13624) and not with driver DNA (LMG 19448^T). These difference products were further screened by means of dot blot hybridisation for their presence in other *B. fumarioli* strains originating from geothermal soils or gelatine (Table 1) and also in other *Bacillus* species that have been associated with gelatine contamination [8]. For each of these species, we included a gelatine isolate and a strain of different origin (Table 1). Of special interest was '*Paenibacillus cookii*' [19], since this species has been isolated, as has *B. fumarioli*, both

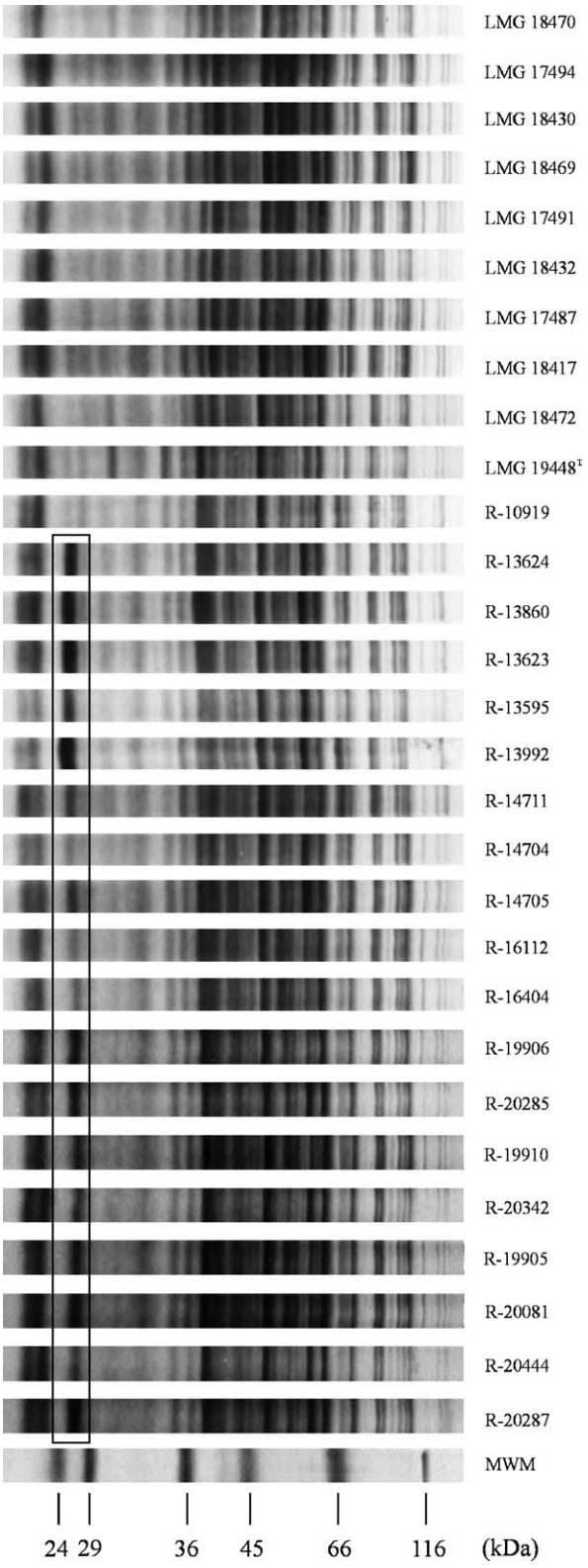

Fig. 2. SDS-PAGE profiles of whole cell proteins of *B. fumarioli* isolates. Gelatine isolates are indicated with an R-number, while Antarctic isolates are indicated with an LMG number. A thick band occurring in profiles of isolates from semi-final gelatine batches is shown, which distinguishes these strains from Antarctic isolates and the isolate from a crude gelatine extract during production (R-10919). A molecular weight marker (MWM) is included.

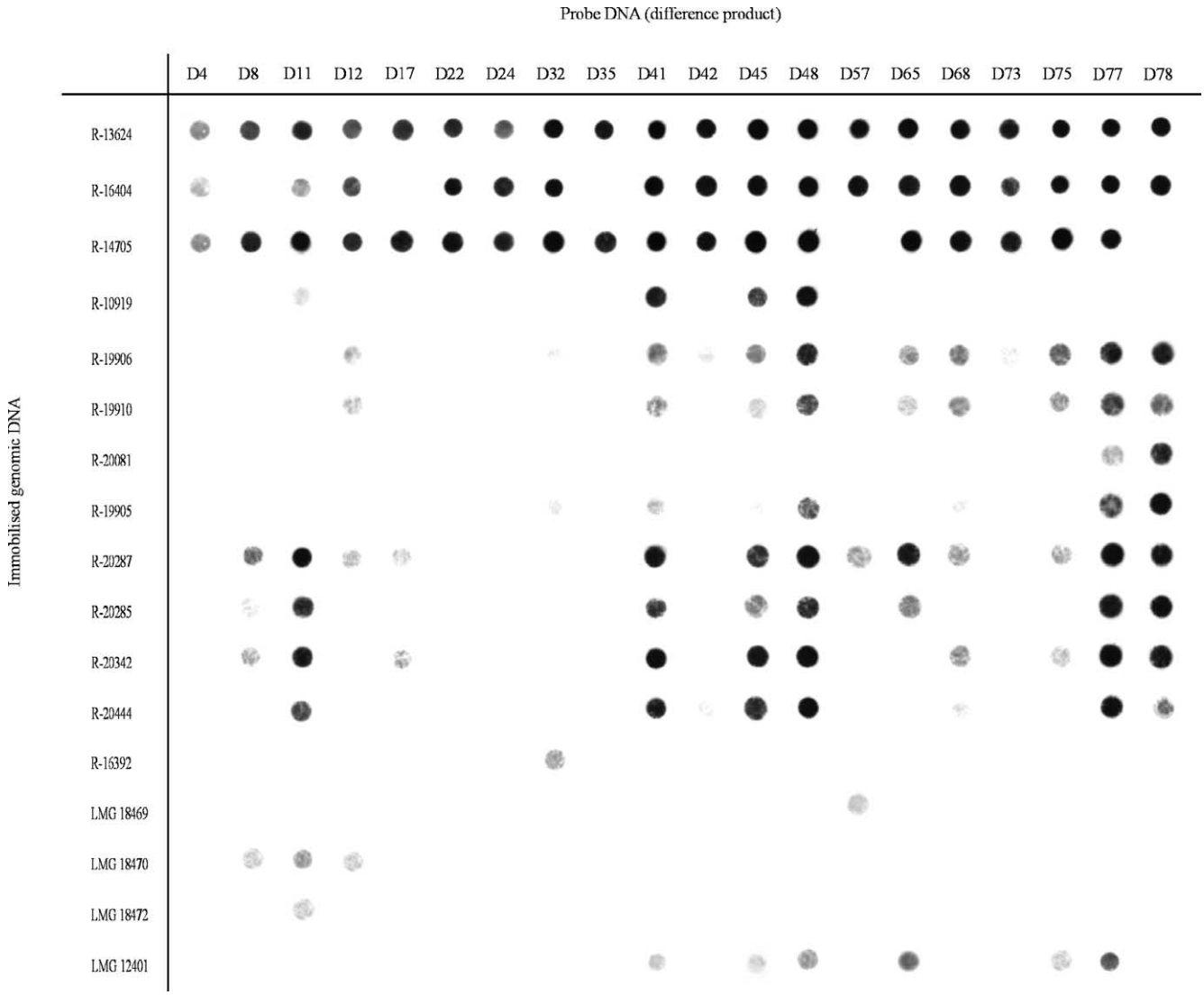

Fig. 3. Dot blot hybridisation of subtractive hybridisation difference products, using *B. fumarioli* strain R-13624 as tester and LMG 19448^{T} as driver, with immobilised genomic DNA of a selection of strains. Strains that did not give any signal were omitted from the figure.

from a geothermal Antarctic environment and from gelatine. Results are shown in Fig. 3. Strains that did not give a signal with any of the difference products, were omitted from the figure. Regarding the Antarctic isolates, only strains LMG 18469, LMG 18470 and LMG 18472 gave signals with a few of the difference products; all of these signals were very weak, however. The signals obtained for the *B. fumarioli* isolates from gelatine were strain-dependent, and none of the difference products hybridised to every gelatine isolate. Concerning the other species, only *B. coagulans* strain LMG 12401 showed some signals, though they were mostly very weak.

Results for the 20 difference products that were sequenced are summarised in Table 2. Nine difference products did not give a match with known proteins. Five difference products showed sequence similarities with a transposase. Two products showed sequence similarities with a nitrate reductase. Other products showed similarities with an integrase, a transcriptional regulator, a transport system permease protein or an endonuclease.

4. Discussion

Genomic and phenotypic techniques illustrate the very close relationship between the *B. fumarioli* isolates from geothermal soils and gelatine. Although both habitats show similarities in their temperature and pH conditions, nutrient availabilities are considered to be very different. While gelatine is a pure animal protein, the organic content of the Antarctic geothermal soils is much lower [4–7]. A striking parallel with these isolations of *B. fumarioli* from such disparate and distant habitats is that of the isolation of *Paenibacillus cookii* from geothermal soil of Candlemas Island and from a European gelatine production plant [19].

Table 2
Results of sequencing of difference products retrieved after SSH

Difference product	Sequence length (bp)	Sequence output	Organism	E-value
D4	579	Hypothetical protein	*Bacillus subtilis*	1.9E-40
D8	292	Transposase	*Oceanobacillus iheyensis*	1.3E-8
D11	696	Hypothetical protein	*'Methanosarcina acetivorans'*	4.1E-7
D12	697	Transposase	*Geobacillus slearothermophilus*	1.5E-20
D17	977	Integrase/recombinase	*Bacillus subtilis*	6.6E-11
D22	900	Hypothetical protein	*Deinococcus radiodurans*	2.4E-14
D24	700	Transcriptional regulator	*Bacillus halodurans*	3.3E-57
D32	781	Hypothetical protein	*Bacillus anthracis*	1.6E-21
D35	516	Hypothetical protein	*Oceanobacillus iheyensis*	0.0001
D41	692	Transposase	*Mycobacterium tuberculosis*	3.1E-7
D42	862	Nitrate reductase	*Bacillus anthracis*	2.2E-9
D45	429	No significant identification		
D48	837	Transposase	*Mycobacterium tuberculosis*	1.6E-22
D57	1071	Hypothetical protein	*Lactobacillus gasseri*	5.00E-80
D65	666	Transposase	*Bacillus halodurans*	7.6E-85
D68	1051	Hypothetical protein	*Clostridium acetobutylicum*	1.00E-21
D73	405	Transport system permease protein	*Clostridium tetani*	6.6E-22
D75	977	Hypothetical protein	*Methanococcus jannaschii*	1.3E-8
D77	564	Nitrate reductase	*Bacillus subtilis*	2.5E-82
D78	*S*04	Endonuclease BsuFI	*Bacillus subtilis*	3.2E-93

The presence of an abundant protein band in isolates from semi-final gelatine batches, a band that was absent or less pronounced in Antarctic isolates, and in the isolate from a crude gelatine extract during production, is of interest. N-terminal sequencing of this protein band revealed significant homology with the phage shock protein A (pspA) of *B. cereus*. The phage shock protein operon consists of at least four genes, designated *pspABCE*. It has been suggested that *psp* genes play a role in competition with other bacteria for survival under energy-limited conditions [28]. The exact physiological role of the individual psp proteins is still not known. The *pspA* gene encodes a 26 kDa protein, which is peripherally associated with the cytoplasmic membrane and has a negative regulatory role in expression of the operon [1]. It is abundantly expressed upon induction and under extreme stress conditions, and can become one of the most highly expressed bacterial proteins [28]. The overexpression of a protein sharing a great deal of homology with pspA in the gelatine isolates may be consistent with the harsh conditions that the organisms would encounter during the gelatine production process, and the absence of this dominant band when culturing in broth for a shorter period may reflect less stressful conditions. Further analysis of the expression of this protein during gelatine production is needed to clarify this issue.

Although most of the retrieved difference products after subtractive hybridisation did not hybridise with Antarctic isolates, and thus seem specific for gelatine isolates, they did not hybridise with all of the gelatine isolates and so they would not appear to have essential roles for survival in gelatine. Some of these corresponding genes may have roles in the colonisation of gelatine, and the pattern of signals retrieved for different gelatine isolates may thus reflect a phenomenon of adaptive evolution linked to this habitat. Since most difference products did not give a match with known proteins, future sequencing work may throw some light on this matter. The observation of 6 transposases and an integrase may indicate significant roles for mobile elements in adaptation to different environments and colonisation of these habitats.

In this paper, we have illustrated the very close relationship between *Bacillus fumarioli* isolates from geographically widely-separated habitats that share similar temperature and pH conditions, but have substantially different organic loads. Differences in genome content and gene expression were observed between gelatine and Antarctic isolates, and this may indicate that adaptive evolution has occurred in the gelatine habitat.

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References

[1] H. Adams, W. Teertstra, M. Koster, J. Tommassen, PspE (phage-shock protein E) of *Escherichia coli* is a rhodanese, FEBS Lett. 518 (2002) 173–176.

[2] P.G. Agron, R.L. Walker, H. Kinde, S.J. Sawyer, D.C. Hayes, J. Wollard, G.L. Andersen, Identification by subtractive hybridization of sequences specific for *Salmonella enterica* serovar *Enteritidis*, Appl. Environ. Microbiol. 67 (2001) 4984–4991.
[3] N.S. Akopyants, A. Fradkov, L. Diatchenko, J.E. Hill, P.D. Siebert, S.A. Lukyanov, E.D. Sverdlov, D.E. Berg, PCR-based subtractive hybridization and differences in gene content among strains of *Helicobacter pylori*, Proc. Nat. Acad. Sci. USA 95 (1998) 13108–13113.
[4] R. Bargagli, P.A. Broady, D.W.H. Walton, Preliminary investigation of the thermal biosystem of Mount Rittmann fumaroles (Northern Victoria Land, Antarctica), Antarctic Sci. 8 (1996) 121–126.
[5] A. Bonaccorso, M. Maione, P.C. Pertusati, E. Privitera, C.A. Ricci, Fumarolic activity at Mount Rittmann volcano (Northern Victoria Land, Antarctica), Mem. Soc. Geol. It. 46 (1991) 453–456.
[6] P. Broady, D. Given, L. Greenfield, K. Thompson, The biota and environment of fumaroles on Mt. Melbourne, Northern Victoria Land, Polar. Biol. 7 (1987) 97–113.
[7] P.A. Broady, Soils heated by volcanism, in: E.I. Friedman (Ed.), Antarctic Microbiology, Wiley–Liss, New York, 1993, pp. 413–432.
[8] E. De Clerck, P. De Vos, Study of the bacterial load in a gelatine production process, focussed on *Bacillus* and related endosporeforming genera, Syst. Appl. Microbiol. 25 (2002) 611–618.
[9] E. De Clerck, T. Vanhoutte, T. Hebb, J. Geerinck, J. Devos, P. De Vos, Isolation, characterisation and identification of bacterial contaminants in semi-final gelatine extracts, Appl. Environ. Microbiol., in press.
[10] E. De Clerck, M. Rodriguez-Diaz, T. Vanhoutte, J. Heyrman, N.A. Logan, P. De Vos, *Anoxybacillus contaminans* sp. nov. and *Bacillus gelatini* sp. nov., isolated from contaminated gelatine batches, Int. J. Syst. Evol. Microbiol., in press.
[11] B. Devreese, F. Vanrobaeys, J. Van Beeumen, Automated nanoflow liquid chromatography/tandem mass spectrometric identification of proteins from *Shewanella putrefaciens* separated by two-dimensional polyacrylamide gel electrophoresis, Rapid Commun. Mass Spectrom. 15 (2001) 50–56.
[12] T. Ezaki, Y. Hashimoto, E. Yabuuchi, Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains, Int. J. Syst. Bacteriol. 39 (1989) 224–229.
[13] W. Gish, D.J. States, Identification of protein coding regions by database similarity search, Nat. Genet. 3 (1993) 266–272.
[14] L.M.F. Herman, M.J.M. Vaerewijck, R.J.B. Moermans, G.M.A.V.J. Waes, Identification and detection of *Bacillus sporothermodurans* spores in 1, 10, and 100 milliliters of raw milk by PCR, Appl. Environ. Microbiol. 63 (1997) 3139–3143.
[15] M. Heyndrickx, L. Vauterin, P. Vandamme, K. Kersters, P. De Vos, Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy, J. Microbiol. Meth. 26 (1996) 247–259.
[16] J. Heyrman, J. Swings, 16S rDNA sequence analysis of bacterial isolates from biodeteriorated murals paintings in the Servilia tomb (necropolis of Carmona, Seville, Spain), Syst. Appl. Microbiol. 24 (2001) 417–422.
[17] P. Jeno, T. Mini, S. Moes, E. Hintermann, M. Horst, Internal sequences from proteins digested in polyacrylamide gels, Anal. Biochem. 224 (1995) 75–82.
[18] N.A. Logan, L. Lebbe, B. Hoste, J. Goris, G. Forsyth, M. Heyndrickx, B.L. Murray, N. Syme, D.D. Wynn-Williams, P. De Vos, Aerobic endospore forming bacteria from geothermal environments in Northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich Archipelago, with the proposal of *Bacillus fumarioli* sp. nov., Int. J. Syst. Evol. Microbiol. 50 (2000) 1741–1753.
[19] N.A. Logan, E. De Clerck, L. Lebbe, A. Verhelst, J. Goris, G. Forsyth, M. Rodriguez-Diaz, M. Heyndrickx, P. De Vos, *Paenibacillus cineris* sp. nov. and *Paenibacillus cookii* sp. nov. from Antarctic volcanic soils and a gelatine processing plant. Int. J. Syst. Evol. Microbiol., in press.
[20] A.J. Mackey, T.A. Haystead, W.R. Pearson, Getting more from less: Algorithms for rapid protein identification with multiple short peptide sequences, Mol. Cell Proteom. 1 (2002) 139–147.
[21] W.R. Pearson, D.J. Lipman, Improved tools for biological sequence comparison, Proc. Nat. Acad. Sci. USA 85 (1988) 2444–2448.
[22] D.G. Pitcher, N.A. Saunders, R.J. Owen, Rapid extraction of bacterial genomic DNA with guanidium thiocyanate, Lett. Appl. Microbiol. 8 (1989) 151–156.
[23] B. Pot, P. Vandamme, K. Kersters, Analysis of electrophoretic whole-organism protein fingerprints, in: M. Goodfellow, A.G. O'Donnell (Eds.), Chemical Methods in Prokaryotic Systematics, Wiley, Chichester, 1994, pp. 493–521.
[24] L. Radnedge, P.G. Agron, K.K. Hill, P.J. Jackson, L.O. Ticknor, P. Keim, G.L. Andersen, Genome differences that distinguish *Bacillus anthracis* from *Bacillus cereus* and *Bacillus thuringiensis*, Appl. Environ. Microbiol. 69 (2003) 2755–2764.
[25] J. Sambrook, E.F. Fritsch, T. Maniatis (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 1989.
[26] E. Stackebrandt, W. Frederiksen, G.M. Garrity, P.A.D. Grimont, P. Kämpfer, M.C.J. Maiden, X. Nesme, R. Rossello-Mora, J. Swings, H.G. Trüper, L. Vauterin, A.C. Ward, W.B. Whitman, Report of the ad hoc committee for the reevaluation of the species definition in bacteriology, Int. J. Syst. Evol. Microbiol. 52 (2002) 1043–1047.
[27] J. Versalovic, M. Schneider, F.J. De Bruijn, J.R. Lupski, Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction, Meth. Mol. Cell. Biol. 5 (1994) 25–40.
[28] L. Weiner, P. Model, Role of an *Escherichia coli* stress-response operon in stationary-phase survival, Proc. Natl. Acad. Sci. USA 91 (1994) 2191–2195.
[29] A. Willems, F. Doignon-Bourcier, J. Goris, R. Coopman, P. De Lajudie, P. De Vos, M. Gillis, DNA–DNA hybridization study of *Bradyrhizobium* strains, Int. J. Syst. Evol. Microbiol. 51 (2001) 1315–1322.
[30] P. Yang, L. Vauterin, M. Vancanneyt, J. Swings, K. Kersters, Application of fatty acid methyl esters for the taxonomic analysis of the genus *Xanthomonas*, Syst. Appl. Microbiol. 16 (1993) 47–71.