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Bacteriology

Characterization of four *Escherichia albertii* isolates collected from animals living in Antarctica and Patagonia

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ABSTRACT. *Escherichia albertii* is a recently discovered species with a limited number of well characterized strains. The aim of this study was to characterize four of the *E. albertii* strains, which were among 41 identified *Escherichia* strains isolated from the feces of living animals on James Ross Island, Antarctica, and Isla Magdalena, Patagonia. Sequencing of 16S rDNA, automated ribotyping, and rep-PCR were used to identify the four *E. albertii* isolates. Phylogenetic analyses based on multi-locus sequence typing showed these isolates to be genetically most similar to the members of *E. albertii* phylogroup G3. These isolates encoded several virulence factors including those, which are characteristic of *E. albertii* (cytolethal distending toxin and intimin) as well as bacteriocin determinants that typically have a very low prevalence in *E. coli* strains (D, E7). Moreover, *E. albertii* protein extracts caused cell cycle arrest in human cell line A375, probably because of cytolethal distending toxin activity.

KEY WORDS: Antarctica, bacteriocins, cytolethal distending toxin, *Escherichia albertii*

Strains of *Escherichia albertii* were originally classified as *Hafnia alvei*-like stains, which were isolated from human stool specimens in the early 1990s and were suspected of being causative agents of diarrhea [1]. Based on DNA-DNA hybridization analyses, the *Hafnia alvei*-like strains were reclassified as a new species–*Escherichia albertii* [15]. Even though *E. albertii* is a relative of *E. coli,* it is phylogenetically distinct from other *Escherichia* species [16, 23, 37, 56].

In general, *Escherichia albertii* is a potential human pathogen with a limited number of characterized isolates. Since 2003, when the new *E. albertii* species was proposed, only 282 isolates have been described and more than half of them (n=144) were associated with diarrhea and/or gastroenteritis in humans [2, 3, 6, 9, 10, 13–15, 17, 20, 21, 24, 25, 31–35, 37, 42, 52, 55–57]. *E. albertii* has also been shown to be responsible for epidemic mortality among birds [33]. It has been isolated from pig, cat, environmental samples, and found as a contaminant of various raw meats [14, 23, 33, 36, 42, 57].

However, the real frequency of *E. albertii* in clinical samples remains unclear. *E. albertii* ferments D-mannitol but not D-xylose and does not produce indole. Because strains of *E. albertii* are not included in the databases of majority of commercial diagnostic tests, *E. albertii* strains are often misidentified as *Hafnia*, *Salmonella*, *Escherichia coli* or *Yersinia ruckeri* [reviewed in 59]. *E. albertii* possesses a specific set of virulence genes including intimin and the *eae*-encoded outer membrane protein, and thus many strains of *E. albertii* might be misidentified as enterohemorrhagic (EHEC) or enteropathogenic *E. coli* (EPEC) [36, 58]. However, compared to the EPEC (and not EHEC), *E. albertii* often encodes cytolethal distending toxin that causes cell cycle arrest in eukaryotic cells, which leads to cell distention and cell death [4, 8, 16, 46, 60].

Escherichia species, as well as many other bacterial species, are known to produce antimicrobial agents called bacteriocins. In the genus *Escherichia*, bacteriocins include colicins and microcins. Although the exact role of bacteriocin production in bacterial populations remains unclear, there is increasing evidence of the role of bacteriocins in bacterial virulence [28, 49], in probiotic phenotype of *E. coli* strains [50], and in colonization of the gastrointestinal tract [11].

Recently, we isolated *E. albertii* from feces of Antarctic animals and published preliminary data regarding characterization [44]. In this work, we have analyzed a larger sample set of isolates and characterized, in greater detail, four isolates of *E. albertii* from

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animals living in Antarctica and Patagonia.

MATERIALS AND METHODS

Samples collection

Fecal specimens and rectal or cloacal swabs from seals (mostly *Leptonychotes weddelli*), penguins (*Pygoscelis adeliae*, *P. papua*, *Spheniscus magellanicus*), skuas (*Stercorarius maccormicki*), and gulls (*Larus dominicanus*) were collected on James Ross Island and Seymour Island, Antarctica, and Isla Magdalena, Patagonia, during austral summers in 2013 and 2014. This sampling was a part of Cultivable Fecal Bacteria Communities study, which was part of the CzechPolar project. The samples were collected using swab/transport tube system E-Swabs (Dispolab, Brno, Czech Republic), kept at 4°C and transported to the Czech Republic for further analyses.

Reference and type strains used in this study were obtained from the Czech Collection of Microorganisms (Masaryk University, Brno, Czech Republic).

Isolation and biochemical identification of strains

Samples were cultivated on Columbia blood agar and sub-cultivated on Columbia blood agar supplemented with 7% sheep blood (Bio-Rad, Praha, Czech Republic) at 30°C for 24 hr. Different colonies revealing the macroscopic morphology typical for enteric bacteria were randomly picked up from smears of faecal samples grown on Endo agar and MacConkey agar. Two commercial identification kits, ENTEROtest 24 (Erba Lachema, Brno, Czech Republic) and Biolog Identification System, GN2 MicroPlate (Biolog, Hayward, CA, U.S.A.), were used (according to the manufacturers' instructions). The list of 41 identified *Escherichia* isolates is shown in Table S1.

16S rDNA and multi-locus sequence typing (MLST) analyses

The 16S rRNA gene, from all 41 *Escherichia* isolates, was amplified as described previously [41]. Additionally, a MLST analysis of *E. albertii* isolates was performed by amplifying and sequencing six housekeeping genes, *asp*C, clpX, *fad*D, *icd*A, *lys*P and *mdh*, using a previously described protocol [16, 33]. All PCR products were sequenced using the Sanger method (Elisabeth® Pharmacon, Brno, Czech Republic and GATC Biotech AG, Konstanz, Germany). Sequences were analyzed using Lasergene software (DNASTAR v.7.1.0., Madison, WI, U.S.A.).

Automated ribotyping, repetitive element PCR (rep-PCR) and pulse-field gel electrophoresis (PFGE)

All 41 isolates, which had been previously classified to the *Escherichia* genus by commercial identification kits, were used for automated ribotyping with the *Eco*RI restriction enzyme and rep-PCR fingerprinting as described previously [53, 54]. PFGE was performed using the *Xba*I enzyme and the standardized PulseNet protocol for *E. coli* O157:H7 (http://www.pulsenetinternational.org/protocols). The dendrograms of automated ribotyping and rep-PCR were constructed with Pearson's correlation coefficient and analysis of PFGE macrorestriction patterns was done with Jaccard similarity coefficient. All dendrograms were constructed with UPGMA clustering method (BioNumerics v. 7.5, Applied Maths, Sint-Martens-Latem, Belgium).

PCR detection of virulence markers

Our set of *E. albertii* isolates and the *E. albertii* type strain, CCM 7160^T, were tested for the presence of 21 virulence markers, which are typical for the *Enterobacteriaceae.* This included genes encoding virulence factors found in enteroaggregative *E. coli* (pCVD432), enterotoxigenic *E. coli* (heat-labile enterotoxin (*lt*) and heat-stable enterotoxin (*st*)), enteroinvasive *E. coli* (invasivity antigen (*ial*) and invasion plasmid antigen H (*ipa*H)), enteropathogenic *E. coli* (colibactin, bundle-forming pillus A (*bfp*A) and intimin (*eae*A)), enterohemorrhagic *E. coli* (enterohemolysin (*ehly*) and Shiga toxins (*stx1*, *stx2*)), diffusely adherent *E. coli* (afimbrial adhesin (*afa*)) and other genes coding virulence factors (α-hemolysin (*α-hly*), aerobactin (*aer*), B unit of the cytolethal distending toxin (*cdt*B), cytotoxic necrosis factor 1 (*cnf*1), type I fimbriae (*fim*A), aerobactin iron transport system (*iuc*C), P-fimbriae (*pap*), S-fimbriae (*sfa*) and uropathogenic specific protein (*usp*)).The primer pair sequences, PCR product lengths and PCR protocols were previously described [5, 19, 22, 24, 26, 38, 39, 43, 59].

Bacteriocin production and identification of bacteriocin types

Detection of bacteriocin production was performed phenotypically as described previously [49] using bacteriocin indicator strains *E. coli* K12 - Row, *E. coli* C6 (φ), *Shigella sonnei* 17, *E. coli* P400, *E. coli* S40 and *E. coli* 5K. For characterization of individual bacteriocin types, PCR amplifications of bacteriocin determinants were performed as described previously [28]. This screening detected most of the known colicins and microcin determinants (n=32). The bacteriocin 'control' producers, used for PCR detection of bacteriocin genes, were previously described in a detail [27, 49].

Whole protein extraction

E. albertii strains examined in this study, *E. albertii* type strain CCM 7160^T, and *E. coli* CCM 4825 (K12) were grown overnight at 37°C in 3 m*l* of TY broth (Himedia, Mumbai, India) and centrifugated at 5,000 g for 10 min. Total bacterial proteins from 1 g of wet bacterial biomass were extracted using B-PER Complete bacterial protein extraction reagent (Thermo Fisher Scientific, Waltham, MA, U.S.A.) according to the manufacturer's recommendations. Final suspension was filtered using 0.45 μm bacterial

Table 1. Characteristics of *Escherichia albertii* isolates analyzed in this study

Isolate	Source	Similarity level −16S rDNA (%)[a)]	MLST[b)]	Detected virulence factor determinants	Detected bacteriocin determinants
P4652	Seal, Antarctica	99.6	99.99% similarity to *E. albertii* in *Corvus* sp.	*eae, cdt*B, *iuc*C	D, E7
P4653	Seal, Antarctica	99.6	99.99% similarity to *E. albertii* in *Corvus* sp.	*eae, cdt*B,*iuc*C	D, E7
P4740	Seal, Antarctica	99.6	99.99% similarity to *E. albertii* in *Corvus* sp.	*eae, cdt*B, *fim*A	D, E7
P5661	Penguin, Patagonia	99.7	Identical to *E. albertii* in *Egretta garzetta*	*eae, cdt*B, *aer, fimA, ipaH*	B, M

a) Similarity was calculated according to the number of single nucleotide variants in 16S rDNA sequences of *E. albertii* isolates compared to the 16S rDNA sequence of *E. albertii* type strain CCM 7160^T (according to GenBank Accession No. AJ508775 in coordinates 58-1409). b) MLST − Multi Locus Sequence Typing based on six concatenated housekeeping genes (*aspC, clpX, fadD, icdA, lysP* and *mdh*). MLST data were compared to the data published in [37].

filters (Nalgene filters, Thermo Fisher Scientific, Waltham, MA, U.S.A.).

Cultivation of cell line A375

The human malignant melanoma cancer cell line, A375 (European Collection of Cell Cultures, Salisbury, U.K.), was used. Cells were grown in RPMI 1640 medium (HyClone Laboratories, Inc., South Logan, UT, U.S.A.) supplemented with 2 mmol l^{-1} L-glutamine (PAA Laboratories, Pasching, Austria), 10% fetal calf serum, penicillin (final concentration of 100 IU ml^{-1}), and streptomycin (final concentration of 100 μg ml^{-1}). Cells were incubated at 37°C under 5% CO_2 in a high-humidity-atmosphere and subcultured three times per week.

Cell cycle analysis

A375 cells were plated in concentration of 7 × 10^4 cells per m*l* and cultivated for 24 and 48 hr. Subsequently, A375 cells were treated with protein extracts (final dilution 1:1,000) for 24 and 48 hr. Both detached and attached cells were harvested into ice-cold PBS, fixed and processed as described previously [47]. A Cytomics FC 500 flow cytometry system (Beckman Coulter, Inc., Prague, Czech Republic) was used for cell cycle analysis. The cell cycle phases were determined using a Multicycle AV for Windows software (Phoenix Flow system, San Diego, CA, U.S.A.).

RESULTS

Identification of Escherichia isolates

As a part of Cultivable Fecal Bacteria Communities study project, we collected, during 2013–2014, 83 swabs of fecal specimens of randomly sampled animals from James Ross (23 seals, 14 penguins and 20 undetermined feces) and Seymour Islands (10 seals and 14 penguins), Antarctica, and Isla Magdalena, Patagonia (two penguins). From eight phenotypically identified genera of enteric bacteria (*Aeromonas, Citrobacter, Edwardsiella, Enterobactre, Escherichia, Leclercia, Raoultella and Serratia*), 41 isolates were classified as members of the *Escherichia* genus using two commercial identification kits, ENTEROtest 24 and Biolog GN2 MicroPlate system. This set of *Escherichia* strains was isolated from perianal or cloacal smears of seals (n=14), penguins (n=13), skuas (n=8), and gulls (n=2), and from the environment (n=4). Samples were collected on James Ross Island (n=25), Seymour Island (n=13), Antarctica, and Isla Magdalena, Patagonia (n=3) (Table S1). In addition to the 37 isolates, which were classified as *E. coli* species using both commercial biochemical kits, four isolates were classified as "atypical/inactive" *E. coli* (9.7%). Compared to the other *E. coli* species, the four isolates were beta-glucuronidase negative and unable to ferment sorbitol and melibiose (data not shown).

The 16S rDNA region (1,352 out of 1,494 bp) in all 41 *Escherichia* isolates was analyzed. While 37 isolates showed 99.5–99.8% nucleotide sequence similarity to the 16S rDNA sequence of *E. coli* (*E. coli* type strain; GenBank Accession No. X80725), the 16S rDNA sequences of the four above mentioned isolates, previously characterized using commercial tests as atypical/inactive *E. coli* (P4652, P4653, P4740 and P5661), showed the greatest similarity (99.6–99.7%) to the 16S rDNA sequence of *E. albertii* (*E. albertii* type strain; GenBank Accession No. AJ508775). Three of the *E. albertii* isolates were obtained from seal feces (James Ross Island, Antarctica) and one *E. albertii* isolate came from the feces of a penguin (Isla Magdalena, Patagonia) (Table 1).

To confirm the classification of *E. albertii* based on 16S rDNA sequencing, two DNA fingerprinting techniques, automated ribotyping and rep-PCR were used to show the differences between the isolates and their similarity to related *Escherichia* spp. The results of both methods clearly grouped isolates P4652, P4653, P4740, and P5661 with the *E. albertii* CCM 7160^T type strain (Fig. 1). Moreover, PFGE of *Xba*I restriction fragments revealed identical fingerprints for isolates P4652 and P4653, suggesting that these two isolates are representatives of the same clone (Fig. 2). The P4652 isolate was deposited in the Czech Collection of Microorganisms (Brno, Czech Republic), under accessional number CCM 8505, as a representative of *E. albertii* strains isolated from seals in Antarctica.

Comparison of Antarctic isolates to previously characterized E. albertii isolates

Based on a genome-wide analysis of 34 *E. albertii* strains isolated from 3 different geographic areas (Japan, Germany, and Brazil) and from 3 different hosts (humans, birds, and cats), *E. albertii* can be further divided into five different phylogroups (G1–G5), as recently described [37]. We compared six concatenated housekeeping gene sequences from *E. albertii* isolates examined

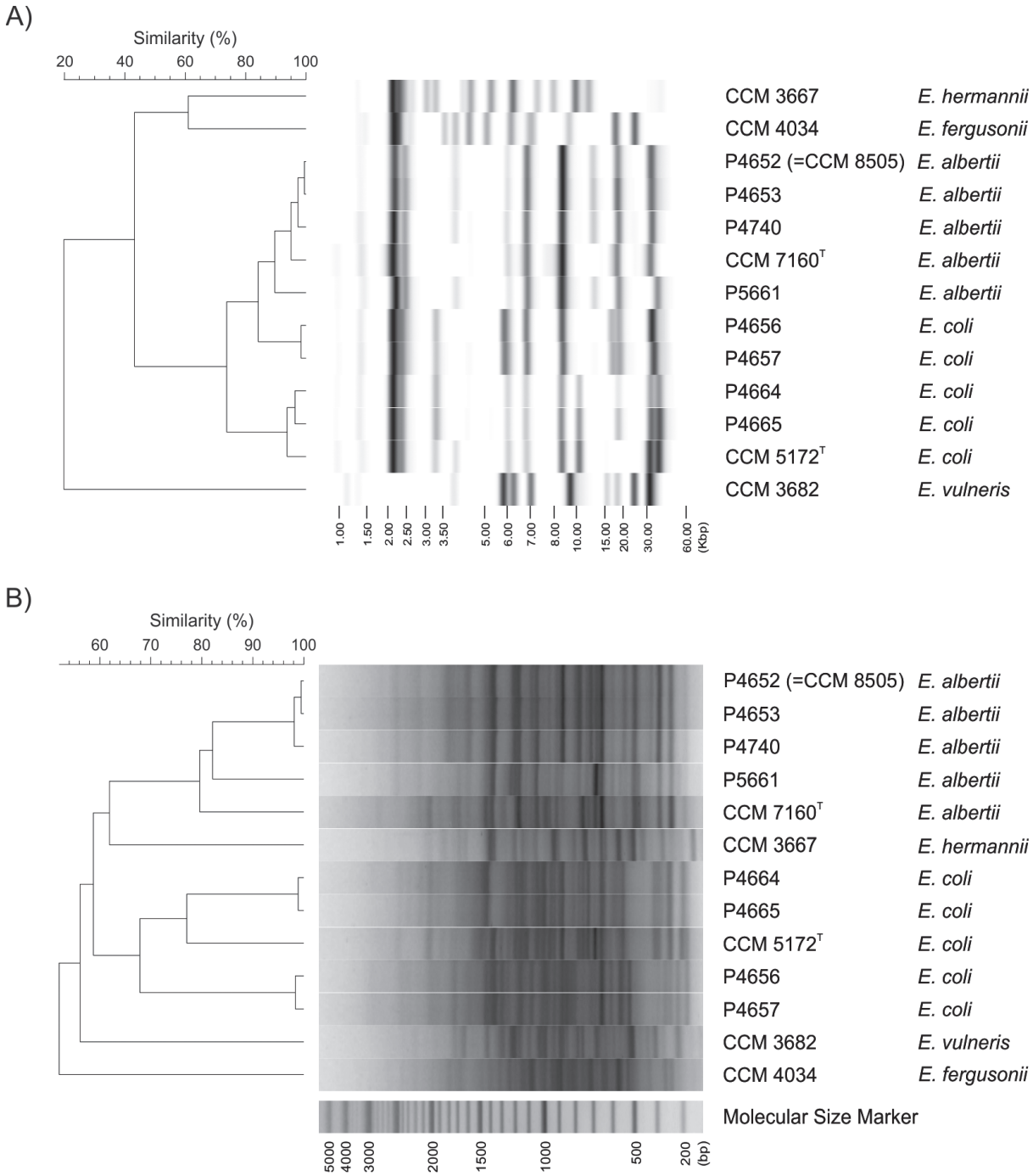

Fig. 1. Dendrograms based on cluster analysis of A) ribotype profiles and B) rep-PCR fingerprints. Four strains previously identified as *E. albertii* by 16S rDNA analysis (P4661, P4652, P4653 and P4740) were used as well as strain CCM 7160^T (type strain of *E. albertii*), strain CCM 5172^T (type strain of *E. coli*), four randomly selected strains examined in this study previously identified as *E. coli* by 16S rDNA analysis (P4656, P4657, P4664 and P4665; Table 1) and *E. vulneris*, *E. hermanni* and *E. fergusonii* reference strains as outgroups.

in this study (with a total length of 2,040 bp) to corresponding sequences present in the 34 draft genomes examined in [37]. While penguin isolate P5661 was completely identical to the silver heron isolate (*Egretta garzetta*) sampled in Japan (NIAH_Bird_8, GeneBank Accession No. BBVQ01000001-BBVQ01000167), seal isolates P4652, P4653 and P4740 were most similar (99.99% of sequence identity) to a raven isolate (*Corvus* sp.) found in Japan (E2675, GeneBank Accession No. BBVT01000001-BBVT01000119). All Antarctic isolates were genetically most similar to the members of phylogroup G3 (99.99% of sequence identity and more) and shared less than 99% of sequence identity with samples from other phylogroups.

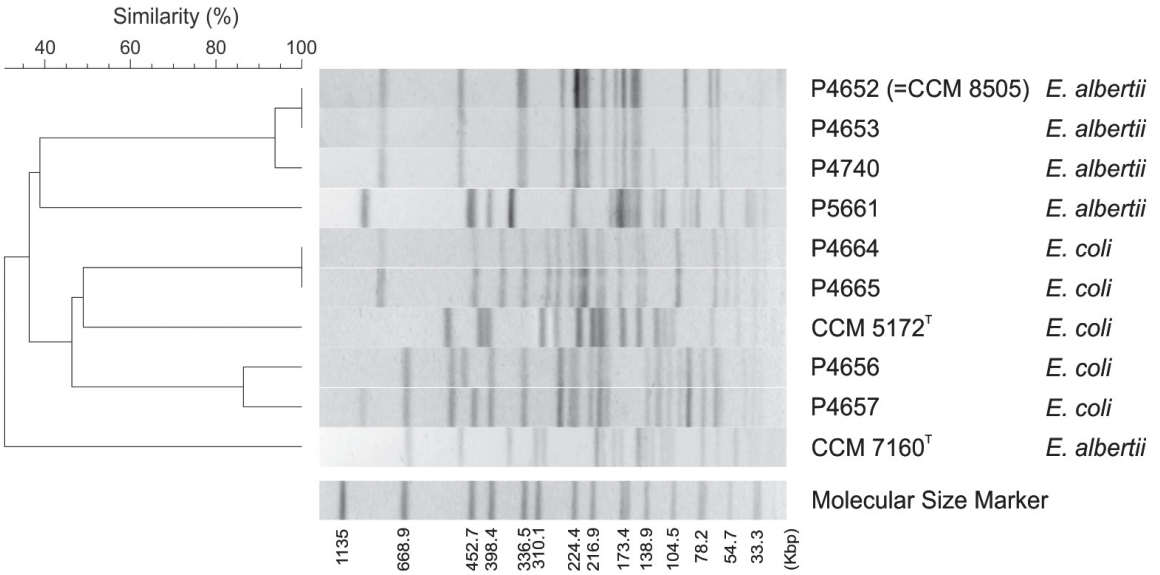

Fig. 2. PFGE dendrogram based on cluster analysis of macro-restriction patterns obtained from investigated *E. albertii* and *E. coli* strains. Four *E. albertii* isolates (P4661, P4652, P4653 and P4740) were used as well as strain CCM 7160^T (type strain of *E. albertii*), strain CCM 5172^T (type strain of *E. coli*), and four randomly selected strains examined in this study previously identified as *E. coli* by 16S rDNA analysis (P4656, P4657, P4664 and P4665; Table 1).

Other characteristics of E. albertii identified in this study

Detection of virulence determinants: Out of 21 tested virulence factor determinants (see Material and Methods section), all isolates were PCR positive for subunit B of cytolethal distending toxin (CDT, *cdt*B) and intimin (*eae*). In addition, the aerobactin determinant (*aer*) was found in P5661, type I fimbriae (*fim*A) in P4740 and P5661, invasion plasmid antigen H (*ipa*H) in P5661, and the aerobactin iron transport system (*iuc*C) in P4652, P4653 and P4740 (Table 1).

Production of antimicrobial agents: While *Escherichia albertii* type strain CCM 7160^T did not produced bacteriocins or phages, Antarctic and Patagonian *E. albertii* isolates inhibited the growth of indicator strains *E. coli* and *S. sonnei*. Using PCR screening, the presence of 32 determinants of known bacteriocin types (i.e. 25 colicin and 7 microcin determinants) was screened and four different bacteriocin types were identified. While colicin D and E7 determinants were found in *E. albertii* isolated from seals in Antarctica, the penguin isolate from Patagonia contained determinants encoding colicins B and M (Table 1).

Analyses of bacterial extracts activity on human cells: Since we detected DNA sequences encoding CDT in all *E. albertii* isolates, which has been found to affect the cell cycle [4, 8, 16, 40, 60], we analyzed cell cycles of human malignant melanoma A375 cells treated with protein extracts from *E. albertii* isolates (P4652, P4653, P4740 and P5661). Unlike the 'control' *E. coli* K12 protein extract, extracts from *E. albertii* isolates as well as lysates from positive controls (*E. albertii* type strain CCM 7160^T) caused accumulation of A375 cells in G2/M transition after 24 and 48 hr of treatment (Fig. 3).

DISCUSSION

Escherichia albertii is considered to be a food-borne pathogen of the human gastrointestinal tract that causes gastroenteritis around the word (Table 2). In this study, we identified three strains of *E. albertii* in feces of seals on James Ross Island, Antarctica, and one strain in feces of a penguin on Isla Magdalena, Patagonia, as *E. albertii* species, using three independent diagnostic tools−16S rDNA sequencing, ribotyping, and rep-PCR.

According to the analyses of six housekeeping genes (*asp*C, *clp*X, *fad*D, *icd*A, *lys*P and *mdh*), all examined *E. albertii* isolates were most similar to the members of phylogroup G3, described in [37]. However, until now, no associations of group G3 and bacterial hosts, with infectious symptoms and geographic areas, have been described [37].

E. albertii isolates are often classified as atypical enteropathogenic *E. coli* (aEPEC). This classification is based on the fact that both groups share a specific set of virulence genes including intimin encoding genes located within the LEE pathogenicity island [45, 58]. Unlike EPEC, *E. albertii* often encodes cytolethal distending toxin. The gene encoding subunit B of CDT (*cdt*B) was detected in all *E. albertii* isolates examined in this study. There are few other bacterial genotoxins; to date, in addition to CDTs, we have the uropathogenic-specific protein (*usp*) and colibactin [4]. Above mentioned results are in concordance with our results showing that Antarctic and Patagonia *E. albertii* whole protein extracts blocked cell cycles in the G2/M phase. Since we detected the *cdt*B subunit in the bacterial DNA and did not find any other DNA determinants coding known genotoxins (*usp* or colibactin), we propose that the cell cycle arrest was caused by CDTs. Except of *eae* and *cdt*B determinants, genes encoding other virulence factors including aerobactin synthesis (*aer*) and aerobactin iron transport system (*iuc*C), and fimbriae type I (*fim*A) were detected.

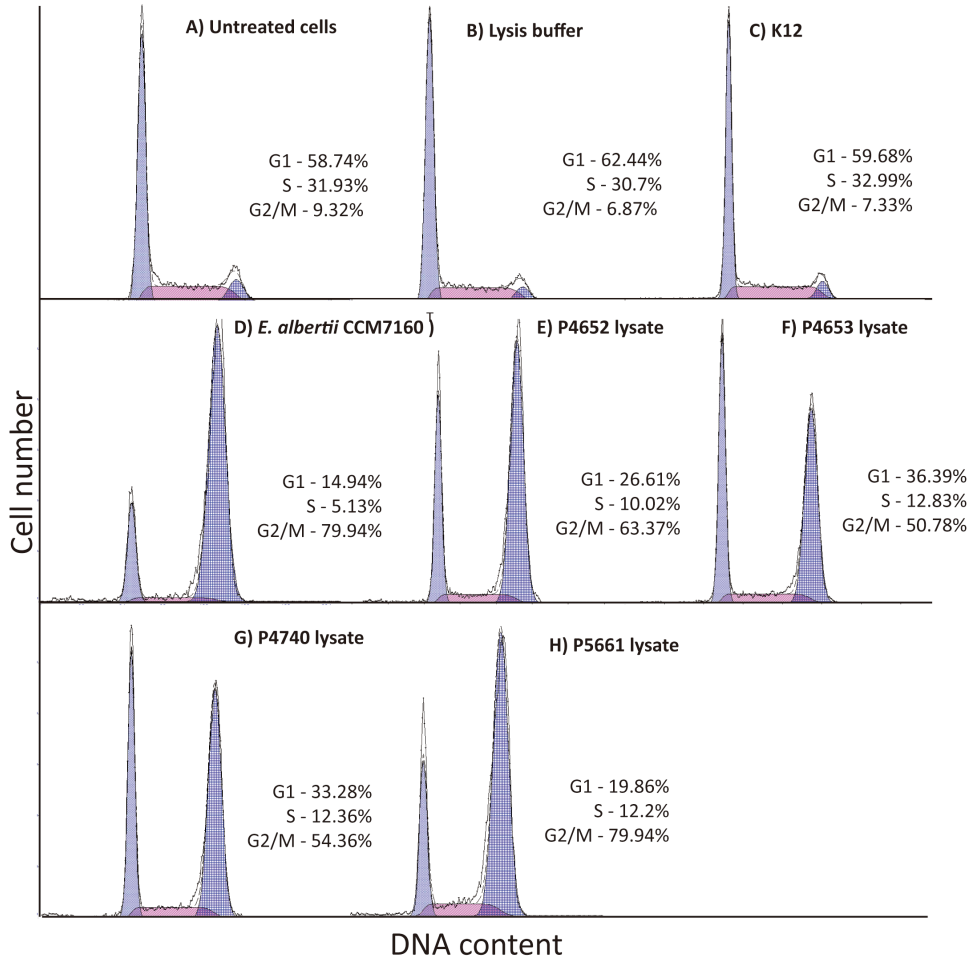

Fig. 3. Cell cycle analysis. All negative controls: non-treated cells (A), cells treated with bacterial lysis buffer (B) and lysate from *E. coli* K12 (non-producing strain; C), did not affect cell cycle of cell line A375, and showed normal distribution of phases. Whole protein extract from *E. albertii* type strain CCM 7160^T (CDT producing strain; D) [16] and extracts from PCR *cdt*B−positive *E. albertii* isolates (P4652, P4653, P4740 and P5661; E-H) caused cell cycle arrest and accumulation of A375 cells in G2/M transition. Only results after 48 hr of treatment are shown.

In addition, the large invasive plasmid (detected by presence of *ipa*H), typical for all virulent *Shigella* and enteroinvasive *E. coli* (EIEC) isolates, was found.

The frequency of bacteriocin determinants in pathogenic *E. coli* isolates is higher compared to commensal *E. coli* [12, 18, 27, 30, 48, 51]. In our study, all four identified *E. albertii* were found to be bacteriocin double-producers (Table 1), encoding colicins B, D, E7, and M. Interestingly, *E. albertii* type strain CCM 7160^T, which was also included in our bacteriocin detection assay, did not produce any tested antimicrobial substances. Production of colicin D is extremely rare in human *E. coli* strains. Occurrence of the colicin D determinant among extraintestinal pathogenic *E. coli* (ExPEC) isolates have revealed two colicin D producers among 407 examined strains (0.5%) [29] and similar analysis among fecal strains of *E. coli* only identified one colicin D determinant among 1,283 examined strains (0.08%) [29]. Similarly, frequency of E7 production in human *E. coli* was shown to be quite low (0.6–2.3%) [12, 27, 29]. On the other hand, colicins B and M are known to be encoded on large plasmids, which are present in many *E. coli*, including commensal (1.1–8.6%) [12, 28], uropathogenic (4.1–12.8%) [7, 49], and other ExPEC (5.0–11.7%) [27, 29]. However, the presence of rare colicin determinants among tested *E. albertii* isolates may correspond to the geographical differences between the tested *E. albertii* and *E. coli* strains [48].

Isolates P4652 and P4653, which came from the different seal feces collected on the same day at small beach nearby Lachman Cape, James Ross Island, Antarctica, shared the following four characteristics: (1) identical 16S rDNA sequences, (2) same sequences of six housekeeping genes (*asp*C, *clp*X, *fad*D, *icd*A, *lys*P and *mdh*), (3) identical ribotype, rep-PCR, and PFGE profiles, and (4) harbored same virulence factors and bacteriocin determinants (Table 1, Figs. 1 and 2). These facts imply that P4652 and P4653 are isolates of the same strain obtained from different faeces of one seal. Antarctic seal isolate P4740 had different ribotype, rep-PCR, and PFGE profiles compared to the P4652 and P4653 isolates. However, P4740 was shown to be phylogenetically related to the P4652 and P4653 isolates, since it possessed identical 16S rDNA sequences, identical sequences of six housekeeping genes, and the presence of identical bacteriocin determinants as the P4652 and P4653 isolates. On the other hand, isolate P5661 was found

Table 2. Strains of *E. albertii* published since 2003

No. of isolates	Host	Geographic area	Clinical impact	Year of isolation	Reference
5	Human	Bangladesh	Diarrhea	1990–1991	[15]
21	Human	Bangladesh	Diarrhea	1990–1993	[52]
1	Drinking water in hospital	Hungary	NA	2005	[9]
7	Birds	U.S.A.	Death	2005–2007	[33]
2	Birds	Canada	Healthy	2005	[33]
5	Birds	Scotland	Death	1998–2000	[20]
9	Birds	Australia	Healthy	2001–2002	[33]
3	Human	Guinea-Bissau	Healthy	1997	[56]
2	Human	U.S.A.	Diarrhea	NA	[33]
7	Birds	Australia	NA	NA	[57]
9	Birds	Korea	Healthy	2009–2010	[34]
6	Human	Japan	Gastroenteritis	2011	[35]
1	Environmental fresh water	Bangladesh	NA	2006	[42]
1	Human	Poland	Diarrhea	NA	[10]
6	Human	Japan	Diarrhea	2003	[2]
1	Swine	Japan	Healthy	2004	[14]
18	Environmental water	Canada	NA	2009	[25]
27	Chicken carcass	U.S.A.	NA	2009–2010	[21]
39	Human	Norway	Diarrhea	2008–2014	[6]
2	Chicken food	Japan	NA	2014	[24]
1	Raw chicken liver	Japan	NA	2013	[3]
14	Human	Japan, Germany, Brazil	Gastroenteritis	1993–2009	[35]
11	Bird	Japan	NA	1993–2009	[35]
1	Cat	Brazil	Healthy	2004	[31]
48	Human	NA	Diarrhea	1997–2007	[32]
30	Raw meat (duck, chicken, mutton)	China	NA	NA	[58]
3	Human	Japan	NA	2008–2009	[37]
1	Human	Australia	Febrile infection	NA	[17]
1	Human	Japan	Diarrhea	2008	[13]

NA; not available.

to be distantly related based on its 16S rDNA sequence, MLST data, ribotyping result, rep-PCR and PFGE profile as well as the presence of different virulence factors and bacteriocin determinants (Table 1 and Figs. 1 and 2). However, since P5661 was isolated from a penguin in Patagonia, the observed differences could simply reflect host and geographical differences.

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