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Genetic Relatedness of *Legionella longbeachae* Isolates from Human and Environmental Sources in Australia

JANICE A. LANSER,[1]* MARK ADAMS,[2] ROBYN DOYLE,[1] NORMA SANGSTER,[1] AND TREVOR W. STEELE[1]

Division of Clinical Microbiology, Institute of Medical and Veterinary Science, Frome Road,[1] and Evolutionary Biology Unit, South Australian Museum, North Terrace,[2] Adelaide, South Australia 5000, Australia

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The genetic relatedness of *Legionella longbeachae* isolated in Australia since 1987 was investigated by restriction fragment length polymorphism (RFLP) analysis and allozyme electrophoresis. Three radiolabeled probes were used in Southern hybridizations for the RFLP studies. They were *Escherichia coli* 16S and 23S rRNA and cloned fragments of *L. longbeachae* selected empirically from genomal banks in lambda and a cosmid. The legionellae included in the study comprised 11 *Legionella longbeachae* serogroup 1 organisms isolated from humans, 28 *L. longbeachae* serogroup 1 isolates from environmental sources, and 3 *L. longbeachae* serogroup 2 environmental isolates. These were compared with the American Type Culture Collection reference strains of both serogroups and some other related *Legionella* species. Results of allozyme and RFLP analysis showed that all the isolates from humans and all but three of the environmental *L. longbeachae* serogroup 1 isolates were closely related. They were also closely related to *L. longbeachae* serogroup 1 ATCC 33462. There was wider variation among the three *L. longbeachae* serogroup 2 environmental isolates. One of these was closely related to *L. longbeachae* serogroup 2 ATCC 33484. RFLP studies with the rRNA probe provided the most discrimination among isolates but did not distinguish between the two serogroups.

Legionella longbeachae was first recognized as a cause of pneumonia in 1981 (14). Since then several sporadic cases have been reported from Sweden (3), Germany (11), Denmark (20), and Canada (9). In May 1987, *L. longbeachae* serogroup 1 was isolated for the first time from a patient in Australia (10). Since then a further 29 confirmed cases of legionellosis caused by *L. longbeachae* serogroup 1 have been detected in South Australia. Twenty-three of these cases occurred in the 3-month period from October 1988 to January 1989. The organism was isolated from respiratory secretions from 11 patients. Subsequently, Steele et al. (19) isolated *L. longbeachae* serogroup 1 from commercial potting mixes and soil around plants in pots obtained from four patients. They were able to show that the organism survived for long periods in potting mixes and that such soils could be a possible source of infection. The restriction fragment length polymorphism (RFLP) studies performed to compare the *L. longbeachae* organisms isolated from patients and soils demonstrated that they were closely related.

The present report extends these studies to describe the genetic relatedness of *L. longbeachae* isolates from a number of environmental sources in Australia and those from patients. Three of the environmental isolates were *L. longbeachae* serogroup 2. All human isolates belonged to *L. longbeachae* serogroup 1.

Several methods have been shown to be useful for the genetic typing of bacteria. They include allozyme electrophoresis (17), monoclonal antibody typing (8), RFLP profiling (6, 21), outer membrane protein analysis (1), and plasmid profiling (2). We used allozyme electrophoresis and RFLP analysis to type the isolates and compare them with *L. longbeachae* serogroup 1 (ATCC 33462) and *L. longbeachae* serogroup 2 (ATCC 33484) and other closely related legionellae.

MATERIALS AND METHODS

Isolation and identification of legionellae. The methods used to isolate and identify *L. longbeachae* from human and environmental samples were those detailed in previous reports (10, 19). Eleven human and 28 environmental isolates of *L. longbeachae* serogroup 1 were included in the study. There were three environmental but no human isolates of *L. longbeachae* serogroup 2. Table 1 describes the environmental sources from which *L. longbeachae* serogroups 1 and 2 were isolated.

Allozyme analysis. (i) Sample preparation. Each isolate was cultured on two plates of charcoal-yeast extract agar base (CM655; Oxoid, Basingstoke, Hampshire, United Kingdom) with α-ketoglutarate and L-cysteine supplement (code SR110; Oxoid) and 0.1% bovine serum albumin in air at 35°C for 3 days. Growth was scraped from the plates into 10 ml of TE (10 mM Tris HCl [pH 8], 1 mM EDTA) buffer, and the cells were suspended by vortexing. The cells were pelleted at approximately 6,000 × *g* for 10 min at 4°C in a JA20 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The supernatant was discarded, and the bacteria were washed in 10 ml of TE buffer and repelleted. The pellet in a small amount of TE buffer was transferred to an Eppendorf tube and centrifuged at 12,000 × *g* for 5 min at 4°C to pack

* Corresponding author.

TABLE 1. *L. longbeachae* isolated from potting soil in Australia

Strain no.	State/ manufacturer[a]	Loci not scorable[b]
Serogroup 1 strains		
248/A1	SA/A	
259/A5	SA/A	*Fum-1*, *Gapd*, *Hbdh*, *Mdh-2*
260/A4	SA/A	
265/B1	SA/A	*Fum-1*, *Galt*, *Gdh*, *Mdh-2*
270/B6a	SA/A	
270/B6b	SA/A	*Gdh*, *Pgk*
271/E4	SA/A	
272/B3	SA/A	
273/E5	SA/A	
280/B4	SA/A	*Fum-1*, *Gapd*, *Pepd*
316/D5	SA/A	
321/G2	SA/A	
324/E1[c]	SA/A	
325/D8	SA/A	
336/E8	SA/A	
348/G5[c,d]	SA/A	
276/B7	SA/B	*Galt*, *Gdh*
292/D2	SA/B	
293/B8	SA/B	
299/F6	SA/C	
350/F1	SA/C	
288/H1	SA/D	
300/C7	Vic/E	
301/C8[c,d]	Vic/F	
349/G6	Vic/G	
297/C5	WA/H	
318/F7[c,d,e]	WA/H	
Serogroup 2 strains		
276/E7[d]	SA/B	
350/F5[d]	SA/C	*Enol*
349/G7[d]	Vic/G	

[a] SA, South Australia; WA, Western Australia; Vic, Victoria.
[b] Allozyme marker not typeable when activity was missing or too weak for reliable interpretation.
[c] Differentiated by lambda probe λIMVSL2.
[d] Differentiated by rRNA probe.
[e] Differentiated by cosmid probe pIMVSL6.

the cells. The supernatant was discarded, and 250 μl of TE buffer with 0.5 mM NADP was added to the cells. The cells were sonicated at setting 6 of a sonifier (B-12; Branson Sonic Power Co., Danbury, Conn.) in six 10-s bursts with the Eppendorf tube packed in ice to keep it cool. Debris was pelleted immediately at 25,000 × *g* for 5 min with a Beckman J18 rotor at 4°C. The supernatant was transferred to glass capillary tubes in aliquots of 10 to 20 μl and stored frozen at −70°C.

All manipulations were performed on ice.

(ii) Electrophoresis. Allozyme electrophoresis was performed on cellulose acetate gels by standard procedures detailed by Richardson et al. (15). Histological stains for more than 60 enzymes were utilized in a search for suitable genetic markers in *L. longbeachae*. Of these, 44 enzymes gave zymograms of sufficient resolution and intensity for reliable genetic interpretation. The details of these 44 enzymes, including Enzyme Commission numbers, abbreviations, and the electrophoretic conditions employed, are described in Table 2. The recipes for the histochemical stains used in this study have been previously published (15) or are listed in Table 3. Reference isolates of *L. cincinnatiensis* (ATCC 43753) and *L. pneumophila* serogroup 1 Philadelphia strain (ATCC 33152) were included on all gels as an aid to allozymic interpretation.

RFLP analysis. (i) Purification and digestion of chromosomal DNA. Whole chromosomal DNA was purified from each isolate by the method of Manning et al. (13). Samples of the preparations were electrophoresed on 0.8% agarose gels to confirm that minimal fragmentation of the chromosomal DNA had occurred.

The DNA was fully digested with *Hin*dIII and *Bam*HI, and the fragments were separated by overnight electrophoresis on 0.8% agarose gels in TAE buffer (12). The fragments were then transferred by the Southern procedure (18) to nylon filter membranes (Biotrace RP; Gelman Sciences, Inc., Ann Arbor, Mich.) which were then baked at 80°C for 2 h and stored at room temperature.

(ii) Derivation of DNA probes. A genomal bank for *L. longbeachae* serogroup 1 (ATCC 33462) was constructed in LambdaGEM-11 (Promega Biotec, Madison, Wis.) according to the instructions of the manufacturer.

Amplified stocks of several clones were prepared and used to infect lawns of *E. coli* LE392 at concentrations which would yield confluent lysis. Bacteriophage was harvested from the plaques by a modification (19) of the method detailed by Maniatis et al. (12). The vector with insert was purified from the phage particles by the method of Maniatis et al. (12).

A second genomal bank was constructed in cosmid pHC79 by the method described by Manning et al. (13). Recombinant plasmids were purified from individual clones selected randomly from the bank by the method of Holmes and Quigley (7).

The vector with insert was labeled with [α-^{32}P]dCTP by nick translation (12). Several clones were tested empirically to find those which gave optimal differentiation of isolates by Southern hybridization.

A third set of RFLP profiles was obtained by Southern hybridization of isolates with *E. coli* 16S and 23S rRNA (Pharmacia LKB Biotechnology, Uppsala, Sweden) end labeled (12) with [γ-^{32}P]dATP (4).

(iii) Hybridization. The filters were hybridized overnight at 42°C in hybridization fluid (50% formamide, 7% sodium dodecyl sulfate, 1% skim milk powder, 5× SSPE [1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, 1 mM EDTA], 200 μg of salmon sperm DNA per ml, radiolabeled recombinant probe DNA or rRNA [5 × 10^5 cpm/ml]). The next day the filters were washed twice for 15 min at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0])–0.1% sodium dodecyl sulfate and then once at 68°C in 1× SSC–0.1% sodium dodecyl sulfate for 45 min and autoradiographed at −70°C.

RESULTS

Allozyme analysis. The 44 enzymes used were encoded by a presumptive 53 loci. Each locus represents a genetic marker which can be used to document genetic variability within *L. longbeachae*. Not all isolates were characterized at all 53 loci. Those that differed are listed in Table 1. As is usual with allozyme electrophoresis, some isolates consistently displayed lower levels of enzyme activity than others, and these isolates were considered not typeable whenever activity was absent or the electrophoretic phenotype was too weak for reliable interpretation. We treated all such occurrences as missing values rather than to postulate the existence of null alleles without supportive genetic or biochemical evidence.

Of the 53 loci examined, 10 markers displayed electropho-

TABLE 2. Details of the enzymes used as genetic markers in this study[a]

Abbreviation	Common name	EC no.	Run buffer	Run time (min)
ACON	Aconitase	4.2.1.3	B	120
ACP	Acid phosphatase	3.1.3.2	D	120
ACYC	Aminoacyclase	3.5.1.14	C	120
ADA	Adenosine deaminase	3.5.4.4	A	120
ADH	Alcohol dehydrogenase	1.1.1.1	B	100
AK	Adenylate kinase	2.7.4.3	B	120
ALADH	Alanine dehydrogenase	1.4.1.1	B	100
ALD	Aldolase	4.1.2.13	D	90
ALDH	Aldehyde dehydrogenase	1.2.1.5	I	90
CAT	Catalase	1.11.1.6	B	100
CS	Citrate synthase	4.1.3.7	D	120
DIA	Diaphorase	1.6.99.?	A	120
ENOL	Enolase	4.2.1.11	A	90
EST	Esterase	3.1.1.1	A	90
FDP	Fructose diphosphatase	3.1.3.11	B	100
FUM	Fumarate hydratase	4.2.1.2	A	120
GALT	Galactose-1-phosphate uridyltransferase	2.7.7.12	C	100
GAPD	Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	B	120
GDH	Glutamate dehydrogenase	1.4.1.3	B	120
GOT	Aspartate aminotransferase	2.6.1.1	B	90
G6PD	Glucose-6-phosphate dehydrogenase	1.1.1.49	A	120
GPI	Glucose-phosphate isomerase	5.3.1.9	D	120
GSR	Glutathione reductase	1.6.4.2	A	100
HBDH	β-Hydroxybutyrate dehydrogenase	1.1.1.30	B	90
HPRT	Hypoxanthine phosphoribosyl transferase	2.4.2.8	C	90
IDH	Isocitrate dehydrogenase	1.1.1.42	B	120
IPP	Inorganic pyrophosphatase	3.6.1.1	C	100
LAP	Leucine aminopeptidase	3.4.11.1	A	100
LEUDH	Leucine dehydrogenase	1.4.1.9	B	120
LYDH	Lysine dehydrogenase	1.4.1.15	B	90
MDH	Malate dehydrogenase	1.1.1.37	A	100
NP	Purine nucleoside phosphorylase	2.4.2.1	A	100
PEPA	Dipeptidase (valine-leucine)	3.4.13.?	A	100
PEPB	Tripeptidase (leucine-glycine-glycine)	3.4.11.?	A	100
PEPD	Prolidase (phenylalanine-proline)	3.4.13.?	A	90
PGK	Phosphoglycerate kinase	2.7.2.3	B	80
PGM	Phosphoglucomutase	5.2.4.2	C	120
PK	Pyruvate kinase	2.7.1.40	C	100
SOD	Superoxide dismutase	1.15.1.1	B	100
SKDH	Shikimate dehydrogenase	1.1.1.25	B	90
THRDH	Threonine dehydrogenase	1.1.1.103	B	100
TPI	Triose-phosphate isomerase	5.3.1.1	A	120
TRK	Transketolase	2.2.1.1	I	90
UK	Unidentified kinase	2.?.?.?	C	100

[a] The unidentified kinase (UK) exhibited activity on gels stained for several kinases (pyruvate kinase, uridine monophosphate kinase, guanylate kinase). The code for the running buffers follows Richardson et al. (15). Stain recipes are presented in Table 3 or given in Richardson et al. (15).

retic patterns typical of the presence of allelic variation. The markers *Ada*, *Cs-1*, *Gpi*, *Gsr*, *Lap*, *Lydh*, *PepB-2*, and *Skdh* each exhibited two allozymes, while *Dia-2* displayed three mobility states. Using these markers, two genetic groups differing at two markers (*Dia-2* and *Gsr*) were evident within the *L. longbeachae* serogroup 2 isolates. In contrast, however, there was no allelic variability expressed among the *L. longbeachae* serogroup 1 isolates ($n = 40$), including the ATCC reference isolate, at any of the 53 loci. Serotype 2 isolates differed from the serotype 1 isolates at 9 to 10 loci (16 to 19% of loci examined).

The outgroups *L. cincinnatiensis* and *L. pneumophila* differed from each other and from *L. longbeachae* at 80 to 95% of loci examined (40 to 50 homologous loci used).

RFLP analysis. Figures 1a, b, and c are triplicates of restriction digests of a representative group of legionellae which showed variable profiles when reacted in Southern hybridizations with the three probes.

All *L. longbeachae* serogroup 1 isolates gave identical RFLP patterns with the cosmid probe designated pIMVSL6 (Fig. 1a) except 318/F7 (lane 6), in which the double band of approximately 6.5 kilobases (kb) was fainter than the others, suggesting a degree of heterogeneity in that sequence. All serogroup 2 isolates possessed identical profiles which were different from the serogroup 1 profiles.

Differences were observed when isolates were hybridized with the probe made from the lambda clone designated λIMVSL2 (Fig. 1b). Variations in the high-molecular-weight bands of approximately 9 to 10 kb were observed in most of the serogroup 1 isolates. *L. longbeachae* serogroup 1 ATCC 33462 (lane 9) had a single 9-kb band, as did 301/C8 (lane 3) and 324/E1 (lane 5). The other Australian strains had a fainter double band in that region, except S348/G5 (lane 2), which was missing bands in the 9- to 10-kb region. Greater variation was seen in isolate 318/F7 (Fig. 1b, lane 6), which did not possess the 1-kb double band seen in the others and

TABLE 3. Histochemical stain recipes for those enzymes not detailed by Richardson et al. (15)

Enzyme	Reagents	Quantity
ACYC	*N*-Acetyl-L-methionine	6 mg
	O-Dianisidine dihydrochloride	6 mg
	L-Amino acid oxidase (0.3 U/mg)	2 mg
	Peroxidase (80 U/mg)	2 mg
	0.1 M Tris hydrochloride (pH 8.6)	2 ml
	0.2 M $MgCl_2$	0.1 ml
ALADH[a]	L-Alanine	10 mg
	0.1 M Tris hydrochloride (pH 8.0)	2 ml
	NAD (20 mg/ml)	0.1 ml
	Thiazolyl blue (MTT) (6 mg/ml)	0.1 ml
	Phenazine methosulfate (PMS) (2 mg/ml)	0.1 ml
CAT	25% Hydrogen peroxide	25 μl
	0.02 M Phosphates (pH 7.0)	5 ml
	Stain gel, blot, incubate gel at 37°C for 10 min, and then counterstain with:	
	O-Dianisidine dihydrochloride	6 mg
	Peroxidase (80 U/mg)	2 mg
	0.1 M Tris hydrochloride (pH 8.6)	2 ml
CS[b]	Acetyl coenzyme A	2 mg
	0.1 M Tris hydrochloride (pH 8.0)	2 ml
	Oxaloacetate (50 mg/ml, pH 8.0)	0.2 ml
	2,6-Dichlorophenol-indophenol (2 mg/ml)	0.1 ml
GALT[b,c]	Galactose-1-phosphate	4 mg
	UDP-glucose	5 mg
	Glucose-1,6-diphosphate	0.5 mg
	0.1 M Tris hydrochloride (pH 8.6)	2 ml
	NADP (20 mg/ml)	0.1 ml
	0.2 M $MgCl_2$	0.1 ml
	Phosphoglucomutase (400 U/ml)	20 μl
	6-Phosphogluconate dehydrogenase (24 U/ml)	50 μl
	Glucose-6-phosphate dehydrogenase (600 U/ml)	10 μl
	2-Mercaptoethanol	10 μl
HPRT[c,d]	Guanine hydrochloride	3 mg
	Adenosine triphosphate	3 mg
	Phosphoenolpyruvate	2 mg
	NADH	3 mg
	Phosphoribosyl pyrophosphate	3 mg
	0.1 M Tris hydrochloride (pH 8.0)	3 ml
	0.2 M $MgCl_2$	0.1 ml
	1 M KCl	0.1 ml
	Guanosine monophosphate kinase (20 U/ml)	50 μl
	Pyruvate kinase (400 U/ml)	20 μl
	Lactate dehydrogenase (5,500 U/ml)	10 μl
IPP	Sodium pyrophosphate	3 mg
	Inosine	5 mg
	Xanthine oxidase (phosphate free)[e]	2 mg
	Nucleoside phosphorylase (phosphate free)[f]	1 mg
	0.1 M Tris hydrochloride (pH 8.0)	2 ml
	0.2 M $MgCl_2$	0.1 ml
	MTT (6 mg/ml)	0.1 ml
	PMS (2 mg/ml)	0.1 ml
LEUDH[a]	As for ALADH, but with L-leucine (8 mg) as substrate	
LYDH[a]	As for ALADH, but with L-lysine (8 mg) as substrate	
SKDH	Shikimic acid	8 mg
	0.1 M Tris hydrochloride (pH 8.0)	2 ml
	NADP (20 mg/ml)	0.1 ml
	MTT (6 mg/ml)	0.1 ml
	PMS (2 mg/ml)	0.1 ml

Continued on following page

TABLE 3—*Continued*

Enzyme	Reagents	Quantity
THRDH[a]	As for ALADH, but with L-threonine (8 mg) as substrate	
TRK[b,c]	Xylulose-5-phosphate	3 mg
	Ribose-5-phosphate	5 mg
	NADH	3 mg
	Thiamine pyrophosphate	1 mg
	0.1 M Tris hydrochloride (pH 7.4)	2 ml
	1 M $MgCl_2$	0.1 ml
	Glycerol-3-phosphate dehydrogenase (340 U/ml)	25 μl
	Triosephosphate isomerase (10,000 U/ml)	10 μl

[a] Modified from Selander et al. (17).
[b] Modified from Harris and Hopkinson (5).
[c] View under UV light.
[d] Modified from Ropers (16).
[e] Boehringher Mannheim product number 1048180.
[f] Boehringher Mannheim product number 1048171.

also had a darker single 3-kb band. 318/F7 was isolated from potting soil made by a Western Australian manufacturer and was not associated with known human cases. 324/E1 (lane 5) also showed an aberrant pattern, missing the 2-kb band. This organism was isolated from potting mix produced by a South Australian manufacturer and was not associated with human infection. As with the pIMVSL6, probes made from λIMVSL2 distinguished between the *L. longbeachae* serogroup 1 and serogroup 2 isolates but not within serogroup 2.

Figure 1c shows the patterns obtained with the rRNA probe. Southern hybridization with this probe produced the clearest differentiation among isolates of any of the techniques used, but it was not able to distinguish between the two serogroups. Most *L. longbeachae* serogroup 1 isolates gave identical profiles. Exceptions were two isolates of *L. longbeachae* serogroup 1 in lane 2 (348/G5) and lane 3 (301/C8) which possessed similar but not identical patterns which differed from the others. These two isolates were also separated from each other and the other *L. longbeachae* serogroup 1 isolates by Southern hybridization with λIMVSL2. These strains were isolated from potting soils obtained from South Australia (S348/G5) and Victoria (301/C8). Isolate 318/F7, which had been shown to be variant by pIMVSL6 and λIMVSL2 probes, was also differentiated from the other strains by the rRNA probe.

The ATCC reference serogroup 1 and 2 strains gave very similar profiles with the rRNA probe. Both had a fainter 4.2-kb band which differentiated them from the Australian *L. longbeachae* serogroup 1 isolates. The Australian *L. longbeachae* serogroup 2 isolates were of two types, and one of the isolates was identical to the ATCC *L. longbeachae* serogroup 2 strain.

All systems were able to distinguish *L. longbeachae* from other *Legionella* species including the serologically related *L. cincinnatiensis*, *L. santicrucis*, and *L. sainthelensi*.

DISCUSSION

The four systems, allozyme typing and RFLP analysis with probes made from rRNA, lambda clone, and cosmid clone, together provided detailed information on the genetic relatedness of the organisms. Typing of isolates by both allozyme electrophoresis and RFLP suggested that the Australian isolates of *L. longbeachae* serogroup 1 from humans were closely related to each other and to most of the Australian environmental isolates. They were also similar to the ATCC reference strain. Minor variability was evident among the human isolates when they were probed with the lambda clones (19), but the other systems did not detect any differences.

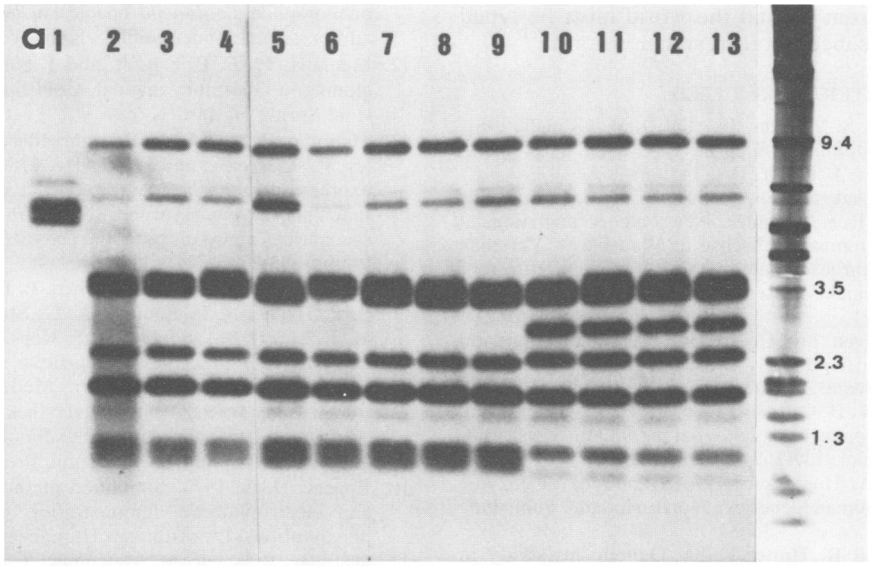
Southern hybridization with rRNA as a probe was best able to detect significant variation among the Australian environmental isolates, differentiating two similar and one unique isolate from the other serogroup 1 strains. One of these (318/F7) was also differentiated by both cloned probes. The other two isolates (S348/G5 and 301/C8) were differentiated by λIMVSL2 but not pIMVSL6.

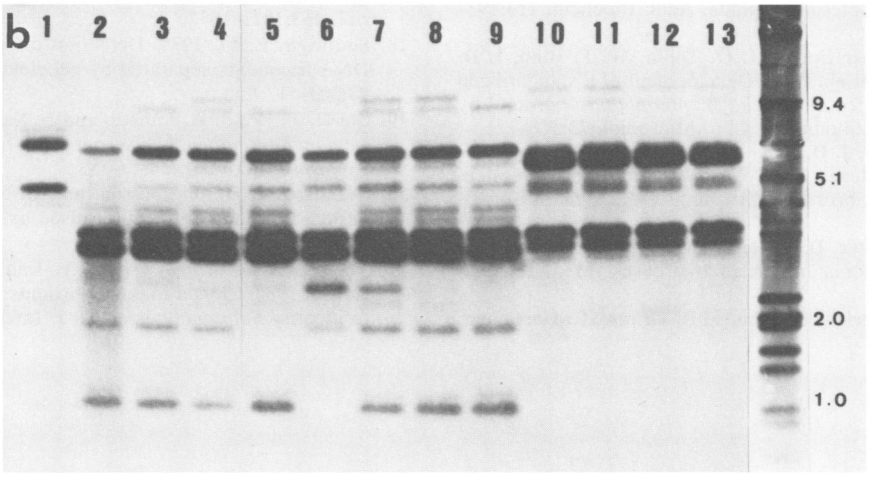
λIMVSL2 provided some differentiation among the *L. longbeachae* serogroup 1 isolates. The cosmid clone chosen for these studies was not able to differentiate well within serogroups but did provide a means of distinguishing the two serogroups of *L. longbeachae* from other species without the requirement for quantitative DNA homology studies.

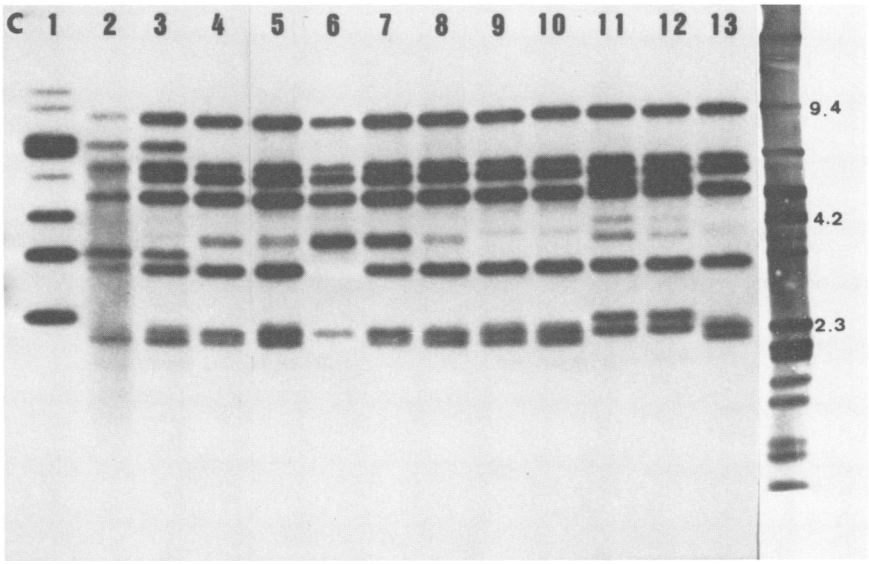
Allozyme electrophoresis was unable to detect differences among the *L. longbeachae* serogroup 1 isolates, although differences were found in the four serogroup 2 isolates.

The close relationship of organisms isolated from countries as far apart as Australia and America is surprising and warrants some comment. Four possible scenarios can be envisaged to explain the result. First, the introduction of isolates to one country or the other may be a recent event. Such an introduction might have resulted from human involvement or may have occurred via other animal vectors, for example, birds. Second, *Legionella* isolates of the species *L. longbeachae* have very low levels of genetic variability. Thus, unrelated isolates will be genetically identical regardless of the time since divergence. A third explanation might be that the majority of the Australian isolates had originated from a single source, being a part of an outbreak, and were by chance similar to ATCC 33462. One final possibility is that the allozyme and DNA characterizations

FIG. 1. Southern hybridization of *Hin*dIII and *Bam*HI digests of genomic DNA. Lanes: 1, *L. cincinnatiensis* ATCC 43753; 2 to 8, isolates 348/G5, 301/C8, 248, 324/E1, 318, human 2, and human 11, respectively; 9, *L. longbeachae* serogroup 1 ATCC 33462; 10, *L. longbeachae* serogroup 2 ATCC 33484; 11 to 13, isolates 276/E7, 349/G7, and 350/F5, respectively. Triplicate Southern transfer filters were hybridized with cosmid probe (pIMVSL6) (a), lambda probe (λIMVSL2) (b), or rRNA probe (c). Numbers on right side show size in kilobases.


a 1 2 3 4 5 6 7 8 9 10 11 12 13
9.4
3.5
2.3
1.3


b 1 2 3 4 5 6 7 8 9 10 11 12 13
9.4
5.1
2.0
1.0


c 1 2 3 4 5 6 7 8 9 10 11 12 13
9.4
4.2
2.3

presented here do not adequately indicate strain relatedness. Many more isolates from around the world must be typed before the true significance of our results is known.

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