TABLE 1 STs of Japanese environmental isolates of *L. pneumophila* serogroup 1

Source environment and ST	No. (%) of isolates	MAb(s) (no. of isolates)
Cooling tower water		
1	37 (74)	Oxford (27), OLDA (10)
154	4 (8)	Oxford (2), OLDA (1), Philadelphia (1)
598	3 (6)	Oxford
150	2 (4)	Oxford
Others	4 (8)	
Total	50 (100)	
Bathwater		
1	6 (12)	OLDA (4), Oxford (2)
129	5 (10)	Bellingham
599	3 (6)	Bellingham
52	2 (4)	OLDA (1), Oxford (1)
86	2 (4)	Bellingham
127	2 (4)	Bellingham
136	2 (4)	Oxford (1), Philadelphia (1)
141	2 (4)	Philadelphia
Others	26 (52)	
Total	50 (100)	
Soil[a]		
48	9 (26)	Bellingham
739	6 (18)	OLDA
22	5 (15)	OLDA
448	3 (9)	OLDA (1), Oxford (1), Benidorm (1)
129	2 (6)	Bellingham
352	2 (6)	Allentown/France
445	2 (6)	OLDA
593	2 (6)	OLDA
Others	3 (9)	
Total	34 (100)[b]	

[a] Excluding an isolate with failed *neuA* amplification.
[b] The sum of percentages is not 100% because each percentage was rounded.

CT and 6 from BW), ST48 (9 from SO and 1 from CT), and ST129 (5 from BW and 2 from SO).

There were no regional differences in the distribution of ST1 in either the CT or the BW isolates (76% [22/29] of CT isolates from the Kanto region and 71% [15/21] from other regions; 14% [3/21] of BW isolates from the Kanto region and 10% [3/29] from other regions). ST1 was not detected among the SO isolates.

The 50 CT isolates were divided into eight STs (IOD = 0.448). The 50 BW isolates were divided into 34 STs (IOD = 0.973). The 35 SO isolates (with one exception that failed *neuA* amplification) were divided into 11 STs (IOD = 0.879).

The minimum-spanning tree illustrates the distribution of the STs (Fig. 2). Thirty of the fifty STs obtained in this analysis were unique to Japan, according to data submitted to the EWGLI SBT database as of March 2012. Twenty of the fifty STs had already also been detected in clinical isolates in Japan and/or abroad, according to the same database. Most SO isolates formed three distinct groups (groups S1, S2, and S3 in Fig. 2). Group S2 had no linkage with other STs. CT isolates formed group C1 and group C2. The two groups were adjacent in the minimum-spanning tree, but even the most related STs (ST161 and ST150) that belonged to group C1 and group C2, respectively, differed in four loci. The BW isolates were dispersed, forming one major group (group B1) and two smaller groups. This finding was supported by neighbor-joining analysis based on a nucleotide sequence comparison of seven concatenated loci of SBT (2,501 bp) of the same isolates as in Fig. 2 (Fig. 3). Figure 3 shows that isolates belonging to each group found in the minimum-spanning tree were also clustered. However, the relationships observed between groups in the dendrogram were different from the minimum-spanning analysis, except for the cluster of group S1 and group C1. Two groups of isolates from cooling tower water (group C1 and group C2) were located distally (unlike Fig. 2). Groups C2, B2, and B3 shared many informative sites between groups, compared to groups C1, S1, S2, S3, and B1, as shown by the bootstrap support value of 74%.

Combining the sequence typing and the MAb subgrouping. Some STs were composed of isolates belonging to different MAb subgroups (and *vice versa*). Thus, ST1 (n = 43) was composed of isolates belonging to the Oxford (n = 29) and OLDA (n = 14) subgroups. ST154 (n = 4) contained the Oxford (n = 2), OLDA (n = 1), and Philadelphia (n = 1) subgroups. ST448 (n = 3) consisted of OLDA, Oxford, and Benidorm isolates. In contrast, all ST48 (n = 9) and all ST129 (n = 5) isolates were Bellingham. By combining the data of SBT and MAb subgrouping, we could divide the 135 isolates into 58 types (IOD = 0.933; Tables 1 and 2).

DISCUSSION

We analyzed *L. pneumophila* SG 1 isolates from three distinct environments using MAb typing and SBT in Japan: cooling tower water, bathwater, and soil. The distributions of MAbs and STs of isolates differed both between the environments and from previous clinical isolates (2).

Of the SG 1 clinical isolates from Japan, 80% had the virulence-associated epitope recognized by MAb 3/1 (2). As for the analyzed 135 environmental isolates, MAb 3/1-positive isolates accounted for only 14%. Similar observations have also been made in studies conducted in other countries (i.e., Germany [3], England and Wales [12], and the United States [15]). Although these data indicated MAb 3/1 as the virulence-associated epitope, our study's MAb 3/1-positive isolates dispersed on the dendrogram by SBT (Fig. 3) in the three kinds of analyzed environments, suggesting the MAb 3/1 epitope is easily lost or gained during adaptation to environments when there is no pressure to retain human pathogenicity. Loss of the MAb 3/1 epitope may bring some advantage for fitness, as MAb 3/1-negative isolates dominated in each environment.

Although 30 of the 50 STs obtained in this analysis were unique to Japan, the EWGLI SBT database indicated that the majority of unique STs have single-locus variants abroad. Among the unique STs, only ST138 and ST162 in group B3, and ST141 have neither single-locus variants nor double-locus variants abroad. ST138 of the Benidorm subgroup is the primary clinical isolate associated with bathwater in Japan (2; unpublished results). Thus, a few STs might be unique to Japan, which is isolated by water.

All of the Japanese ST1 strains were of the MAb 3/1-negative OLDA or Oxford subgroups, whereas the ST1 strains in the EWGLI database are divided into nine MAb types. This distribution of MAb types within ST1 may be a regional difference. On the other hand, a regional difference did not always apply. All nine ST48 from our results were of the Bellingham subgroup, and according to the EWGLI SBT database prior to May 2011, all of the MAb-typed ST48 strains submitted thus far were Bellingham. Since May 2011, however, Camperdown and OLDA strains containing ST48 have been deposited. If more strains could be ana-

TABLE 2 STs and MAb subtypes of 135 Japanese environmental isolates of *L. pneumophila* serogroup 1[a]

Strain	Origin	MAb subgroup	MAb 3/1	Allele no.							ST[b]	Yr
				flaA	*pilE*	*asd*	*mip*	*mompS*	*proA*	*neuA*		
NIIB 267	B	OLDA	Neg.	1	4	3	1	1	1	1	1	2000
NIIB 273	B	OLDA	Neg.	1	4	3	1	1	1	1	1	2000
NIIB 277	B	Oxford	Neg.	1	4	3	1	1	1	1	1	2000
NIIB 720	B	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 766	B	OLDA	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 772	B	OLDA	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 270	B	OLDA	Neg.	1	10	3	1	1	1	1	52	2000
NIIB 271	B	Oxford	Neg.	1	10	3	1	1	1	1	52	2000
NIIB 275	B	Oxford	Neg.	2	3	18	5	5	1	2	92	2000
NIIB 886	B	Allentown/France	Pos.	2	6	17	14	12	8	11	788	2001
NIIB 1090	B	Bellingham	Neg.	2	10	3	3	9	4	11	545	2005
NIIB 729	B	Bellingham	Neg.	2	10	3	6	9	4	11	**614**	2005
NIIB 370	B	Philadelphia	Pos.	2	12	3	6	8	14	9	**141**	2002
NIIB 885	B	Philadelphia	Pos.	2	12	3	6	8	14	9	**141**	2004
NIIB 158	B	Bellingham	Neg.	3	13	1	10	14	9	11	**127**	
NIIB 925	B	Bellingham	Neg.	3	13	1	10	14	9	11	**127**	2004
NIIB 160	B	Bellingham	Neg.	6	6	15	28	4	14	11	129	
NIIB 231	B	Bellingham	Neg.	6	6	15	28	4	14	11	129	
NIIB 887	B	Bellingham	Neg.	6	6	15	28	4	14	11	129	2002
NIIB 889	B	Bellingham	Neg.	6	6	15	28	4	14	11	129	2004
NIIB 890	B	Bellingham	Neg.	6	6	15	28	4	14	11	129	2005
NIIB 888	B	Camperdown	Neg.	6	6	15	28	4	4	11	**164**	2002
NIIB 712	B	Camperdown	Neg.	6	6	15	28	9	14	11	**601**	2005
NIIB 929	B	Benidorm	Pos.	6	7	15	3	4	14	2	**165**	2004
NIIB 715	B	Benidorm	Pos.	6	10	11	28	4	14	9	**602**	2005
NIIB 1073	B	Knoxville	Pos.	6	10	15	3	19	4	11	**604**	2005
NIIB 1197	B	Heysham	Neg.	6	10	15	3	21	4	3	**606**	2005
NIIB 707	B	Benidorm	Pos.	6	10	15	13	17	14	11	**122**	2005
NIIB 128	B	Bellingham	Neg.	6	10	15	28	4	4	11	**125**	
NIIB 743	B	Bellingham	Neg.	6	10	15	28	44	14	11	**201**	2005
NIIB 1213	B	Bellingham	Neg.	6	10	15	28	4	14	11	278	2005
NIIB 229	B	Philadelphia	Pos.	6	10	17	6	9	4	9	**136**	
NIIB 295	B	Oxford	Neg.	6	10	17	6	9	4	9	**136**	2000
NIIB 278	B	Bellingham	Neg.	6	10	17	28	19	4	6	**599**	
NIIB 696	B	Bellingham	Neg.	6	10	17	28	19	4	6	**599**	2005
NIIB 699	B	Bellingham	Neg.	6	10	17	28	19	4	6	**599**	2005
NIIB 710	B	Bellingham	Neg.	6	10	19	28	19	14	11	**600**	2005
NIIB 230	B	Philadelphia	Pos.	6	10	21	6	9	4	9	**137**	
NIIB 733	B	Benidorm	Pos.	6	10	21	13	17	14	11	**131**	2005
NIIB 126	B	Oxford	Neg.	6	16	14	3	21	14	3	124	
NIIB 159	B	Bellingham	Neg.	7	6	17	3	14	11	11	128	
NIIB 1109	B	Bellingham	Neg.	7	6	17	28	36	11	11	**86**	2005
NIIB 1115	B	Bellingham	Neg.	7	6	17	28	36	11	11	**86**	2005
NIIB 805	B	Bellingham	Neg.	7	8	17	3	14	11	11	**603**	2005
NIIB 1044	B	Bellingham	Neg.	7	10	17	3	13	9	11	**605**	2005
NIIB 268	B	Allentown/France	Pos.	8	10	3	10	2	1	6	**610**	2000
NIIB 1206	B	Benidorm	Pos.	10	12	7	3	16	18	6	**138**	2005
NIIB 594	B	Benidorm	Pos.	10	22	7	3	16	9	6	**162**	2001
NIIB 891	B	Oxford	Neg.	11	14	16	1	15	13	2	159	2005
NIIB 1099	B	OLDA	Neg.	12	8	11	23	29	26	2	260	2005
NIIB 65	C	Oxford	Neg.	1	4	3	1	1	1	1	1	1996
NIIB 121	C	OLDA	Neg.	1	4	3	1	1	1	1	1	
NIIB 122	C	OLDA	Neg.	1	4	3	1	1	1	1	1	
NIIB 124	C	OLDA	Neg.	1	4	3	1	1	1	1	1	
NIIB 182	C	Oxford	Neg.	1	4	3	1	1	1	1	1	1997
NIIB 217	C	OLDA	Neg.	1	4	3	1	1	1	1	1	1986
NIIB 223	C	OLDA	Neg.	1	4	3	1	1	1	1	1	1986
NIIB 224	C	Oxford	Neg.	1	4	3	1	1	1	1	1	1986
NIIB 225	C	OLDA	Neg.	1	4	3	1	1	1	1	1	1986
NIIB 226	C	OLDA	Neg.	1	4	3	1	1	1	1	1	1986
NIIB 228	C	OLDA	Neg.	1	4	3	1	1	1	1	1	1986
NIIB 237	C	Oxford	Neg.	1	4	3	1	1	1	1	1	1996
NIIB 239	C	Oxford	Neg.	1	4	3	1	1	1	1	1	1993
NIIB 418	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2004
NIIB 547	C	OLDA	Neg.	1	4	3	1	1	1	1	1	2001
NIIB 563	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2001
NIIB 568	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2001
NIIB 586	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2001
NIIB 597	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2001
NIIB 697	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 717	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 722	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 725	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 732	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 739	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 742	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 744	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 758	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 764	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 802	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005

(Continued on following page)

TABLE 2 (Continued)

Strain	Origin	MAb subgroup	MAb 3/1	Allele no.							ST[b]	Yr
				flaA	*pilE*	*asd*	*mip*	*mompS*	*proA*	*neuA*		
NIIB 1048	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 1050	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 1052	C	OLDA	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 1057	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 1082	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 1201	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2005
NIIB 1592	C	Oxford	Neg.	1	4	3	1	1	1	1	1	2006
NIIB 946	C	Bellingham	Neg.	5	2	22	27	6	10	12	48	2005
NIIB 552	C	Oxford	Neg.	11	4	3	1	1	1	1	**161**	2001
NIIB 595	C	Oxford	Neg.	11	14	16	1	15	13	1	150	2001
NIIB 1207	C	Oxford	Neg.	11	14	16	1	15	13	1	150	2005
NIIB 694	C	Oxford	Neg.	11	14	16	10	15	13	11	**598**	2005
NIIB 746	C	Oxford	Neg.	11	14	16	10	15	13	11	**598**	2005
NIIB 1098	C	Oxford	Neg.	11	14	16	10	15	13	11	**598**	2005
NIIB 590	C	Oxford	Neg.	11	14	16	16	15	13	2	154	2001
NIIB 591	C	Oxford	Neg.	11	14	16	16	15	13	2	154	2001
NIIB 778	C	OLDA	Neg.	11	14	16	16	15	13	2	154	2005
NIIB 1077	C	Philadelphia	Pos.	11	14	16	16	15	13	2	154	2005
NIIB 1269	C	Oxford	Neg.	11	14	16	16	15	13	1	**607**	2005
NIIB 611	C	OLDA	Neg.	18	4	3	1	1	1	1	**163**	2001
NIIB 2366	S	OLDA	Neg.	2	3	6	10	2	1	6	22	2001
NIIB 2388	S	OLDA	Neg.	2	3	6	10	2	1	6	22	2001
NIIB 2403	S	OLDA	Neg.	2	3	6	10	2	1	6	22	2001
NIIB 2404	S	OLDA	Neg.	2	3	6	10	2	1	6	22	2001
NIIB 2409	S	OLDA	Neg.	2	3	6	10	2	1	6	22	2001
NIIB 2375	S	Oxford	Neg.	2	3	18	10	2	1	6	448	2001
NIIB 2395	S	OLDA	Neg.	2	3	18	10	2	1	6	448	2001
NIIB 2338	S	Benidorm	Neg.	2	3	18	10	2	1	6	448	2001
NIIB 2380	S	Allentown/France	Pos.	2	3	18	10	25	5	6	**740**	2001
NIIB 2363	S	OLDA	Neg.	2	3	18	13	2	1	6	445	2001
NIIB 2392	S	OLDA	Neg.	2	3	18	13	2	1	6	445	2001
NIIB 2339	S	OLDA	Neg.	2	3	40	13	2	1	6	593	2001
NIIB 2373	S	OLDA	Neg.	2	3	40	13	2	1	6	593	2001
NIIB 2355	S	OLDA	Neg.	5	1	22	26	6	10	12	45	2001
NIIB 2332	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2335	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2346	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2383	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2398	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2399	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2405	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2406	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2408	S	Bellingham	Neg.	5	2	22	27	6	10	12	48	2001
NIIB 2371	S	Bellingham	Neg.	6	6	15	28	4	14	11	129	2001
NIIB 2411	S	Bellingham	Neg.	6	6	15	28	4	14	11	129	2001
NIIB 2370	S	OLDA	Neg.	6	10	23	10	18	14	0[c]	J6[d]	2001
NIIB 2342	S	OLDA	Neg.	12	8	11	2	10	12	2	739	2001
NIIB 2356	S	OLDA	Neg.	12	8	11	2	10	12	2	739	2001
NIIB 2381	S	OLDA	Neg.	12	8	11	2	10	12	2	739	2001
NIIB 2386	S	OLDA	Neg.	12	8	11	2	10	12	2	739	2001
NIIB 2390	S	OLDA	Neg.	12	8	11	2	10	12	2	739	2001
NIIB 2394	S	OLDA	Neg.	12	8	11	2	10	12	2	739	2001
NIIB 2327	S	Allentown/France	Pos.	12	8	11	13	10	12	2	**352**	2001
NIIB 2353	S	Allentown/France	Pos.	12	8	11	13	10	12	2	**352**	2001
NIIB 2343	S	Philadelphia	Pos.	21	14	29	30	15	29	6	**741**	2001

[a] Origins: B, bathwater; C, cooling tower water; S, soil. MAb 3/1: Pos., positive; Neg., negative.

[b] Underlining indicates an ST first reported from Japan and later reported from other countries. Bold facing indicates STs unique to Japan as of 8 March 2012.

[c] Allele number "0" means a failed amplification.

[d] J6, an arbitrary number allocated to unique six-allele profile without *neuA*.

lyzed, we predict that different MAb types within many ST groups would be detected.

The isolates from soil were divided into three groups (Fig. 2) by the spanning tree analysis and only had two common STs that were detected in different environments: ST48 with an isolate from cooling tower water and ST129 with an isolate from bathwater. These findings indicate that these bacteria generally inhabit the soil but are able to contaminate water sources. Further investigations of more isolates from soil may identify STs that link the three groups or that have more corresponding STs with isolates from water environments. Nine of the 11 STs of soil isolates were also detected in clinical isolates, in contrast to only 11 of 34 in bathwater and 3 of 8 from cooling tower water. These findings support the possibility that soil is one of the infectious sources of legionellosis.

In Canada, the distribution of STs in strains from natural water sources was noted as significantly different compared to strains from a manufactured environment (22). We note a similar finding in the present study. The water of Japanese public baths is often derived from hot springs. The characteristics of hot spring water, namely, chemical features such as pH and temperature, are highly variable, whereas the water from hot or cold water systems and cooling towers tend to have rather similar characteristics due to similar water treatment procedures. In our results, STs and MAb types of isolates from bathwater both differed from and were more varied than those of cooling tower water. These features might be

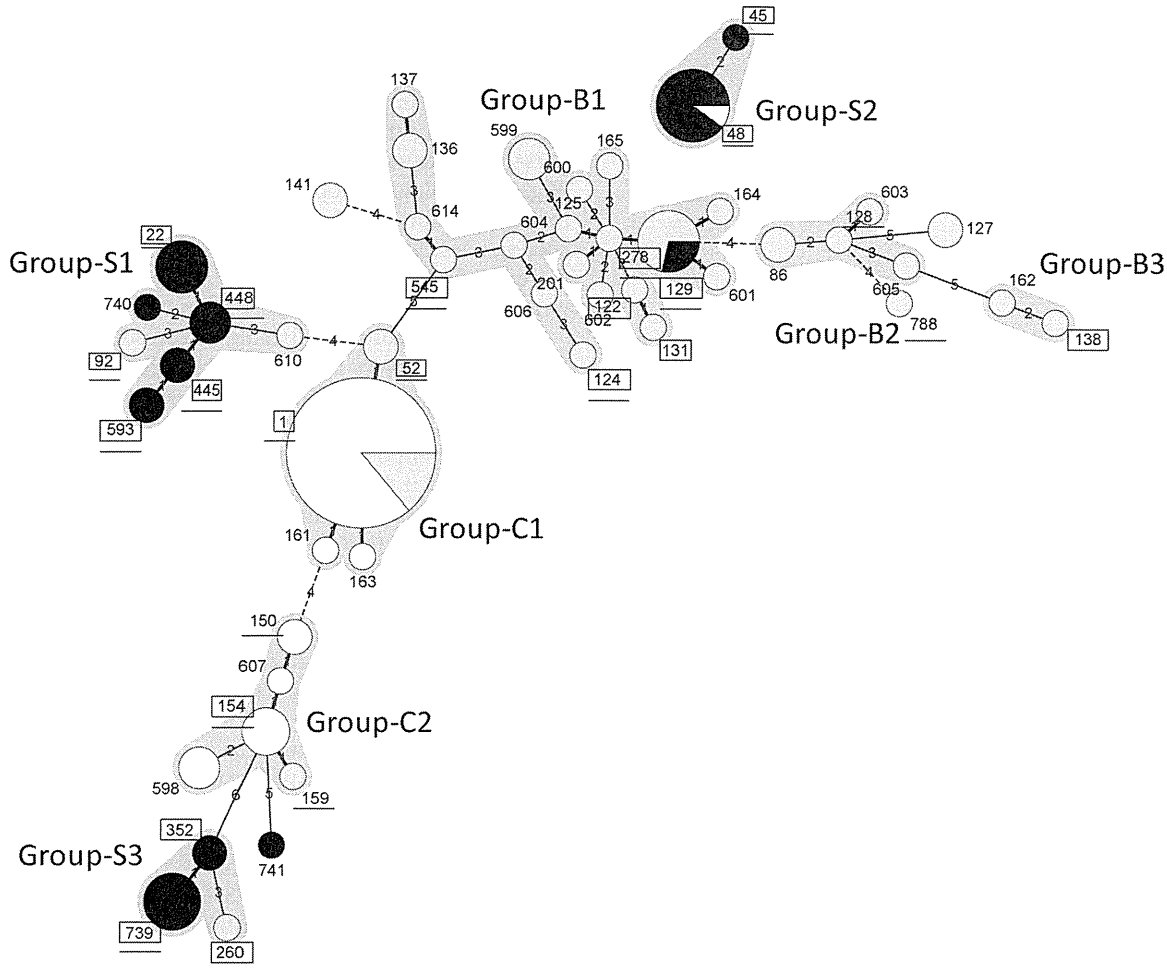

FIG 2 Minimum-spanning tree showing how the *L. pneumophila* isolates, with seven determined alleles, are distributed in terms of their STs. The ST number is shown beside the circle. An underlined ST number indicates that the ST also has been reported abroad, and a boxed ST number indicates that clinical isolates with the same STs were detected. The size of the circle indicates the number of isolates. The white parts of the circles (pie charts) indicate isolates derived from cooling tower water ($n = 50$), the gray indicates isolates derived from bathwater ($n = 50$), and the black indicates isolates derived from soil ($n = 34$). Short thick branches connect single-locus variants, thin branches connect double- or triple-locus variants, broken branches connect four-locus variants, and thinner branches connect five- or six-locus variants. The numbers of locus variants are proportional to the length of branches. Groups that were generated with single-, double-, and triple-locus variants are indicated by differently shaded backgrounds. Groups C1 and C2 had their major isolates derived from cooling tower water, groups B1, B2, and B3 had their major isolates derived from bathwater, and groups S1, S2, and S3 had their major isolates derived from soil.

related to the kind of host amoebae, which adapt to and inhabit various environments (20, 23). It has been shown that the growth of *L. pneumophila* in some species of host amoebae depends on bacterial genetic background (4, 8). Some isolates with particular STs adapted for amoebae that live in bathwater may be infectious to humans.

L. pneumophila SG 1 isolates in cooling tower water in Japan were divided into two genetic groups (group C1 and group C2; Fig. 3). Recombination events may have occurred between members of group C1 and group C2. For example, ST161 (*flaA11*, *pilE4*, *asd-3*, *mip-1*, *mompS1*, *proA1*, and *neuA1*) was a recombinant between ST1 (*flaA1*, *pilE4*, *asd-3*, *mip-1*, *mompS1*, *proA1*, and *neuA1*) and ST154 (*flaA11*, *pilE14*, *asd-16*, *mip-16*, *mompS15*, *proA13*, and *neuA2*), which was a predicted primary founder, and ST150 (*flaA11*, *pilE14*, *asd-16*, *mip-1*, *mompS15*, *proA13*, and *neuA1*) was also a recombinant between ST1 (with adjacent alleles, *mip-1* and *neuA1*) and ST154, shortening the distance between the two groups on the minimum-spanning tree (Fig. 2).

The IOD (0.886) of the 135 environmental isolates was lower than described in our previous report based on clinical isolates (0.979 [2]). These findings were similar to those reported in Canada and the United States (15, 22). The lower diversity observed among environmental isolates compared to clinical isolates may be due to the high prevalence of ST1 (22). ST1 is the most prevalent ST in the world (3, 6–7, 12, 15–16, 22, 26). We have also shown that the majority of environmental isolates, especially from cooling tower waters in Japan (37/50, or 74%), are ST1. Similar results were shown in South Korea (46/68 [67.6%] of SG 1 isolates from cooling tower water were ST1), which is adjacent to Japan (16). In a Canadian study, 34.2% of *L. pneumophila* strains from manufactured environments and 7.7% of *L. pneumophila* isolates from natural water sources (lakes and hot springs) were SG 1 and ST1. Among the Canadian strains, five of six SG 1 isolates from cooling tower waters were ST1 (22). In a U.S. study, ST1 accounted for 40% of the *L. pneumophila* SG 1 environmental isolates; however, the types of environments were not indicated. In

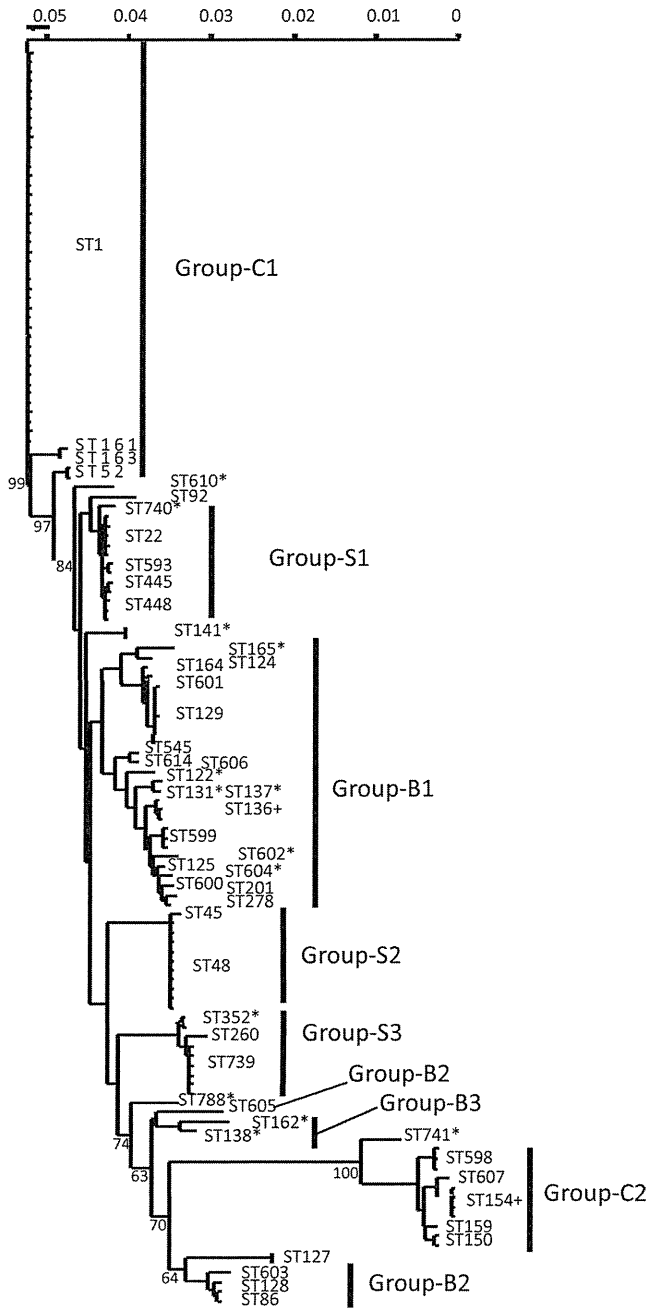

FIG 3 Phylogenetic tree of *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA* concatenated sequences from *L. pneumophila* serogroup 1 isolates determined by the neighbor-joining method. Bootstrap support values for nodes outside groups higher than 50% are shown. The scale bar indicates genetic distances between sequences. The groups correspond to those in Fig. 2. STs of isolates that are MAb 3/1 positive are denoted by asterisks (*), and STs of a part of isolates that are MAb 3/1 positive are denoted by plus symbols (+).

Singapore in tropical southeast Asia, the IOD of environmental *L. pneumophila* isolates was found to be 0.970, and three (two from a cooling tower and one from a water tank) of 16 SG 1 isolates were ST1 (17). In a study conducted in England and Wales, 154 of 276 *L. pneumophila* isolates, including 29 isolates derived from cooling tower water, were SG 1, and 54/154 (35%) SG 1 environmental isolates were ST1 (12). In our study, ST1 accounted for 74 and 12% of the environmental SG 1 isolates from cooling tower water and bathwater, respectively, whereas no ST1 was found in isolates from soil. Isolates with ST1 have adapted to water environments, especially in manufactured water systems such as cooling towers, and have been detected around the world. The ability of ST1 isolates to adapt to natural water sources such as lakes and hot springs might be rather low. Moreover, they might be unfit to survive well in soil environments. The predominant ST, ST1, of isolates from cooling tower water induced an insufficient IOD, 0.448, whereas the discrimination powers for isolates from bathwater and soil were sufficiently significant (0.973 and 0.879, respectively).

Handling of potting soil could be considered a risk factor for legionellosis. Surveys in several countries have detailed various *Legionella* species, including *L. pneumophila* SG1, that were isolated from potting soil or composted materials (5, 18, 25). SBT analysis on composted material isolates in United Kingdom revealed that their *L. pneumophila* SG1 isolates belonged to ST84 (18). Seven alleles belonging to ST84 were unshared by soil isolates in our study (except for one allele, *flaA12*, which was), although ST84 has been detected in clinical isolates in Japan (2) and other countries, according to the EWGLI database. Groups S1, S2, and S3, mainly formed by isolates derived from soil, were distant phylogenetically from groups of isolates derived from water environments. Only ST129 soil isolates shared the B1 group with isolates from a water environment. Although some isolates from cooling tower water and bathwater were included in groups S1, S2, and S3, this might imply that some part of these *L. pneumophila* subpopulations primarily inhabits soil, occasionally mutating and becoming fit to contaminate water environments. Recently, indigenous soil samples were collected in Thailand, and 115 *Legionella* isolates, including 2 *L. pneumophila* SG1 isolates, were identified (24; EWLGI database). One ST identified from the *L. pneumophila* SG1 soil sample isolates related to group S1 and the other to group S2, supporting the idea that most soil isolates belong to particular groups. It is also interesting that the most prevalent ST1 isolates from water samples were not isolated from soil in our study, suggesting the possibility of habitat segregation of *L. pneumophila*. To elucidate this possibility, we need to investigate more environmental isolates from both soil and water.

ACKNOWLEDGMENTS

We thank Norman K. Fry (Respiratory and Systemic Infection Laboratory, Health Protection Agency) for assigning the newly identified alleles.

This study was supported by the Health and Labor Sciences research grant H22-kenki-014 (to F.K.) and partially supported by Ministry of Education, Culture, Sports, Science, and Technology grant KAKENHI 23590530 (to J.A.-M).

The Working Group for *Legionella* in Japan included Mie Sasaki, Miyagi Prefectural Institute of Public Health and Environment; Mikako Hosoya, Niigata Prefectural Institute of Public Health and Environmental Sciences; Yuko Watanabe, Toshiro Kuroki, Kanagawa Prefectural Institute of Public Health; Masamichi Wada, Nagano Environmental Conservation Research Institute; Hitoshi Doi, Osaka Prefectural Institute of Public Health; and Koichi Murakami, Fukuoka Institute of Health and Environmental Sciences.

REFERENCES

1. **Amemura-Maekawa J, et al.** 2008. Distinct difference of *flaA* genotypes of *Legionella pneumophila* between isolates from bath water and cooling tower water. Microbiol. Immunol. 52:460–464.
2. **Amemura-Maekawa J, et al.** 2010. Characterization of *Legionella pneumophila* isolates from patients in Japan according to serogroups, mono-

clonal antibody subgroups, and sequence types. J. Med. Microbiol. **59**: 653–659.
3. **Borchardt J, Helbig JH, Lück PC.** 2008. Occurrence and distribution of sequence types among *Legionella pneumophila* strains isolated from patients in Germany: common features and differences to other regions of the world. Eur. J. Clin. Microbiol. Infect. Dis. **27**:29–36.
4. **Buse HY, Ashbolt NJ.** 2011. Differential growth of *Legionella pneumophila* strains within a range of amoebae at various temperatures associated with in-premise plumbing. Lett. Appl. Microbiol. **53**:217–224.
5. **Casati S, Conza L, Bruin J, Gaia V.** 2010. Compost facilities as a reservoir of *Legionella pneumophila* and other *Legionella* species. Clin. Microbiol. Infect. **16**:945–947.
6. **Cazalet C, et al.** 2008. Multigenome analysis identifies a worldwide distributed epidemic *Legionella pneumophila* clone that emerged within a highly diverse species. Genome Res. **18**:431–441.
7. **Chasqueira MJ, Rodrigues L, Nascimento M, Marques T.** 2009. Sequence-based and monoclonal antibody typing of *Legionella pneumophila* isolated from patients in Portugal during 1987–2008. Euro. Surveill. **14**: 409–412.
8. **Dey R, Bodennec J, Mameri MO, Pernin P.** 2009. Free-living freshwater amoebae differ in their susceptibility to the pathogenic bacterium *Legionella pneumophila.* FEMS Microbiol. Lett. **290**:10–17.
9. **Fields BS.** 1996. The molecular ecology of legionellae. Trends Microbiol. **4**:286–290.
10. **Furuhata K, Okabe Y, Dogasaki C, Hara M, Fukuyama M.** 2002. Isolation of *Legionella* spp. from soils in Japan. Bokin Bobai **30**:555–561. (In Japanese.)
11. **Gaia V, et al.** 2005. Consensus sequence-based scheme for epidemiological typing of clinical and environmental isolates of *Legionella pneumophila.* J. Clin. Microbiol. **43**:2047–2052.
12. **Harrison TG, Afshar B, Doshi N, Fry NK, Lee JV.** 2009. Distribution of *Legionella pneumophila* serogroups, monoclonal antibody subgroups and DNA sequence types in recent clinical and environmental isolates from England and Wales (2000–2008). Eur. J. Clin. Microbiol. Infect. Dis. **28**: 781–791.
13. **Helbig JH, et al.** 2002. Pan-European study on culture-proven Legionnaires' disease: distribution of *Legionella pneumophila* serogroups and monoclonal subgroups. Eur. J. Clin. Microbiol. Infect. Dis. **21**:710–716.
14. **Hunter PR, Gaston MA.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26**:2465–2466.
15. **Kozak NA, et al.** 2009. Distribution of *lag-1* alleles and sequence-based types among *Legionella pneumophila* serogroup 1 clinical and environmental isolates in the United States. J. Clin. Microbiol. **47**:2525–2535.
16. **Lee HK, Shim JI, Kim HE, Yu JY, Kang YH.** 2010. Distribution of *Legionella* species from environmental water sources of public facilities and genetic diversity of *L. pneumophila* serogroup 1 in South Korea. Appl. Environ. Microbiol. **76**:6547–6554.
17. **Lim YH, et al.** 2011. Environmental surveillance and molecular characterization of *Legionella* in tropical Singapore. Trop. Biomed. **28**:149–159.
18. **McCabe S, Brown A, Edwards GF, Lindsay D.** 2011. Enhanced isolation of *Legionella* species from composted material. Clin. Microbiol. Infect. **10**:1517–1520.
19. **National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour, and Welfare.** 2000. Legionellosis, April 1999-July 2000. IASR (Infectious Agents Surveillance Report) **21**:186–187.
20. **Patterson WJ, Hay J, Seal DV, McLuckie JC.** 1997. Colonization of transplant unit water supplies with *Legionella* and protozoa: precautions required to reduce the risk of legionellosis. J. Hosp. Infect. **37**:7–17.
21. **Ratzow S, Gaia V, Helbig JH, Fry NK, Lück PC.** 2007. Addition of *neuA*, the gene encoding *N*-acylneuraminate cytidylyl transferase, increases the discriminatory ability of the consensus sequence-based scheme for typing *Legionella pneumophila* serogroup 1 strains. J. Clin. Microbiol. **45**:1965–1968.
22. **Reimer AR, Au S, Schindle S, Bernard KA.** 2010. *Legionella pneumophila* monoclonal antibody subgroups and DNA sequence types isolated in Canada between 1981 and 2009: laboratory component of national surveillance. Eur. J. Clin. Microbiol. Infect. Dis. **29**:191–205.
23. **Stockman LJ, Wright CJ, Visvesvara GS, Fields BS, Beach MJ.** 2011. Prevalence of *Acanthamoeba* spp. and other free-living amoebae in household water, Ohio, U.S.A.—1990–1992. Parasitol. Res. **108**:621–627.
24. **Travis TC, et al.** 2012. Survey of *Legionella* species found in Thai soil. Int. J. Microbiol. **2012**:218791.
25. **Velonakis EN, et al.** 2010. First isolation of *Legionella* species, including *L. pneumophila* serogroup 1, in Greek potting soils: possible importance for public health. Clin. Microbiol. Infect. **16**:763–766.
26. **Zhou H, et al.** 2010. Optimization of pulsed-field gel electrophoresis for *Legionella pneumophila* subtyping. Appl. Environ. Microbiol. **76**:1334–1340.