

# A Multilocus Sequence Typing Scheme Implies Population Structure and Reveals Several Putative Novel *Achromobacter* Species

Theodore Spilker,<sup>a</sup> Peter Vandamme,<sup>b</sup> and John J. LiPuma<sup>a</sup>

Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, Michigan, USA,<sup>a</sup> and Laboratory of Microbiology, Ghent University, Ghent, Belgium<sup>b</sup>

The genus *Achromobacter* currently is comprised of seven species, including *Achromobacter xylosoxidans*, an opportunistic and nosocomial pathogen that displays broad-spectrum antimicrobial resistance and is recognized as causing chronic respiratory tract infection in persons with cystic fibrosis (CF). To enable strain typing for global epidemiologic investigations, to clarify the taxonomy of “*Achromobacter*-like” strains, and to elucidate the population structure of this genus, we developed a genus-level multilocus sequence typing (MLST) scheme. We employed *in silico* analyses of whole-genome sequences of several phylogenetically related genera, including *Bordetella*, *Burkholderia*, *Cupriavidus*, *Herminiimonas*, *Janthinobacterium*, *Methylibium*, and *Ralstonia*, for selecting loci and designing PCR primers. Using this MLST scheme, we analyzed 107 genetically diverse *Achromobacter* isolates cultured from biologic specimens from CF and non-CF patients, 1 isolate recovered from sludge, and an additional 39 strains obtained from culture collections. Sequence data from these 147 strains, plus three recently genome-sequenced *Achromobacter* strains, were assigned to 129 sequence types based on seven loci. Calculation of the nucleotide divergence of concatenated locus sequences within and between MLST clusters confirmed the seven previously named *Achromobacter* species and revealed 14 additional genogroups. Indices of association showed significant linkage disequilibrium in all of the species/genogroups able to be tested, indicating that each group has a clonal population structure. No clear segregation of species/genogroups between CF and non-CF sources was found.

When the genus *Achromobacter* was first proposed, it included *Achromobacter xylosoxidans* as its solitary species (17). *Achromobacter ruhlandii* and *Achromobacter piechaudii* subsequently were reassigned to this genus from the genus *Alcaligenes* (16), and thereafter, *Achromobacter xylosoxidans* subsp. *denitrificans* was reclassified as *Achromobacter denitrificans* (4). More recently, *Achromobacter spanius*, *Achromobacter insolitus*, and *Achromobacter marplatensis* were described as novel members of the genus (5, 7). These *Achromobacter* species vary with respect to their preferred environmental niches and impact on human health. *A. xylosoxidans* can be isolated from various water sources and is well recognized as an opportunistic human pathogen capable of causing a variety of infections, including endophthalmitis, keratoconjunctivitis, catheter-associated bloodstream infection, endocarditis, pneumonia, meningitis, and peritonitis (1, 12, 14). The remaining *Achromobacter* species are considered inhabitants of soil and have been much less frequently associated with human infection (4, 5, 7, 9).

*Achromobacter* infections are particularly noteworthy in persons with cystic fibrosis (CF). Although potential virulence factors remain to be elucidated and the role that respiratory tract infection with *Achromobacter* plays in contributing to lung disease in CF remains unclear, existing data indicate that airway infection in CF patients can be transient or chronic (10, 15). The incidence of *Achromobacter* infection in this patient population may be increasing; approximately 6.0% of CF patients were reported to have had *Achromobacter* infection in 2005, with the highest rate (8.1%) being seen among persons between the ages of 18 and 25 years (10).

It is a challenge to better understand the epidemiology, ecology, and clinical impact of human infection with *Achromobacter* because the taxonomy and population structure of these species are poorly defined, and consequently, accurate species identifica-

tion is problematic. *Achromobacter* species are frequently misidentified by commercial identification systems, being most commonly mistaken for *Alcaligenes*, *Bordetella*, *Burkholderia*, *Cupriavidus*, *Pandoraea*, *Ralstonia* and *Stenotrophomonas* species (2, 11). By using repetitive-element PCR (rep-PCR)-based genotyping employing the BOX A1R primer (BOX-PCR), we have noted remarkable diversity among strains we previously identified as *A. xylosoxidans* using a species-specific PCR assay designed to target signature 16S rRNA sequences in this species (11).

The objective of this study was to develop a multilocus sequence typing (MLST) scheme for *Achromobacter* to elucidate the taxonomy and population structure of this genus. We took advantage of an extensive collection of diverse “*Achromobacter*-like” strains, as well as several recently available whole-genome sequences of strains belonging to related species.

## MATERIALS AND METHODS

**Bacterial strains.** The 150 strains included in this study are listed in the supplemental material (see Table S1). A total of 115 isolates recovered from culture of biologic specimens from patients with and without CF were obtained from the strain collection of the *Burkholderia cepacia* Research Laboratory and Repository (University of Michigan). All of these strains tested positive in a PCR assay previously designed to target *Achromobacter* 16S rRNA (11) and were selected, based on repetitive-element

Received 27 March 2012 Returned for modification 4 May 2012

Accepted 30 June 2012

Published ahead of print 11 July 2012

Address correspondence to John J. LiPuma, jlipuma@umich.edu.

Supplemental material for this article may be found at <http://jcm.asm.org/>.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.00814-12

TABLE 1 Genes used for the MLST scheme with primers used for amplification

Gene	Primer sets (5' → 3') <sup>a</sup>	Temp (°C)	Amplicon size (bp)	Gene fragment size (bp) <sup>b</sup>
<i>nusA</i>	P1, GCCTGCAAGARCCCGACAAGP2, GTCCATSGCGTGTCTTC	59	795	355
<i>rpoB</i>	P1, TGCCMTGGAACGGYTACAACP2, GGCCAGRTASACCTTGATCATCTT	57	791	413
<i>eno</i>	P1, ATGCCCGTGCCSATGATGAAP2, TCAGGGTGCCGATCTGGTTG	57	613	214
<i>gltB</i>	P1, TGCAACCGGGCAAGATGTP2, TCGGACACGATCAGGATGTT	57	685	241
<i>lepA</i>	P1, CTAYAACCTGAACCTGATCGACAC P2, GCGACTTSGGCGTGAACAC	57	524	347
<i>nuoL</i>	P1, CATGCACCAYRACCAGGACATP2, CGCGAACGCGTAGTGATAGATG	56	880	230
<i>nrdA</i>	P1, GAACTGGATTCCCGACCTGTTCP2, TTCGATTTGACGTACAAGTTCTGG	56	954	449

<sup>a</sup> Standard mixed oligonucleotide bases for primer sequences are listed in bold.

<sup>b</sup> Sequence length used in MLST analysis for each locus.

PCR genotyping (3), to represent distinct strains (i.e., to avoid including duplicate strains in the sample set). An additional strain was recovered from sludge. Thirty-one reference strains, which included representatives of the seven named *Achromobacter* species, were obtained from the BCCM/LMG (Ghent, Belgium) and ATCC (Manassas, VA) culture collections. DNA sequences from the three remaining *Achromobacter* strains were obtained from NCBI (<http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi>).

**Culture of bacteria and preparation of DNA.** All bacterial cultures were incubated aerobically at 32°C for 24 h on Mueller-Hinton agar. For DNA preparation, a single colony was suspended in 20 µl of lysis buffer containing 0.25% (vol/vol) sodium dodecyl sulfate and 0.05 N NaOH. After heating for 15 min at 95°C, 180 µl of high-pressure liquid chromatography (HPLC)-grade H<sub>2</sub>O was added. The suspension was centrifuged at 13,300 rpm for 5 min, and the supernatant was stored at 4°C.

**Selection of MLST loci.** At the outset of this project, no *Achromobacter* whole-genome sequences were available. Thus, we initially analyzed annotated DNA sequences of phylogenetically related (based on 16S rRNA sequence analysis) genera available at NCBI, including the following 19 strains, representing seven genera: *Bordetella avium* 197N, *Bordetella bronchiseptica* RB50, *Bordetella pertussis* Tohama I, *Bordetella parapertussis* 12822, *Bordetella petrii* DSM 12804, *Burkholderia ambifaria* MC40-6, *Burkholderia cenocepacia* J2315, *Burkholderia mallei* ATCC 23344, *Burkholderia phytofirmans* PsJN, *Cupriavidus metallidurans* CH34, *Cupriavidus necator* H16, *Cupriavidus pinotubonensis* JMP134, *Cupriavidus taiwanensis* LMG 19424, *Hermiiniimonas arsenicoxydans* ULPAs1, *Janthinobacterium* sp. Marseille, *Methylobium petroleiphilum* PM1, *Ralstonia pickettii* 12D, *Ralstonia pickettii* 12J, and *Ralstonia solanacearum* GMII1000.

DNA sequences were aligned with MAUVE 2.3.0 build 98(c) using default parameters with MUSCLE 3.6 to identify large contiguous blocks (LCBs) that were present in all genomes and that had approximately 70% homology. LCBs were analyzed, using the annotated genome of *B. bronchiseptica* RB50 as a reference, to identify candidate MLST genes. Candidate genes from all 19 sequenced strains were aligned using Clustal W, and the resulting dendrograms were each compared to a dendrogram based on the respective 16S rRNA genes from these strains. If the two dendrograms indicated different phylogenies, then the gene was eliminated as a possible MLST candidate. Candidate genes were limited to those with only a single copy in the genome, and in the case of species with multiple chromosomes (e.g., *Burkholderia* spp.), candidate genes were also limited to those located on the primary chromosome. Genes were removed as candidates if a paralog or homolog with a nucleotide identity of ≥80% was identified in any one of the 19 sequenced strains. Ultimately, this process identified

seven genes for inclusion in the MLST scheme: *eno*, *gltB*, *lepA*, *nrdA*, *nuoL*, *nusA*, and *rpoB*.

**Amplification and DNA sequencing.** The MLST locus within each of the selected genes ranged in length from 214 bp (*eno*) to 449 bp (*nrdA*) (Table 1). PCR primers for each locus were manually designed to target sequences ~50 to 200 bases upstream or downstream from the sequence of interest and then checked for hairpin turns and primer dimers using Primer Select (DNASar, Madison, WI). Amplification of targeted DNA was carried out in 25-µl reaction volumes, each containing final concentrations of the following reagents: 2 mM MgCl<sub>2</sub>, 50 mM KCl, 20 mM Tris-Cl (pH 8.4), 250 µM (each) deoxynucleoside triphosphates (ISC Bio-Express, Kaysville, UT), 0.4 µM (each) primer (IDT Technology, Coralville, IA), 5% (vol/vol) dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO), 1 M betaine monohydrate (Sigma), 2 U of *Taq* polymerase (Invitrogen, Carlsbad, CA), and 2 µl of whole-cell bacterial lysate, adjusted to 25 µl by the addition of HPLC-grade H<sub>2</sub>O. Amplification was carried out in a PTC-100 (Bio-Rad, Hercules, CA) thermal controller. After an initial denaturation for 2 min at 95°C, 30 cycles were completed, with each consisting of 30 s at 94°C, 30 s at the appropriate annealing temperature (Table 1), and 60 s at 72°C. A final extension of 5 min at 72°C was applied with an infinite hold at 8°C. Amplified PCR products were purified using the Qiagen QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) by following the manufacturer's instructions. DNA sequencing was carried out with an Applied Biosystems ABI model 3730 sequencer using the protocols provided by the manufacturer (PE Applied Biosystems, Foster City, CA) by using the BigDye Terminator cycle sequencing ready reaction kit.

Sequence chromatograms were visualized and edited with Chromas version 2.31 (Technelysium Pty. Ltd.). All sequences were aligned and appropriate cutoffs were applied using MegAlign (DNASar); trimmed sequences were saved individually. Concatenation of sequences was performed with SeqEdit (DNASar).

**Allele and ST assignment.** Alignments of sequences from each gene were performed using Clustal V. A different number was assigned to each unique allele, and a sequence type (ST) was assigned to each unique allelic profile. These are available in the *Achromobacter* MLST database (<http://pubmlst.org/achromobacter/>).

**Phylogenetic analyses.** Alignment of concatenated MLST sequences (2,249 bp) for each isolate was performed using MegAlign with Clustal W to generate a phylogenetic tree, with 1,000 bootstrap replications using the default parameters in MegAlign. Statistical analyses of allelic profiles, number of polymorphic sites, frequencies of alleles, GC content, and the ratio of nonsynonymous to synonymous substitutions (*dN/dS*) were calculated using START (<http://pubmlst.org/software/analysis/start/>). The

TABLE 2 Analysis of the seven MLST loci in the 150 strains studied

Gene	No. of alleles	No. of polymorphic sites	% GC content	Simpson's index ( <i>D</i> )	<i>dN/dS</i>
<i>nusA</i>	62	87	64.6	0.973	0.0136
<i>rpoB</i>	70	52	62.8	0.959	0.0448
<i>eno</i>	64	47	60.6	0.973	0.0469
<i>gltB</i>	53	45	62.9	0.963	0.0350
<i>lepA</i>	69	93	64.9	0.978	0.0946
<i>nuoL</i>	58	54	62.2	0.976	0.1042
<i>nrdA</i>	65	77	58.2	0.980	0.0473

standardized index of association ( $I_A$ ) was calculated using LIAN 3.5 (<http://pubmlst.org>), and significance ( $P = 0.05$ ) was established under the null hypothesis of linkage equilibrium ( $H_0: V_D = V_e$ ) by performing 1,000 Monte Carlo resamplings. Simpson's index of diversity was calculated using the comparing partitions program (available at <http://darwin.phylovis.net/ComparingPartitions/index.php?link=Tool>).

For validation of distinctness of sequence similarity clusters in the population, the  $k$  parameter, which is the ratio of the intergroup divergence to the mean of the intragroup divergence, was calculated as previously described (13). Ratios greater than 2 were considered distinct sequence similarity clusters. Subgroups within clusters were analyzed separately and designated "a" or "b."

All sequence data and related information were deposited in the *Achromobacter* MLST database (<http://pubmlst.org/achromobacter/>).

## RESULTS

**Allelic variation in *Achromobacter*.** Analysis of all 150 strains revealed 129 STs (see Table S1 in the supplemental material). The number of alleles at each locus ranged from 53 (*gltB*) to 70 (*rpoB*), while the number of polymorphic sites ranged from 45 (*gltB*) to 93 (*lepA*) (Table 2). Simpson's index of diversity ranged from 0.959 (*nuoL*) to 0.980 (*nusA*), indicating a high level of discrimination. Low *dN/dS* values indicated the absence of strong positive selection pressure at these loci and supported their suitability for use in population genetic analyses.

***Achromobacter* species and genogroup delineations.** Alignment of concatenated MLST sequences from all 150 isolates revealed interspecies/genogroup divergence rates ranging from 1.6% to 5.3%. Therefore, a rate of 1.6% was used as the cutoff for defining the lower limit of any single species or genogroup (Fig. 1). Maximum intraspecies divergence was 2.1%. Applying this as the cutoff in the concatenated MLST dendrogram (rooted with *Bordetella* reference strains) identified at least 14 additional genogroups, most of which had distinctness of population ratio ( $k$ ) parameters of  $\geq 2$  (Fig. 2). *A. xylosoxidans* and *A. marplatensis* could not be considered separate sequence similarity clusters, with  $k$  values of 1.68, despite both being taxonomically validly named species. The only other genogroup failing to meet the distinctness of population ratio was *Achromobacter* genogroup 5 ( $k = 1.58$ ); however, the closest genogroup, *Achromobacter* genogroup 7, contained only 2 strains. The five remaining previously named

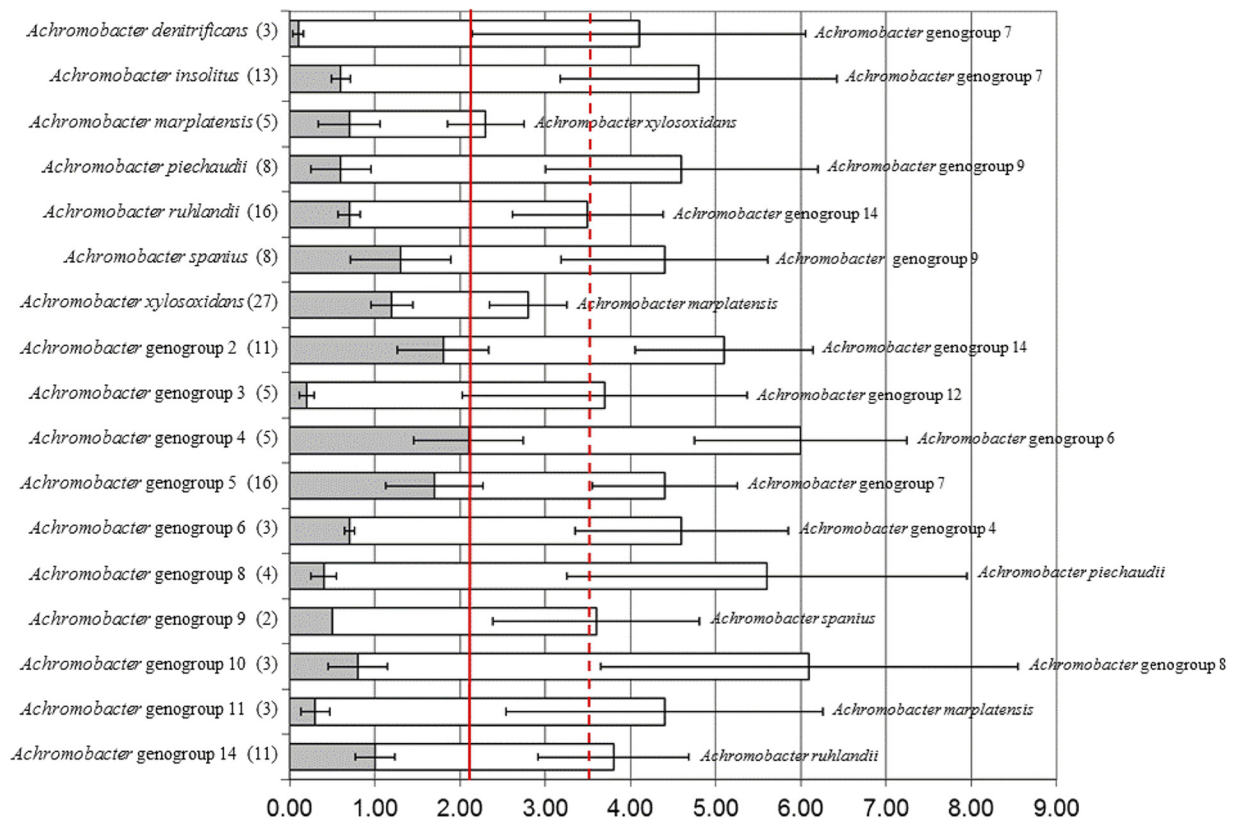


FIG 1 Nucleotide divergence of concatenated MLST loci. Shaded bars represent divergence within a species/genogroup; open bars represent the divergence between species/genogroup and its closest neighbor (listed to the right), as defined by percentage similarity. Error bars represent the standard errors of the shaded and open bars. The solid line represents maximum intraspecies divergence; the dashed line represents the average interspecies divergence.

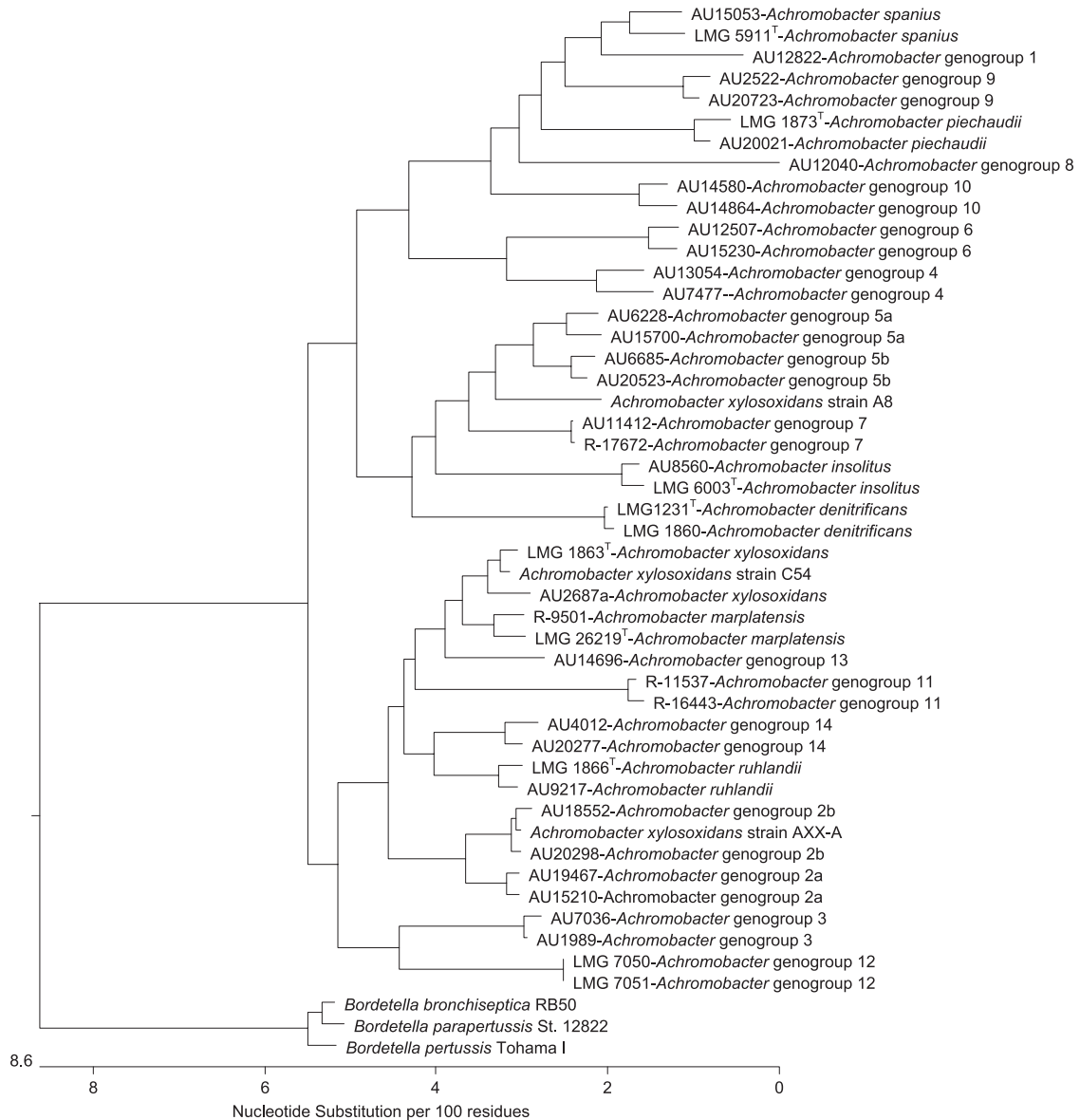


FIG 2 Dendrogram of concatenated MLST loci sequences containing two strains of each *Achromobacter* species or genogroup (unless only a single strain was available) and rooted with *Bordetella* species.

*Achromobacter* species and new genogroups had  $k$  parameters indicating distinctness (range, 2.36 to 40.00).

**Presence of distinct subgroups.** The concatenated MLST tree revealed that *Achromobacter* genogroups 2 and 5 contained distinct subgroups, each labeled a and b. Using bootstrapping with 1,000 replicates, *Achromobacter* genogroups 2a and 2b showed 100% support that distinct nodes would be maintained. Similarly, *Achromobacter* genogroups 5a and 5b showed 93.8% support that distinct nodes would be maintained. Intradivergence and interdivergence levels, as well as  $k$  parameters, indicated that genogroups 2a and 2b and genogroups 5a and 5b could be considered separate clusters, with  $k$  parameters of 3.27 and 2.62, respectively. These data provide evidence that these two genogroups contain distinct diverging subgroups.

**Linkage disequilibrium.** Assessment of the population struc-

ture of *Achromobacter* was investigated by calculating the  $I_A$  for each species or genogroup. All species/genogroups showed  $I_A$  values differing from zero, indicating that all were in linkage disequilibrium. However, five groups (genogroups 1, 7, 9, 12, and 13) did not include enough strains for reliable testing of significance between the observed variance and maximum trial variance based on the criteria proposed by Kaiser et al. (8). Among the remaining 16 species/genogroups, all showed significant evidence of linkage disequilibrium based on the number of strains analyzed, while 14 maintained significance when analyzed for number of STs only (see Table S2 in the supplemental material).

## DISCUSSION

*Achromobacter* species are increasingly recognized as nosocomial and opportunistic human pathogens, particularly in persons with



CF, in whom respiratory tract infection likely contributes to progressive lung disease (10). The broad-spectrum antibiotic resistance of most clinical isolates limits effective therapy of infection, and prevention of infection and identification of virulence factors are challenged by an incomplete understanding of the ecology, epidemiology, and taxonomy of *Achromobacter* species. Unfortunately, little progress has been made in these areas during the past decade. The novel species *A. insolitus* and *A. spanius* were added to the genus in 2003, and *A. marplatensis* was proposed as yet another novel species in 2010, bringing the total number of validly named species in this genus to seven (7). However, our ongoing analyses of bacterial isolates recovered primarily from human specimens have identified a variety of “*Achromobacter*-like” strains that cannot be placed into one of these seven species with confidence, suggesting the existence of several additional distinct taxa. These strains, along with strains from each of the seven named species, tested positive in a previously described PCR assay targeting 16S rRNA gene sequences intended to be specific for *A. xylosoxidans* (11). Genotyping analyses of such strains using a rep-PCR typing method (3) indicated a high level of genetic diversity and identified several clusters, again suggestive of distinct species.

In order to delineate the taxonomy and epidemiology of *Achromobacter* species, we sought to devise an MLST scheme that would not only provide strain genotyping but also enable reliable genus-level phylogenetic analyses. We took advantage of the whole-genome sequences available for 19 strains from several related genera and employed a strategy to select candidate MLST genes that would predict phylogenetic relationships among these genera concordant with those predicted by analysis of their complete 16S rRNA gene sequences. Limiting MLST genes to those located on the primary chromosome in species with multiple chromosomes (e.g., *Burkholderia* spp.) avoided genes with potentially greater rates of evolutionary change that may be found on secondary chromosomes (6).

We applied this MLST scheme to examine a large collection of putative *Achromobacter* strains, primarily recovered from human sources and selected based on previous genotyping analysis to represent a diverse strain set. The analysis reiterated the high level of diversity in this set, reflected by a large number of alleles and polymorphic sites, as well as a Simpson index of diversity of at least 0.959 in all seven loci. Our results revealed the existence of 14 distinct genogroups in addition to the seven currently named species. Analysis of the concatenated MLST sequences from the 150 strains included in the study demonstrated that these 21 species/genogroups had low (maximum, 2.1%) intragroup and high (average, 3.54%) intergroup sequence divergence levels. The  $k$  parameter, a measure of distinctness between two groups, was high ( $>2$ ) for most pairwise sets; the value between genogroup 5 and genogroup 7 was limited by the inclusion of only two strains in genogroup 7. Interpretation of the low  $k$  value (1.68%) between *A. xylosoxidans* and *A. marplatensis* was similarly limited by the presence of only two distinct strains in the latter species. This species has been shown, nevertheless, to be a distinct *Achromobacter* species on the basis of polyphasic analyses, including DNA-DNA hybridization values and phenotypic characteristics (7). The concatenated MLST analysis also identified two distinct subgroups in both genogroup 2 and genogroup 5. Sequence divergence levels, as well as  $k$  values, indicated that these four subgroups most likely represent distinct taxa. Confirmation of this will require further

taxonomic analyses and would corroborate that the design of our MLST scheme provides phylogenetically sound data.

The  $I_A$ , a summary statistic based on the variance of pairwise distances between strains in a set, revealed values different from zero for all 21 species/genogroups. However, because the expected value of  $I_A$  depends on sample size, testing of the significance of the  $I_A$  was not reliable in the five genogroups that each contained fewer than three strains. We found significant evidence for linkage disequilibrium in all remaining groups, including the subgroups in genogroups 2 and 5, consistent with a clonal epidemic population structure.

Our results have important implications in efforts to better understand the epidemiology, ecology, clinical microbiology, and virulence of *Achromobacter* species. Advances in each of these areas are predicated on a clearer appreciation of the taxonomy and population structure of this genus. For example, we have found that, based on a preliminary analyses of a large set of *Achromobacter* isolates recovered from respiratory cultures from CF patients in the United States, approximately 1/3 are not *A. xylosoxidans*, although essentially all were initially identified as this species by commercial identification systems. It appears *A. ruhlandii* will comprise a considerable minority of *Achromobacter* species recovered from CF patients. Our analysis also shows that, among the *A. xylosoxidans* strains for which genome sequencing has been performed, only strain C54 is actually a member of this species; strain AXX-A is placed in genogroup 2b, while strain A8 cannot be confidently placed in any of the 21 species/genogroups identified in our study.

Our MLST scheme provides a sequence database and method for genotyping *Achromobacter* strains for global epidemiologic studies and for assigning strains to defined or new genogroups. Ongoing work is aimed at developing less labor-intensive methods to delineate each of the species/genogroups in this genus.

## ACKNOWLEDGMENT

This work was supported by the Cystic Fibrosis Foundation.

## REFERENCES

1. Aisenberg G, Rolston KV, Safdar A. 2004. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989–2003). *Cancer* 101:2134–2140.
2. Brisse S, et al. 2002. Comparative evaluation of the BD Phoenix and VITEK 2 automated instruments for identification of isolates of the *Burkholderia cepacia* complex. *J. Clin. Microbiol.* 40:1743–1748.
3. Coenye T, Spilker T, Martin A, LiPuma JJ. 2002. Comparative assessment of genotyping methods for epidemiologic study of *Burkholderia cepacia* genomovar III. *J. Clin. Microbiol.* 40:3300–3307.
4. Coenye T, et al. 2003. *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Ruger and Tan 1983 as *Achromobacter denitrificans* comb. nov. *Int. J. Syst. Evol. Microbiol.* 53:1825–1831.
5. Coenye T, Vancanneyt M, Falsen E, Swings J, Vandamme P. 2003. *Achromobacter insolitus* sp. nov. and *Achromobacter spanius* sp. nov., from human clinical samples. *Int. J. Syst. Evol. Microbiol.* 53:1819–1824.
6. Cooper VS, Vohr SH, Wrocklage SC, Hatcher PJ. 2010. Why genes evolve faster on secondary chromosomes in bacteria. *PLoS Comput. Biol.* 6:e1000732.
7. Gomila M, et al. 2011. *Achromobacter marplatensis* sp. nov., isolated from a pentachlorophenol-contaminated soil. *Int. J. Syst. Evol. Microbiol.* 61:2231–2237.
8. Kaiser S, Biehler K, Jonas D. 2009. A *Stenotrophomonas maltophilia* multilocus sequence typing scheme for inferring population structure. *J. Bacteriol.* 191:2934–2943.
9. Kiredjian M, Holmes B, Kersters K, Guilvout I, De Ley J. 1986. *Alcal-*

- igenes piechaudii*, a new species from human clinical specimens and the environment. *Int. J. Syst. Evol. Microbiol.* 36:282–287.
10. LiPuma JJ. 2010. The changing microbial epidemiology in cystic fibrosis. *Clin. Microbiol. Rev.* 23:299–323.
  11. Liu L, et al. 2002. Ribosomal DNA-directed PCR for identification of *Achromobacter* (*Alcaligenes*) *xylosoxidans* recovered from sputum samples from cystic fibrosis patients. *J. Clin. Microbiol.* 40:1210–1213.
  12. Oh JY, Shin YJ, Wee WR. 2005. A case of epidemic keratoconjunctivitis complicated by *Alcaligenes xylosoxidans* infection. *Korean J. Ophthalmol.* 19:233–234.
  13. Palys T, Nakamura LK, Cohan FM. 1997. Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int. J. Syst. Bacteriol.* 47:1145–1156.
  14. Robert PY, et al. 2008. *Alcaligenes xylosoxidans* endophthalmitis following phacoemulsification and intraocular lens implantation. *Ophthalmic Surg. Lasers Imaging* 39:500–504.
  15. Rønne Hansen C, Pressler T, Høiby N, Gormsen M. 2006. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients; a retrospective case control study. *J. Cyst. Fibros.* 5:245–251.
  16. Yabuuchi E, Kawamura Y, Kosako Y, Ezaki T. 1998. Emendation of genus *Achromobacter* and *Achromobacter xylosoxidans* (Yabuuchi and Yano) and proposal of *Achromobacter ruhlandii* (Packer and Vishniac) comb. nov., *Achromobacter piechaudii* (Kiredjian et al.) comb. nov., and *Achromobacter xylosoxidans* subsp. *denitrificans* (Ruger and Tan) comb. nov. *Microbiol. Immunol.* 42:429–438.
  17. Yabuuchi E, Yano I. 1981. *Achromobacter* gen. nov. and *Achromobacter xylosoxidans* (ex Yabuuchi and Ohyama 1971) nom. rev. *Int. J. Syst. Evol. Microbiol.* 31:477–478.