Reassessment of *Stenotrophomonas maltophilia* Phenotype[▽]

Lisa A. Carmody, Theodore Spilker, and John J. LiPuma*

Department of Pediatrics and Communicable Disease, University of Michigan Medical School, Ann Arbor, Michigan 48109

Received 1 November 2010/Returned for modification 1 December 2010/Accepted 22 December 2010

Standard microbiology references describe *Stenotrophomonas maltophilia* as oxidase negative and variable with respect to utilization of lactose and sucrose. Analysis of a collection of 766 *S. maltophilia* isolates indicated that approximately 20% are oxidase positive and that this species should be reevaluated for other phenotypes, including oxidative fermentation of lactose and sucrose.

Stenotrophomonas maltophilia is an emerging opportunistic pathogen of particular importance owing to its intrinsic multi-drug resistance. Typically found in water and soil and on plants (7), it is also frequently isolated as a contaminant of medical devices and hospital water sources, faucets, and sinks (3). *S. maltophilia* is not usually highly virulent in healthy persons, although it can be a considerable source of morbidity and mortality for immunocompromised and hospitalized patients (7). The species is also commonly associated with respiratory tract infection in persons with cystic fibrosis (CF) (6), although the impact on outcomes in this population is unclear (8).

S. maltophilia was initially classified in the genus *Pseudomonas* (5) and then *Xanthomonas* (12) before being placed into the new *Stenotrophomonas* genus (10). Misidentification of *S. maltophilia* is not uncommon (1, 3). A species-specific PCR assay targeting the 23S rRNA gene has been developed as a genotypic method for identification (14); however, phenotypic identification still presents a challenge for the clinical microbiology laboratory. The ninth edition of the *Manual of Clinical Microbiology* describes *S. maltophilia* as oxidase negative and variable with respect to utilization of lactose and sucrose (9). In contrast, a considerable minority of *S. maltophilia* isolates analyzed by the *Burkholderia cepacia* Research Laboratory and Repository (BcRLR; University of Michigan, Ann Arbor) were found to be oxidase positive, and most have been found to be negative for oxidative fermentation of lactose and sucrose. We describe these findings, which should allow improved phenotypic identification of this species.

Isolates referred to the BcRLR for species identification were evaluated by polyphasic analyses. All strains were grown aerobically for 24 h at 32°C on nonselective Mueller Hinton agar in ambient air. Bacterial DNA was prepared as described previously (11). Briefly, a single CFU was suspended in 20 μ l of lysis buffer containing 0.25% (vol/vol) sodium dodecyl sulfate and 0.05 N NaOH. After the mixture was subjected to heating for 15 min at 95°C, 180 μ l of high-performance liquid chromatography-grade water (Fisher, Pittsburgh, PA) was added.

A 23S rRNA gene-directed PCR assay was performed as previously described (14). Sequencing and analysis of the 16S rRNA gene were also performed as previously described (11), but with the following modifications. A BLASTN search (<http://blast.ncbi.nlm.nih.gov>) was performed using the complete 16S rRNA gene sequence to obtain a tentative species identification. DNA sequences were aligned using MegAlign (DNASTAR, Madison, WI) and trimmed to contain the same number of nucleotides (1,428 bp), allowing equal weighting in a ClustalV-based tree. An average of 126 bp was removed from the combined 5' and 3' ends of the full-length 16S rRNA sequences due to primer location and DNA sequence editing. The trimmed sequences were then aligned with 16S rRNA gene sequences from a panel of 11 *Stenotrophomonas*, *Xanthomonas*, and *Pseudomonas* reference strains available in the NCBI database. These strains included *Stenotrophomonas nitritireducens* L2 (DSM 12575^T), *Stenotrophomonas rhizophila* e-p10 (LMG 24537^T), *Stenotrophomonas acidaminiphila* amx 19 (ATCC 700916), *Stenotrophomonas humi* R-32729^T, *Stenotrophomonas koreensis* strain TR6-01 (LMG 23369^T), *Stenotrophomonas terrae* R-32768 (LMG 23958^T), *Stenotrophomonas maltophilia* LMG 958^T, *Xanthomonas campestris* pv. *campestris* LMG 568^T, *Xanthomonas axonopodis* pv. *axonopodis* LMG 538^T, *Xanthomonas oryzae* pv. *oryzae* LMG 5047^T, and *Pseudomonas dokdonensis* Yoon DS-16 LMG 24163^T (*S. dokdonensis* basonym). Isolates that clustered with the *S. maltophilia* LMG 958^T type strain were classified as *S. maltophilia*. DNA sequence identity values among the *S. maltophilia* strains ranged from 100% to 98.9%. The next closest species was *Stenotrophomonas humi*, with an identity value of 97.4%.

Isolates identified as *S. maltophilia* by PCR and DNA sequence analyses were examined for the presence of cytochrome c oxidase by the use of a Remel BactiDrop Oxidase test kit (Remel Products, Lenexa, KS). Briefly, a small amount of bacteria was collected on a sterile cotton swab and one drop of oxidase reagent was applied to the bacteria. Development of a violet to deep purple color within 10 s to 30 s was considered to represent a positive result. Oxidative fermentation of lactose and sucrose was determined using BAM M117 medium. Oxidative-fermentative test medium was prepared per the guidelines presented on the FDA website (<http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM063487>) with the following modifications. The medium pH was adjusted to 6.95, and inoculated tubes were

* Corresponding author. Mailing address: Department of Pediatrics and Communicable Disease, University of Michigan Medical School, 8323 MSRB III, SPC 5646, 1150 W. Medical Center Dr., Ann Arbor, MI 48109. Phone: (734) 615-4616. Fax: (734) 764-4279. E-mail: jlipuma@umich.edu.

[▽] Published ahead of print on 29 December 2010.

incubated at 37°C. Isolates were considered to have returned a positive result when the media turned yellow within 24 h; a color change at the air-medium interface only was not considered to represent a positive result.

A total of 831 isolates referred to the BcRLR from 152 clinical microbiology laboratories in 42 U.S. states during a 12-year period were identified as *S. maltophilia* by 23S rRNA-directed PCR. Among the 766 isolates tested for oxidase activity, 154 (20%) gave positive results. Of these oxidase-positive *S. maltophilia* isolates, 124 (80.5%) had not been correctly identified by the referring microbiology laboratories, while 356 (58.2%) of the oxidase-negative strains had not been identified as *S. maltophilia*. Most of the strains that had not been correctly identified as *S. maltophilia* by the referring laboratories either had not been classified to the species level (i.e., had been identified only as “Gram negative rod” or “nonfermenter”) (64%) or were identified as *B. cepacia* complex (28%). Of 516 strains tested for utilization of lactose and sucrose, seven (1.4%) utilized lactose and 11 (2.1%) utilized sucrose.

A review of the source literature for the data presented in the ninth edition of the *Manual of Clinical Microbiology*, which characterizes *S. maltophilia* as oxidase negative, indicated that these results were derived from an analysis of 73 isolates. These isolates were reported as being uniformly oxidase negative on the basis of direct application of the oxidase reagent to colonies cultured on rabbit's blood agar (13). A footnote to the table describing these results indicates, however, that when tested by Kovacs' method, 14 (19.2%) of the 73 isolates gave oxidase-positive results. The more sensitive Kovacs' test is comparable to the Remel BactiDrop Oxidase test kit we employed and yielded results consistent with our finding that 154 (20.1%) of the 766 *S. maltophilia* strains tested were oxidase positive. Of note, the manufacturers of Kovacs' reagent indicate that the test should not be performed with bacteria recovered from selective or differential media, such as blood agar. The fermentation of glucose, as found in rabbit's blood agar media, can inhibit oxidase activity (9).

The ninth edition of the *Manual of Clinical Microbiology* reports that acid production by oxidative fermentation of lactose and sucrose by *S. maltophilia* is variable but is produced by the majority of strains, indicating that 60% and 63% of 73 strains tested were positive for these phenotypes, respectively. Our findings reveal much lower rates of utilization of these carbohydrates by *S. maltophilia*. The reason(s) for this disparity in results is not entirely clear. However, the data described in the *Manual of Clinical Microbiology* were based on a report published around the time when the taxonomy of *S. maltophilia* was being revised. It is possible that inaccurate differentiation of *S. maltophilia* from related species (e.g., *Xanthomonas* sp.) contributed to the phenotypic results reported.

Lack of species specificity of the 23S rRNA gene-directed PCR assay reported by Whitby et al. (14) (and used by the BcRLR for the identification of *S. maltophilia*) has been previously reported. Using a modification of that assay in which an additional 15 PCR cycles were applied, Foster et al. (4) described positive reactions with one strain each of *Stenotrophomonas acidaminiphila*, *Stenotrophomonas rhizophila*, *Stenotrophomonas nitritireducens*, *Xanthomonas axonopodis* pv. *Citri*, and *Xanthomonas campestris* pv. *campestris*.

To better assess the accuracy of this PCR assay in identifying the strains referred to us as *S. maltophilia*, we sequenced the 16S rRNA gene from 21 of the isolates identified as *S. maltophilia* on the basis of the 23S rRNA-directed PCR assay. This subset of isolates was selected to include representatives of every phenotype observed among the *S. maltophilia* isolates in our collection. BLASTN analysis of the 16S rRNA gene sequences indicated that all 21 isolates had the highest identity to *S. maltophilia*. In addition, the 16S rRNA sequences from the 21 isolates clustered exclusively with the *S. maltophilia* type strain in a tree that included other *Stenotrophomonas* species. This is consistent with the observation that *Xanthomonas* species and *Stenotrophomonas* species other than *S. maltophilia* have been recovered only very rarely from human specimens (2).

The results described herein are based on an analysis of isolates that were derived primarily (although not exclusively) from cultures of respiratory specimens from persons with cystic fibrosis. It is also likely that isolates referred to the BcRLR from clinical microbiology laboratories are biased toward those that were difficult to identify with commercially available test systems. Regardless of the source of isolates, however, our findings indicate that *S. maltophilia* should be considered variable with respect to oxidase activity and that the rates of utilization of sucrose and lactose by *S. maltophilia* strains have been overestimated. Incorporating this revised information into identification algorithms (particularly the change in oxidase reactivity, which plays a key role in identification of aerobic Gram-negative bacteria) should improve the identification of *S. maltophilia* based on phenotypic assessment. The improved understanding of the taxonomy of *Stenotrophomonas* and related genera provides an opportunity for a more comprehensive reassessment of the phenotypes of these species.

This work was supported by the U.S. Cystic Fibrosis Foundation.

REFERENCES

- Burdge, D. R., M. A. Noble, M. E. Campbell, V. L. Krell, and D. P. Speert. 1995. *Xanthomonas maltophilia* misidentified as *Pseudomonas cepacia* in cultures of sputum from patients with cystic fibrosis: a diagnostic pitfall with major clinical implications. *Clin. Infect. Dis.* **20**:445–448.
- Coenye, T., J. Goris, T. Spilker, P. Vandamme, and J. J. LiPuma. 2002. Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. *J. Clin. Microbiol.* **40**:2062–2069.
- Denton, M., and K. G. Kerr. 1998. Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. *Clin. Microbiol. Rev.* **11**:57–80.
- Foster, N. F., G. B. Harnett, T. V. Riley, and B. J. Chang. 2008. Cross-reaction of *Stenotrophomonas* and *Xanthomonas* species in a 23S rRNA gene-directed PCR for detection of *S. maltophilia*. *J. Clin. Microbiol.* **46**:4111–4113.
- Hugh, R., and E. Ryschenkow. 1961. *Pseudomonas maltophilia*, an *Alcaligenes*-like species. *J. Gen. Microbiol.* **26**:123–132.
- LiPuma, J. J. 2010. The changing microbial epidemiology in cystic fibrosis. *Clin. Microbiol. Rev.* **23**:299–323.
- Looney, J. W., M. Narita, and K. Mühlemann. 2009. *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *Lancet Infect. Dis.* **9**:312–323.
- Marzuillo, C., et al. 2009. Molecular characterization of *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients and the hospital environment. *Infect. Control Hosp. Epidemiol.* **30**:753–758.
- Murray, P. R., E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Tenover (ed.). 2007. *Manual of clinical microbiology*, 9th ed. ASM Press, Washington, DC.
- Palleroni, N. J., and J. F. Bradbury. 1993. *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. *Int. J. Syst. Bacteriol.* **43**:606–609.

11. **Spilker, T., T. Coenye, P. Vandamme, and J. J. LiPuma.** 2004. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. *J. Clin. Microbiol.* **42**:2074–2079.
12. **Swings, J., P. De Vos, M. Van den Mooter, and J. de Ley.** 1983. Transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov. *Int. J. Syst. Bacteriol.* **33**:409–413.
13. **Weyant, R. S.** 1996. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria, 2nd ed. Williams & Wilkins, Baltimore, MD.
14. **Whitby, P. W., et al.** 2000. Identification and detection of *Stenotrophomonas maltophilia* by rRNA-directed PCR. *J. Clin. Microbiol.* **38**: 4305–4309.