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, MI 48109, USA.

Abstract word count: 50

Text word count: 1481

*Corresponding author. Mailing address: 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
Abstract

Standard microbiology references describe *Stenotrophomonas maltophilia* as oxidase negative and variable for utilization of lactose and sucrose. Analysis of a collection of 766 *S. maltophilia* isolates indicates 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, 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 genus *Stenotrophomonas* (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 based 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 for utilization of lactose and sucrose (9). In contrast, a considerable minority of *S. maltophilia* isolates analyzed by the *Burkholderia cepacia* Research Lab and Repository (BcRLR; University of Michigan, Ann Arbor) have been found to be oxidase positive, and most have been negative for oxidative fermentation of lactose and sucrose. We describe these findings, which should allow for improved phenotypic identification of this species.

Isolates referred to the BcRLR for species identification were evaluated by polyphasic analyses. All strains were grown for 24 h at 32°C aerobically on nonselective Mueller Hinton agar in ambient air. Bacterial DNA was prepared as described previously (11).
Briefly, a single CFU was suspended in 20 µl of lysis buffer containing 0.25% (v/v) sodium dodecyl sulfate and 0.05 N NaOH. After heating for 15 min at 95°C, 180 µl 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 was 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 (1428 bp) allowing for equal weighting in a ClustalV-based tree. An average of 126 bp were 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 eleven Stenotrophomonas, Xanthomonas, and Pseudomonas reference strains available in the NCBI database. These strains included Stenotrophomonas nitritireducens L2 (DSM 12575T), Stenotrophomonas rhizophila e-p10 (LMG 24537T), Stenotrophomonas acidaminiphila amx 19 (ATCC 700916), Stenotrophomonas humi R-32729T, Stenotrophomonas koreensis strain TR6-01 (LMG 23369T), Stenotrophomonas terrae R-32768 (LMG 23958T), S. maltophilia LMG 958T, Xanthomonas campestris pv. campestris LMG 568T, Xanthomonas axonopodis pv. axonopodis LMG 538T, Xanthomonas oryzae pv. oryzae LMG 5047T and Pseudomonas dokdonensis Yoon DS-16 LMG 24163T (S. dokdonensis-basonym). Isolates that clustered with the S.
maltophilia type strain LMG 958\textsuperscript{T} were identified as \textit{S. maltophilia}. DNA sequence identity within the \textit{S. maltophilia} strains ranged from 100\% to 98.9\%. The next closest species was \textit{Stenotrophomonas humi} with an identity of 97.4\%.

Isolates identified as \textit{S. maltophilia} by PCR and DNA sequence analyses were examined for the presence of cytochrome c oxidase using the Remel BactiDrop\textsuperscript{TM} 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 a positive result. Oxidative fermentation of lactose and sucrose was determined using BAM Media M117. Oxidative-Fermentative Test Medium was prepared as per the FDA website (http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM063487) with the following modifications. Media pH was adjusted to 6.95 and inoculated tubes were incubated at 37\textdegree C. Isolates were considered positive if the media turned yellow within 24 h; a color change at the air/media interface only was not considered a positive result.

A total of 831 isolates referred to the BcRLR during a 12 year period from 152 clinical microbiology laboratories in 42 U.S. states were identified as \textit{S. maltophilia} by 23S rRNA-directed PCR. Among the 766 isolates tested for oxidase activity, 154 (20\%) were positive. Of these oxidase-positive \textit{S. maltophilia}, 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 \textit{S. maltophilia}. Most of the strains that had not been correctly identified as \textit{S. maltophilia} by the referring laboratories were
either not classified to the species level (i.e., identified only as “gram negative rod” or “non-fermenter”) (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 (Table 1).

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, indicates that these results were derived from an analysis of 73 isolates. These isolates were reported as being uniformly oxidase negative based on 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 were oxidase-positive. The more sensitive Kovac’s test is comparable to the Remel BactiDrop™ Oxidase test kit we employed, and yielded results consistent with our finding that 20.1% of *S. maltophilia* strains tested were oxidase-positive. Of note, the manufacturers of Kovac’s 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 is 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 positive in 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
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 (and used by the BcRLR for the identification of *S. maltophilia*) has been reported. Using a modification of this assay in which an additional 15 PCR cycles were applied, Foster et al (4) described positive reactions with one strain each of *Stenotrophomonas acidaminiphilia*, *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* based on the 23S rRNA-directed PCR assay. This subset of isolates was selected to include representatives of every phenotype observed among the *S. maltophilia* 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 *Stenotrophomonas* and *Xanthomonas* 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 culture of respiratory specimens from persons with cystic fibrosis. It is also likely that isolates referred to the BcRLR from clinical
microbiology laboratories are biased towards 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 for 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.

REFERENCES


<table>
<thead>
<tr>
<th>Test</th>
<th>Number positive / number tested (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase</td>
<td>154 / 766 (20.1%)</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>11 / 516 (2.1%)</td>
</tr>
<tr>
<td>Lactose</td>
<td>7 / 516 (1.4%)</td>
</tr>
</tbody>
</table>

**TABLE 1.** Phenotypic characteristics of *Stenotrophomonas maltophilia* recovered from clinical specimens.