

## SHORT COMMUNICATION

## Identification of Environmental *Serratia plymuthica* Strains with the New Combo Panels Type 1S

José Vivas, José Antonio González, Luis Barbeyto, Luis A Rodríguez/+

Departamento de Biología Funcional y Ciencias de la Salud, Facultad de Ciencias, Universidad de Vigo, Campus de Ourense, Las Lagunas 32004, Spain and Laboratorio de Microbiología, Hospital Cristal Piñor, Ourense, Spain

*Automated systems are required when numerous samples need to be processed, offering both high throughput and test of a multiple simultaneously. This study was performed to compare the MicroScan WalkAway automated identification system in conjunction with the new MicroScan Combo Neg Panels Type 1S with conventional biochemical methods for identifying ten environmental Serratia plymuthica strains. High correlation between both methods were observed for all the 21 tests evaluated, and the MicroScan system was found capable of correctly identifying all S. plymuthica strains tested. In all tests, the percentage of correlation was 100%, except in raffinose test (91%).*

Key words: *Serratia plymuthica* - MicroScan Combo Panels 1S - identification

The *Serratia* genus includes different species, such as *S. marcescens*, which is frequently involved in human and animal infections, and *S. plymuthica* a saprophytic fermentative, non-motile gram-negative rod, that produces red pigment (prodigiosin), classified as an uncommon cause of human (Reina et al. 1992, Carrero et al. 1995, Ramos et al. 1995) and animal infection (Nieto et al. 1990, Austin & Stobie 1992). *S. plymuthica* is found in soil (Kalbe et al. 1996), and has been isolated from different types of food (Lopez Sabater et al. 1996, Lyhs et al. 1998).

Most of the *S. plymuthica* strains described so far have been isolated from fresh water and fish suggesting that may be a potential opportunistic pathogen for animals and humans (Nieto et al. 1990, Rodríguez et al. 1990).

Automated systems improve both speed and high throughput for the identification of pathogen from environmental samples. The MicroScan Walkaway (Dade MicroScan Inc. Sacramento, CA) is an automated, commercially available system for rapid identification of gram negative bacilli and has received favorable reports relative to identification of clinical (Kelly & Leicester 1992, Bascomb et al. 1997) and environmental bacteria (Odumeru et al. 1999, Súa et al. 1999). Automated

systems have been used with some *Serratia* species, however, the ability to detect *S. plymuthica* from clinical and environmental samples has never been evaluated (Rhoads et al. 1995). The MicroScan Combo Negative Panels type 1S are designed to identify aerobic or anaerobic facultative gram negative bacilli at the specific level using fluorogenic substrates as a pH indicator of bacterial enzymatic activity. The purpose of this study was to evaluate the ability of the MicroScan WalkAway system in conjunction with the new Combo Negative type 1S panels to identify ten *S. plymuthica* strains isolated from fresh water.

Water samples were obtained from the rivers Miño, Barbaña and Sil, and collected in sterile glass bottles. *S. plymuthica* was recovered from water samples using 0.2 µm membrane filters (Albet, Spain). Ten *S. plymuthica* strains (VV1 to VV10) isolated from fresh water samples were selected for testing. The strains were identified in parallel using the MicroScan WalkAway system and by standard reference procedures (Nieto et al. 1984, Amos 1985). The strains were routinely cultured on trypticase soy agar (TSA, Cultimed) at 37°C for 24 h, and stored on TSA slants at 4°C under mineral oil and frozen at -70°C with 15% glycerol. A *S. plymuthica* K1R isolated from rainbow trout (*Oncorhynchus mykiss*) was included as reference strain. MicroScan WalkAway has been described previously (Súa et al. 1999). Conventional MicroScan Negative Combo type 1S panels were inoculated with the strains by the turbidity standard technique. The panels were incubated for 24 h at 35°C within MicroScan WalkAway system.

+Corresponding author. Fax: 988-387001. E-mail: lalopez@uvigo.es

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All procedures were performed according to the manufacturer's directions (Dade International Inc. 1997).

*Comparison of biochemical tests* - The following of D-glucose, sucrose, D-sorbitol, raffinose, L-rhamnose, L-arabinose, M-inositol, D-adonitol, and melibiose; urease; Hydrogen sulfide production (H<sub>2</sub>S); indole production; decarboxylation of lysine, and ornithine; arginine dihydrolase; tryptophan deaminase (TDA); esculin hydrolysis; Vogues-Proskauer (VP); utilization of citrate; o-nitrophenyl-β,D-galactopyranoside (ONPG); and OF-glucose tests were compared.

The MicroScan identification patterns showed a correct identification of all the strains tested as *S. plymuthica*: positive for glucose, sucrose, inositol and arabinose fermentation, citrate utilization, esculin, VP, ONPG and OF-glucose tests; variable for raffinose fermentation and negative for lysine, arginine and ornithine, sorbitol, adonitol, rhamnose and melibiose fermentation, H<sub>2</sub>S and indole production, and urease and TDA tests.

Probability percentages for the identification using MicroScan System, indicated that nine of the strains tested were: *S. plymuthica* 98.5%, *S. liquefaciens* 0.8% and *Enterobacter agglomerans* 0.6%. In the case of the VVP3 strain, the positive result for the raffinose fermentation, produced an identification of *S. plymuthica* 98.3%, *S. rubidea* 1% and *S. liquefaciens* 0.7%, equally valid.

In the comparison between MicroScan WalkAway system and conventional laboratory tests to evaluate important biochemical characteristics for the identification of *S. plymuthica* strains, all the tests presented a correlation of the 100% except the raffinose test (91%).

Although *S. plymuthica* infections are relatively rare in humans and animals, his clinical and veterinarian importance is well documented. Some studies on the reliability of automated systems for bacterial identification have shown reliable results for gram negative bacteria although *S. plymuthica* strains, were not included between the species of Enterobacteriaceae family tested.

Overall, MicroScan WalkAway system in conjunction with the new Combo Negative type 1S panels proved to be very useful and reliable in identifying environmental *S. plymuthica* strains. This system identified correctly all the strains tested, and, in all the twenty one analyzed tests, the correlation between the MicroScan system and the conventional test was a 100% with the unique exception of raffinose test showed no incidence in the final identification of *Serratia* to specific level.

In conclusion, the MicroScan Walkaway in conjunction with the Combo Negative type 1S panels, was able to identify *S. plymuthica* environmen-

tal isolates including the reference strain *S. plymuthica* K1R.

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