Comparative proteomic analysis of collection and clinical-isolate strains of *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia is a Gram-negative pathogen with emerging nosocomial incidence [1]. It has been associated with several clinical syndromes, primarily in relation to the opportunistic infection of immunocompromised patients. Although not an inherently virulent pathogen, its ability to colonise respiratory-tract epithelial cells and medical-device surfaces makes it a ready coloniser of hospital settings.

Antibiotic treatment of *S. maltophilia* is greatly hampered by extensive drug resistance [2]. Besides more specific resistance mechanisms, its capacity to form biofilms confers the bacterium additional protection in front of the immune system, antibiotics and disinfectants [3].

The genomes of two different strains have been recently sequenced and compared [4] but no expression-pattern studies have been performed yet. In this communication, we present a comparative proteome analysis of four strains with different infective capacities, ATCC 13637 and the new clinical isolates M30, UV74 and E77.

Materials & Methods

Bacterial strains and growth

Three new *S. maltophilia* strains were isolated. M30 from a patient with decubitus ulcer. E77 from sputum and UV74 from patient with a vascular ulcer .ATCC 13637 and clinical isolated S. *maltophilia* strains were cultured in LB media to reach exponential growth. **Total protein extract:** 20ml of culture were washed with PBS and resuspended in lysis solution (8M urea, 2M thiourea, 2.5% CHAPS, 2% ASB-14, 40mM Tris-HCL pH 8.8, 0.5% IPG). Then, samples were disrupted by sonication and centrifuged. **DIGE labeling:** Samples were labeled as follows: Clinical strains with Cy3 (green), ATCC 13637 strain with Cy5 (red) and the internal standard with Cy2 (Blue). 50 µg of each sample were mixed and the resulting 150 µg were loaded onto the IPG strips. **2-D Gel Electrophoresis:** The IPG strips were 24cm long and the pH range was from 3 to 10 lineal and 4 to 7. The sample was loaded with the cup-loading system. The second dimension was performed in 12.5% polyacrylamide gels. Gels were performed in triplicate for statistical analysis. **Image analysis**: Images were acquired immediately using a Typhoon scanner (GE Healthcare) and the image analysis was carried out with Samespots software (Nonlinear dynamics). **In-Gel Tryptic Digestion:** After image analysis silver staining of the gels was performed. The selected spots were excised from the acrylamide gel, and immediately destained and digested. **MALDI-MS Analysis:** 1µl of sample was mixed with 1µl of HCCA. This mixture was analyzed with an Ultraflex MALDI-TOF (Bruker). For PMF analysis, the MASCOT search engine (Matrix Science) was used with the following parameters: one missed cleavage permission, 50 ppm measurement tolerance, and at least five matching peptide masses. In the searches, methionine oxidation and cystein carbamidomethylation were taken into account as variable modifications.

Conclusions

A differential expression analysis of the proteomes of newly isolated clinical strains of *S. maltophilia* and the laboratory strain ATCC 13637 has been performed (Figure 1). Eye-inspection of the gel images readily suggests the existence of substantial differences between the two proteomes under the experimental conditions. The identification of differentially expressed proteins in these strains shall provide

the basis for the interpretation of their phenotypic differences at a biomolecular level, with a special focus on infection and resistance mechanisms.

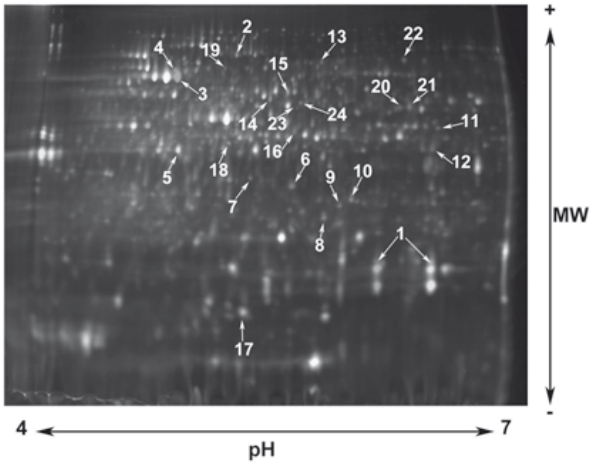

Figure 1. *DIGE of the M30 and the ATCC 13637 S. maltophilia strains*

On average, around 1500 spot features were detected on each analytical gel. Statistical analysis of gels allowed the detection of about 300 spots with significant changes between the clinical isolates and ATCC strains. We have identified 24 differentially expressed proteins. Interestingly, some of these proteins (Spot nº 9, 16 and 21) are involved in the fatty acid metabolism. Moreover, a protein involved in the poliketide sugar unit biosynthesis has also been identified (Spot nº 18). The upregulation of these proteins in the some of the clinical strains suggests a different membrane lipopolysaccharide composition, which in turn could be related with biofilm formation and pathogenesis. A number of proteases are upregulated in the clinical strains (Spot nº 2, 14, 22 and 23) that could also be potentially relevant to infection. Other identified proteins are involved in protein synthesis (Spot nº 3, 4, 5, 13 and 17) and in amino acid metabolism (spot nº 12, 15 and 20).

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