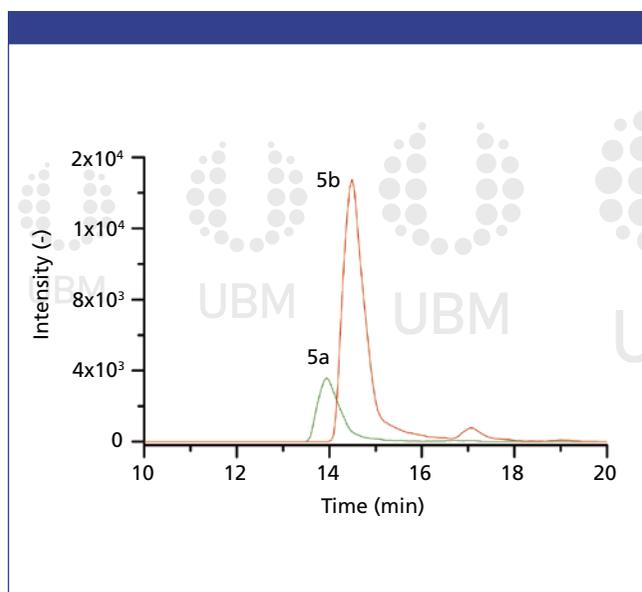


**Figure 2:** Extracted ion chromatograms of the iron(III) complexes of the pyoverdines with an acyl side chain derived from malic acid (1) or malic acid amide (3), and proline pyoverdine (5).



**Figure 3:** Extracted ion chromatogram showing the iron complex (5a) and the aluminium complex (5b) of the proline pyoverdine (5).

HESI II source, operated in positive ionization mode (ESI+).

**Column:** 150 × 2.1 mm, 3.5 μm 100 Å, iHILIC®-Fusion (P/N 110.152.0310, HILICON AB, Sweden)

**Eluent:** A) acetonitrile; B) ammonium bicarbonate (5 mM, pH 7) and acetonitrile (95:5, v/v);

**Gradient Elution:** 0–0.75 min, 80% A; 0.75–20 min, from 80% A to 40% A; 20–25 min, 40% A; 25–27 min, from 40% A to 80% A; 27–40 min, 80% A

**Flow Rate:** 0.3 mL/min

**Column Temperature:** 40 °C

**Injection Volume:** 5 μL

***P. taiwanensis* VLB120 Cell Supernatants:** Cell supernatants of the *P.*

*taiwanensis* VLB120 bacteria were provided by Dr. Till Tiso at iAMB (Institute of Applied Microbiology) and ABBt (Aachen Biology and Biotechnology, RWTH Aachen University, Aachen, Germany) and purified by solid-phase extraction as described in reference 5. The samples for injection contained five pyoverdines that differ in their acyl side chain and in the peptide moiety (6), shown in Figure 1.

## Results and Conclusion

The different siderophores of the *P. taiwanensis* VLB120 bacteria and their non-covalent iron and aluminium complexes can be simultaneously separated and determined by an iHILIC®-Fusion column hyphenated with electrospray ionization mass spectrometry (ESI-MS) detection (Figures 2 and 3). Pyoverdines with various acyl side chains exhibit different levels of hydrophilic partitioning and electrostatic interaction with the stationary phase. The extracted ion chromatograms in Figure 3 also demonstrate that the iHILIC®-Fusion column is even capable of separating the iron(III) and aluminium(III) complexes of each siderophore. Because of the smaller radius, aluminium has a stronger polarizing effect on the siderophores. Therefore, aluminium(III) complex has higher retention on the HILIC stationary phase. This work demonstrates that intact non-covalent metal complexes can be separated and identified by a HILIC-MS method using an iHILIC®-Fusion column.

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**HILICON AB**

Tvistevägen 48, SE-90736, Umeå, Sweden

Tel.: +46 (90) 193469

E-mail: info@hilicon.com

Website: www.hilicon.com