

## Purification and Structural Analysis of QS-inhibiting Compounds from *Staphylococcus delphini*

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**[Abstract]** The knowledge that many pathogens rely on cell-to-cell communication mechanisms known as quorum sensing, opens a new disease control strategy: quorum quenching. Here we present a purification protocol for molecules excreted by a group of Gram-positive zoonotic pathogen bacteria, the '*Staphylococcus intermedius* group', that suppress the quorum sensing signaling and inhibit the growth of a broad spectrum of Gram-negative beta- and gamma-proteobacteria. These compounds were isolated from *Staphylococcus delphini* (*S. delphini*). They represent a new class of quorum quenchers with the chemical formula *N*-[2-(1*H*-indol-3-yl)ethyl]-urea and *N*-(2-phenethyl)-urea, which we named yayurea A and B, respectively. These substances can be isolated and purified from the culture supernatant using this upscalable purification method.

### Materials and Reagents

1. *Staphylococcus delphini* DSMZ20771 strain (DSM number: 20071)  
*Note: DSMZ stands for Deutsche Sammlung von Mikroorganismen und Zellkulturen.*
2. Tryptic Soy Broth (TSB) (Sigma-Aldrich, catalog number: T8907)
3. Amberlite XAD-16 resin (Sigma-Aldrich, catalog number: 1-0379)
4. Methanol
5. Acetic acid (Merck KGaA)
6. Amberlite IRC 50 cation exchange resin (SERVA Electrophoresis GmbH, catalog number: 40501)
7. Sodium hydroxide
8. Ethanol
9. 50 mM and 1 M sodium phosphate buffer
10. SP sepharose cation exchange column (GE Healthcare, catalog number: 17-5161-01)
11. Sodium chloride
12. Trifluoroacetic acid (TFA) (Sigma-Aldrich, catalog number: T6508)
13. Phosphoric acid for HPLC (Sigma-Aldrich, catalog number: 79606)

14. Acetonitrile for HPLC (Mallinckrodt Baker, catalog number: 9012)

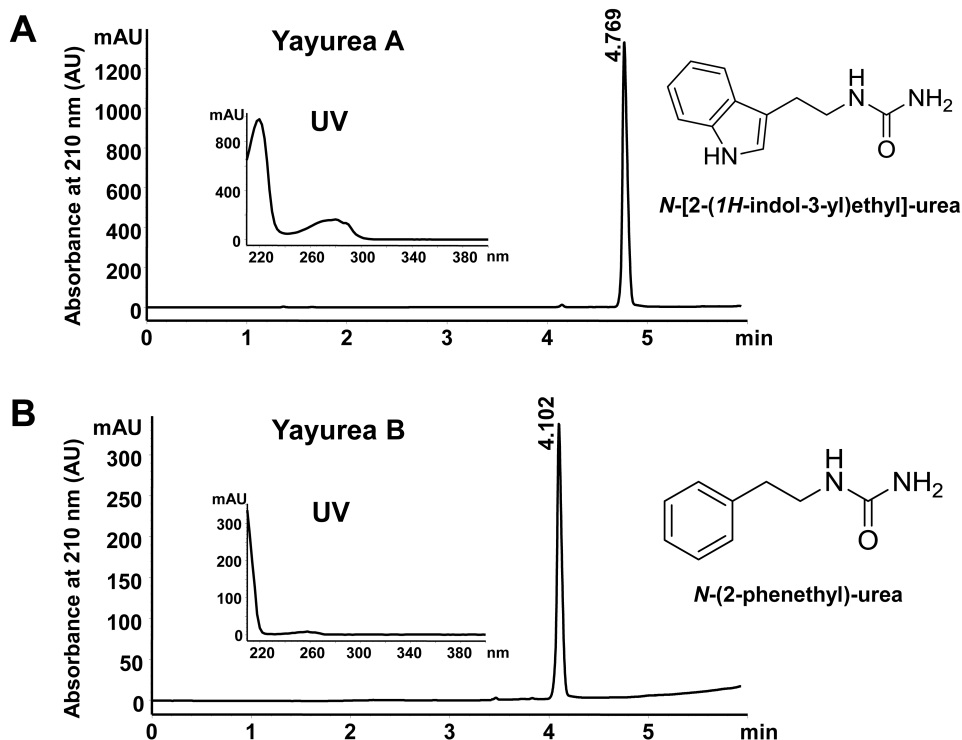
### **Equipment**

1. 37 °C shaking incubator (Infors AG)
2. Centrifuge (Eppendorf)
3. Rotary evaporator (BÜCHI Labortechnik AG)
4. Äkta FPLC equipped with P-900, UV-900, PH/C-900 (GE Healthcare)
5. Preparative HPLC System equipped with Bischoff HPLC compact pump QC-P 2250 and Multiwavelength detector QC-1157 (Bischoff)
6. Nucleosil 100 C-18 (8 x 250 mm column) (MACHEREY-NAGEL, catalog number: 715332.80)
7. Agilent 1200 series HPLC system (Agilent)
8. Waters XBridge C18 (5 mm, 4.6 x 150 mm column) (Waters, part number: 186003116)

### **Procedure**

1. *S. delphini* is cultivated in 100 ml TSB at 37 °C on a shaking incubator at 150 rpm for 20 h.
2. Cells are centrifuged at 5,000 rpm (4,500 x g) at 4 °C for 10 min and the supernatant is applied on to a column filled with 10 ml Amberlite XAD-16 resin.
3. The column is first washed with 5 bed volumes each of milliQ water, then with 40% and 60% methanol and finally eluted with 80% methanol containing 5% acetic acid at a flow rate of 10 bed volumes per hour.
4. The eluate is evaporated using a rotary evaporator until all methanol is removed.
5. The eluate is resuspended with 50 ml water and the pH adjusted to 7.0 with 1 M NaOH. It is then applied on to a column filled with 10 ml Amberlite IRC-50 cation exchange resin.
6. The column is washed first with water, then with 70% ethanol and eluted with 80% ethanol acidified with 5% acetic acid each with 5 bed volumes at a flow rate of 10 bed volumes per hour.
7. The eluate is concentrated using a rotary evaporator until all methanol is removed.
8. The eluate is diluted with water and adjusted to a final concentration of 50 mM sodium phosphate using 1 M sodium phosphate buffer and pH is lowered to 4.2 using 85% phosphoric acid.
9. In the third purification step, the eluate is separated on a 5 ml SP sepharose cation exchange column with a linear 0 to 1 M NaCl gradient in 50 mM sodium phosphate buffer on an Äkta purifier FPLC at a flow rate of 3 ml/min.

10. The final purification and desalting of each peak is carried out by reversed phase preparative HPLC (RP-PHPLC) on a nucleosil 100 C-18, 8 x 250 mm column with a linear water acetonitrile (containing 0.1% TFA) gradient of 0% to 60% in 25 min.
11. Purified compounds are lyophilized and stored at -20 °C.
12. Qualitative analysis is carried out on an Agilent 1200 HPLC system and a RP-HPLC Waters xBridge C18, 5 mm, 4.6 x 150 mm column. Compounds are eluted with a 15 min linear gradient of aqueous phosphoric acid (0.1% vol/vol) to acetonitrile at a flow rate of 1.5 ml/min and detected at 210 nm.



**Figure 1.** RP-HPLC profile, UV-spectrum and structures of the two QS-inhibitors purified from *S. delphini*. A. QS-inhibitor, *N*-[2-(1*H*-indol-3-yl)ethyl]-urea (yayurea A). B. QS-inhibitor, *N*-(2-phenethyl)-urea (yayurea B). RP-HPLC is carried out on an Agilent 1200 and WatersXBridge C18, 5 mm, 4.6 x 150 mm column; compounds are eluted with a 15 min linear gradient of 0.1% phosphoric acid to acetonitrile at a flow rate of 1.5 ml/min.

## **Acknowledgments**

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## **References**

1. Chu, Y. Y., Nega, M., Wölfle, M., Plener, L., Grond, S., Jung, K. and Götz, F. (2013). [A new class of quorum quenching molecules from \*Staphylococcus\* species affects communication and growth of gram-negative bacteria.](#) *PLoS Pathog* 9(9): e1003654.