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Characterization of H₂S positive and negative strains of *Shewanella baltica* isolated from spoiled whiting (*Merlangius merlangus*)

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Introduction

Among the numerous *Shewanella* species described in marine animals and sea water, *Shewanella putrefaciens* has been described as the spoiling agent for fresh fish stored on melting ice (Gram & Dalgaard, 2002).

Nonetheless *S. putrefaciens* is more likely a bacterial group composed of four sub-groups than a single species.

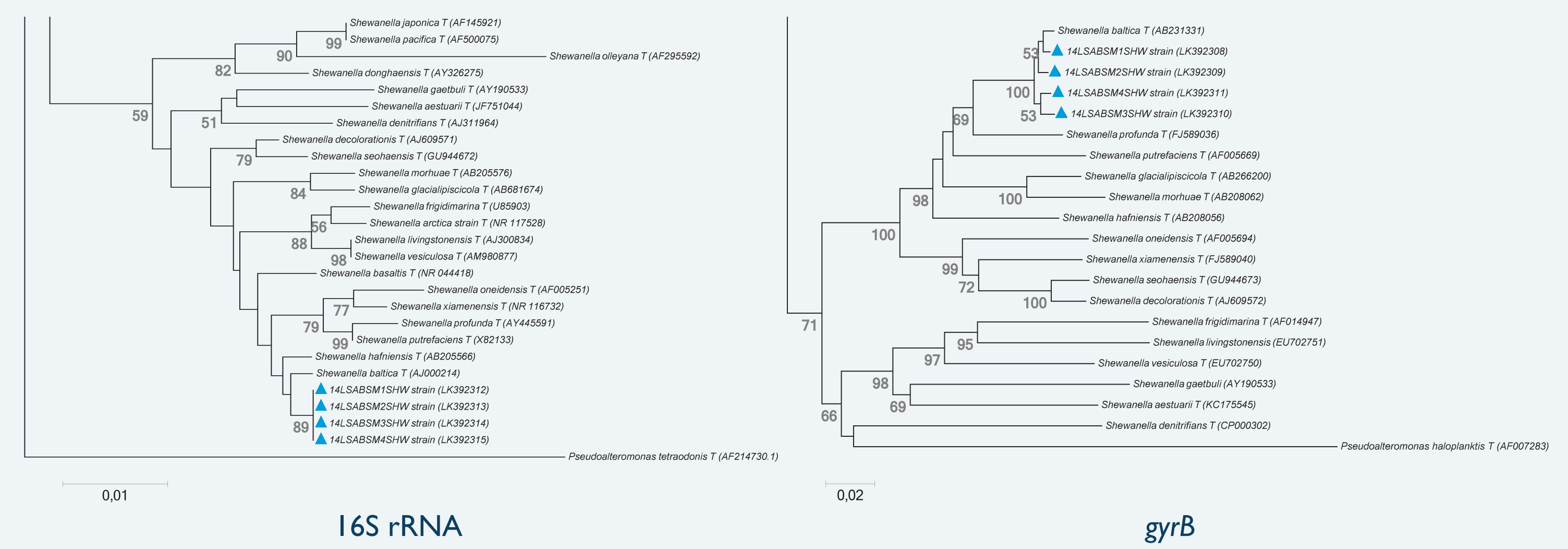
S. putrefaciens group II, reclassified as *Shewanella baltica*, is a very heterogeneous group recently characterized by MultiLocus Sequence Typing (MLST) in nine clades (Ziemke *et al.*, 1998; Deng *et al.*, 2014).

S. baltica has been described as a potential spoilage organism and the most H₂S producing bacteria during storage of marine fish (Vogel *et al.* 2005).

➔ The present study describes the phenotypic, genotypic and proteomic characterization of four *S. baltica* strains isolated from spoiled whiting (*Merlangius merlangus*).

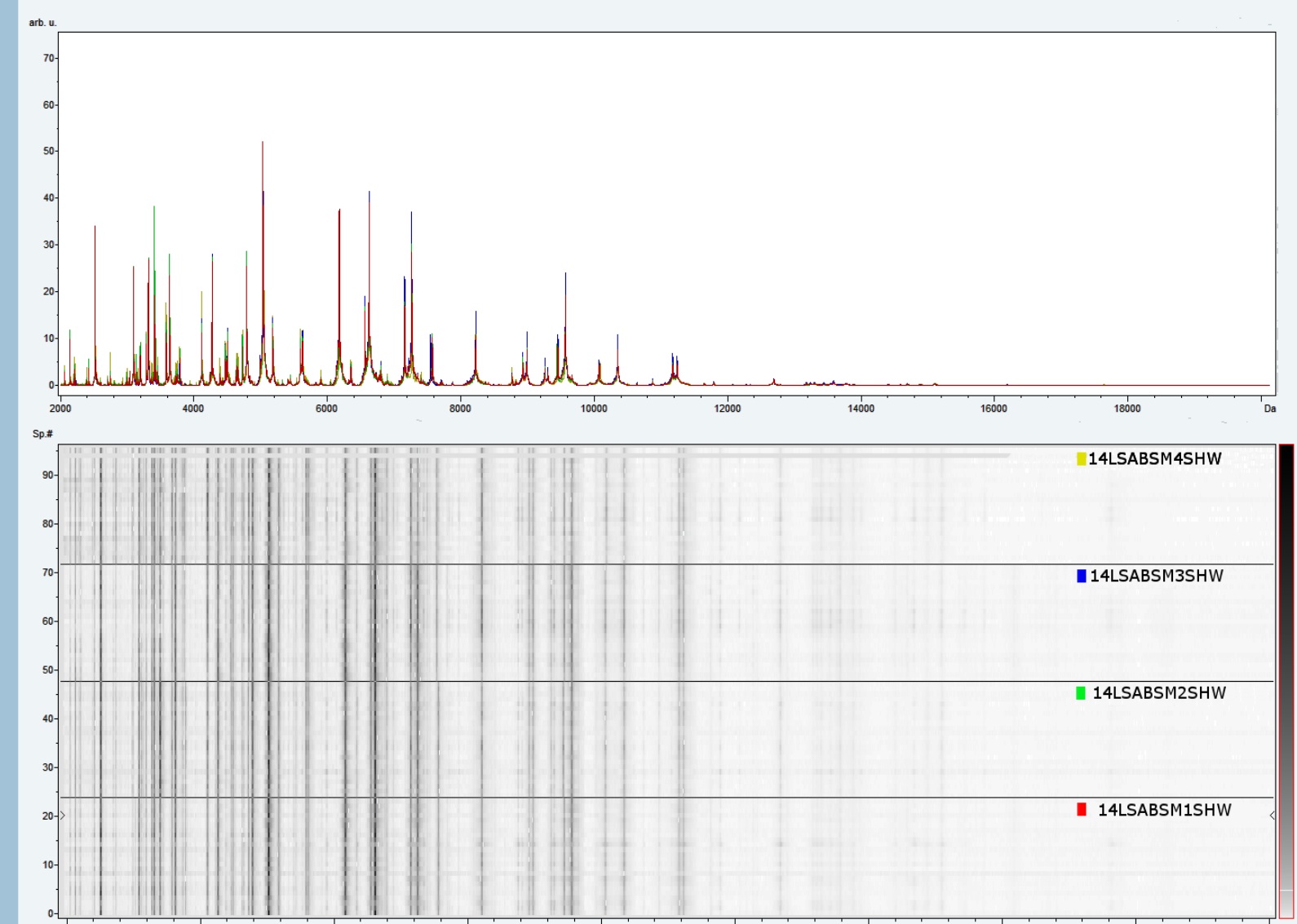


Genotypic approaches



All the strains were identified as *S. baltica* by both 16S rRNA and *gyrB* partial sequencing. The second gene, *gyrB*, gave more informations on the diversity of the isolated isolates.

Proteomic approaches



| Strains | Mean | %RSD | Identification on |
|--------------|-------|------|--------------------------|
| I4LSABSM1SHW | 2.402 | 2.8% | <i>S. baltica</i> OSS155 |
| I4LSABSM2SHW | 2.355 | 2.7% | <i>S. baltica</i> OSS155 |
| I4LSABSM3SHW | 2.330 | 2.0% | <i>S. baltica</i> OSS155 |
| I4LSABSM4SHW | 2.294 | 3.1% | <i>S. baltica</i> OSS155 |

Overall, isolates display homogeneous MALDI-TOF profiles.

MALDI-TOF study led to good identification scores (≥ 2.3) and low %RSD among the 10 replicates per strain.

Using the Brüker database, isolates were identified as closer to *S. baltica* OSS 155 MALDI-TOF profile.

More deeply, some differences have been highlighted between the four isolates :

• Ion 3025 m/z differentiate I4LSABSM1SHW & I4LSABSM3SHW from I4LSABSM2SHW & I4LSABSM4SHW.

• Ions 3359 m/z, 3767 m/z, & 3780 m/z differentiate I4LSABSM2SHW from I4LSABSM4SHW.

• Ions 3767 m/z, 3780 m/z, 7536 m/z & 7562 m/z differentiate I4LSABSM1SHW from I4LSABSM3SHW.

Confirmation of genotypic identification by the MALDI-TOF study. This technology allowed to visualize some slight differences between the proteomic profiles of the strains.

Materials & Methods

Phenotypic and Biochemical

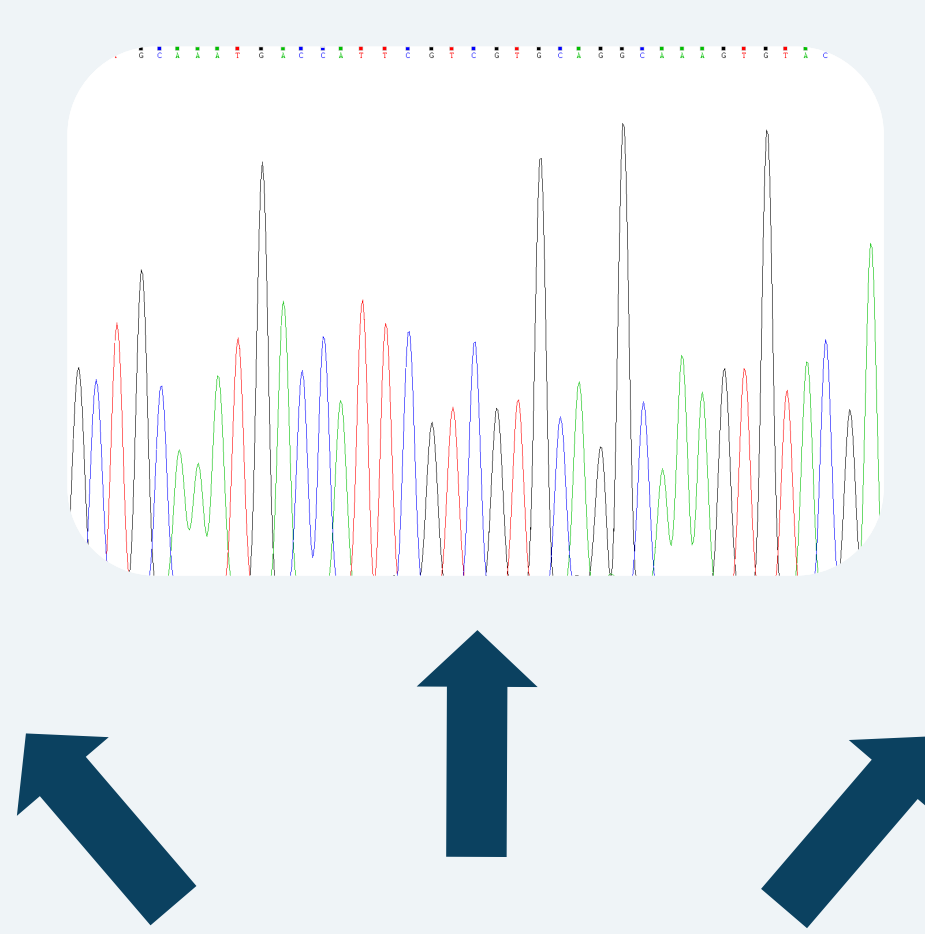
Bacterial cultures and biochemical tests

Genotypic

16S rRNA and *gyrB* genes sequencing

Proteomic

MALDI-TOF profiles



Three approaches

Four strains of *Shewanellaceae*

(I4LSABSM1SHW, I4LSABSM2SHW, I4LSABSM3SHW & I4LSABSM4SHW)

Isolated from a spoiled whiting (*Merlangius merlangus*) and selection on tryptone soya agar yeast extract with 1.5 % (w/v) NaCl (TSA-Ye-NaCl).

Results

Phenotypic and Biochemical approaches

Strains growth characteristics:

| | | I4LSABSM1SHW | I4LSABSM2SHW | I4LSABSM3SHW | I4LSABSM4SHW |
|---|-------|--------------|--------------|--------------|--------------|
| Aerobic growth (TSA-Ye medium) | (n=2) | | | | |
| | 4°C | + | + | + | + |
| | 8°C | + | + | + | + |
| | 15°C | + | + | + | + |
| | 25°C | + | + | + | + |
| | 30°C | + | + | + | + |
| Anaerobic growth (TMAO-TSA-Ye medium) | (n=2) | | | | |
| | 25°C | + | + | + | + |

Aerobic growth was achieved on TSA-Ye-NaCl plates for 72h (4°C), 48h (8°C) and 24h (15°C, 25°C, 30°C and 37°C). Anaerobic growth was carried out on TSA-Ye-NaCl plates supplemented with 40 mM TMAO for 24h at 25°C, anaerobic conditions having been generated thanks to Genbag system.

Strains grew on a large range of temperature (4-30°C) and are able to grow anaerobically, in presence of TMAO, with the development of the specific fish off-odor.

Strains biochemical patterns:



Heterogeneous biochemicals patterns observed. One strain is surprisingly H₂S negative, two strains synthesize an ornithine decarboxylase and all the strains, except one, are able to use sucrose and arabinose.

Conclusions

Phenotypic and biochemical approaches failed to accurately identify the four strains of *Shewanellaceae*.

Genotypic approach, by sequencing the two genes 16s rRNA and *gyrB*, allowed to identify the whole isolates as members of *S. baltica* species.

Proteomic approach, using the MALDI-TOF, confirmed the conclusion of the genotypic study and highlights slight differences between strains.

Isolate I4LSABSM1SHW, unable to produce H₂S, displayed an atypical phenotype that is why it was filed in the collection of the Institut Pasteur with the identifier CIP 110804.

H₂S production might not be the most appropriate screening parameter for isolation of *Shewanella* isolates.

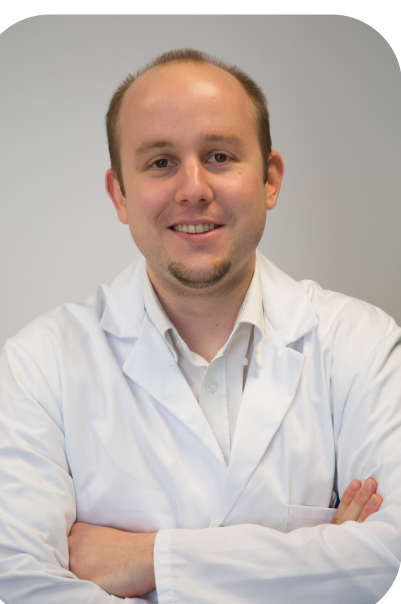
The spoiling potential of I4LSABSM1SHW has to be characterized.

In-depth reading...

Dehaut, A., Midelet-Bourdin, G., Brisabois, A., & Duflos, G. (2014). Phenotypic and genotypic characterization of H₂S-positive and H₂S-negative strains of *Shewanella baltica* isolated from spoiled whiting (*Merlangius merlangus*). *Letters in applied microbiology*, 59(5), 542-548.



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