Effect of salt on the BVOC production of three Greenlandic halotolerant bacteria

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Greenland is a source of novel, extremophilic organisms whose potential is yet to be fully exploited. In this study, **three novel, halotolerant strains** (*Nesterenkonia halotolerans* strain CF4.12, *N. aurantiaca* strain CMS1.6, and *Oceanobacillus sp.* strain CF4.6) isolated from soil samples of Peary Land, Northern Greenland, were cultured under **different salt concentrations** (5, 10 and 15% w/v), and their production of **biogenic volatile organic compounds** (BVOC) was analysed using a dynamic headspace (DHS) setup, sorbent Tenax NA tubes and GC-MS. The three strains produce distinct BVOC blends. Furthermore, salt has a statistically significant effect on the BVOC production of *N. aurantiaca*. Both *Nesterenkonia* strains were found to produce compounds with biotechnological interest as biofuels, namely 2- and 3-methyl-butanol, and 2-methyl butenal. This work highlights the importance of extremophiles in the green transition and their potential future applications.

MATERIALS AND METHODS

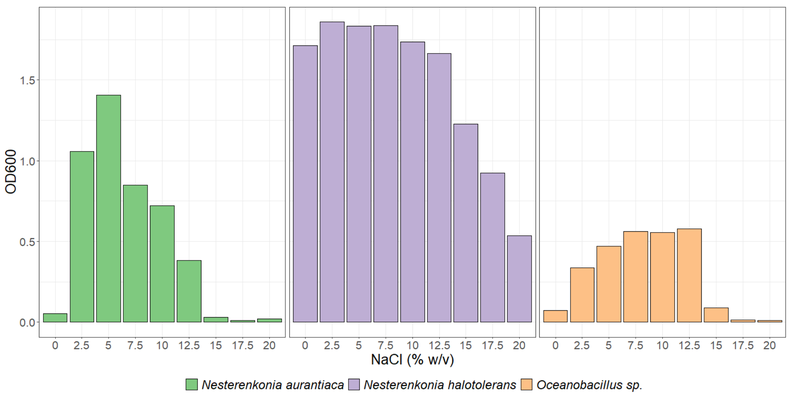


Figure 1. Growth of the three strains used in this study after 70 hours in different salt concentrations in modified HM medium **(1)** , measured using 96 well plates and a plate reader. The three strains were selected for their different responses to NaCl concentration.

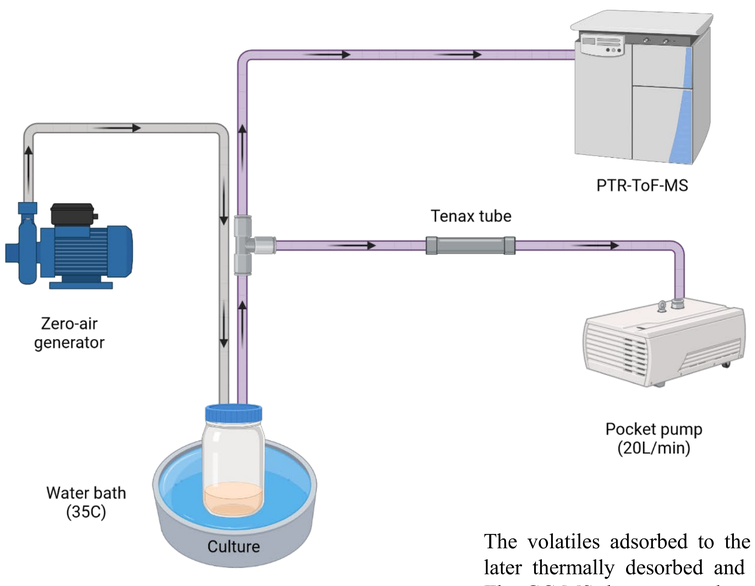


Figure 2. DHS setup used to sample the volatiles produced by the strains. A zero-air generator was used to produce VOC-free air. Three conditions were tested: 5, 10 and 15% NaCl (w/v). After the cultures were grown aerobically in modified HM medium **(1)** at 25ºC until the late exponential phase, the headspace was sampled for 10 minutes at 35ºC. Negative controls were also incubated to subtract the volatiles during the data analysis.

The volatiles adsorbed to the Tenax NA tube were later thermally desorbed and analysed via GC-MS. The GC-MS data was analysed with PARADISe **(2)**. The compounds were identified based on MS data and the NIST database, and were quantified using premixed standards ran before and after the samples. The PTR-MS data is currently being analysed. Every sample was run in triplicate. Unfortunately, all three replicates of *Oceanobacillus sp.* at 5% NaCl were lost due to a machine error.

RESULTS

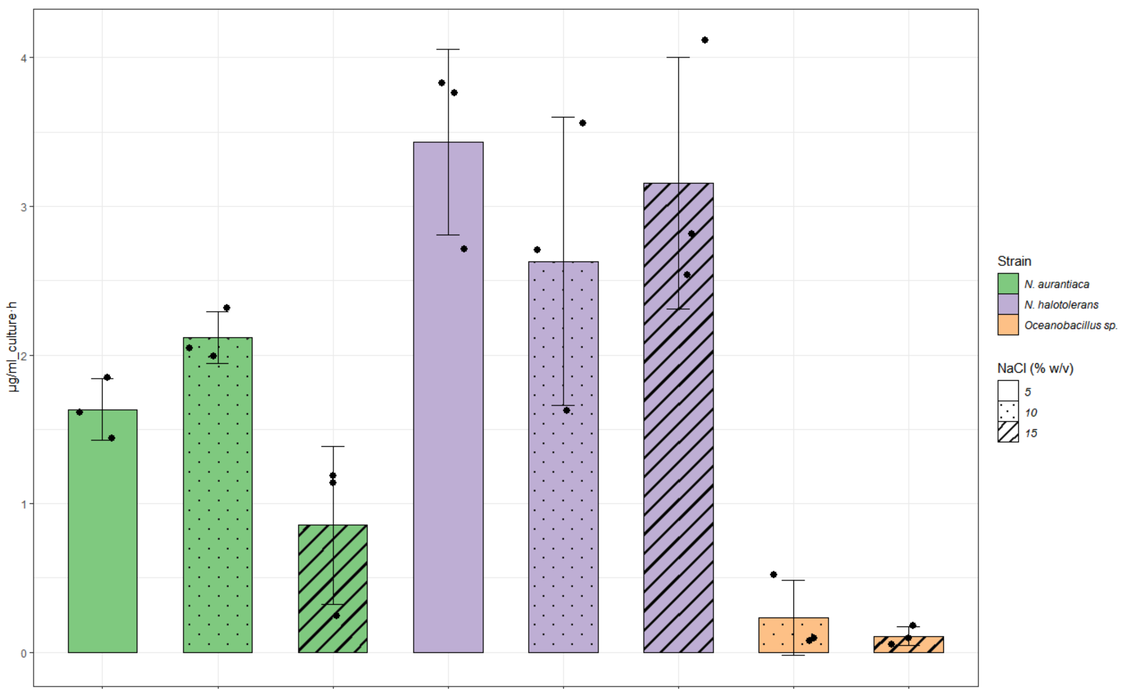


Figure 4. Total VOC emissions of the strains, in micrograms per millilitre of culture per hour.

Emission rates		
Test	R value	Significance
Between strains	0,7292	1e-4
NaCl within *N. aurantiaca*	0,6543	3,5e-3
Between *Nesterenkonia* strains	0,6543	1e-4
VOC blend (%)		
Between strains	0,7059	1e-4
NaCl within *N. aurantiaca*	0,572	6,9e-3
Between *Nesterenkonia* strains	0,692	1e-4

Table 1. Similarity between samples (ANOSIM) (n= 3), for either the absolute emission rates, or the volatile blend. Only the statistically significant (p <0,01) results are shown.

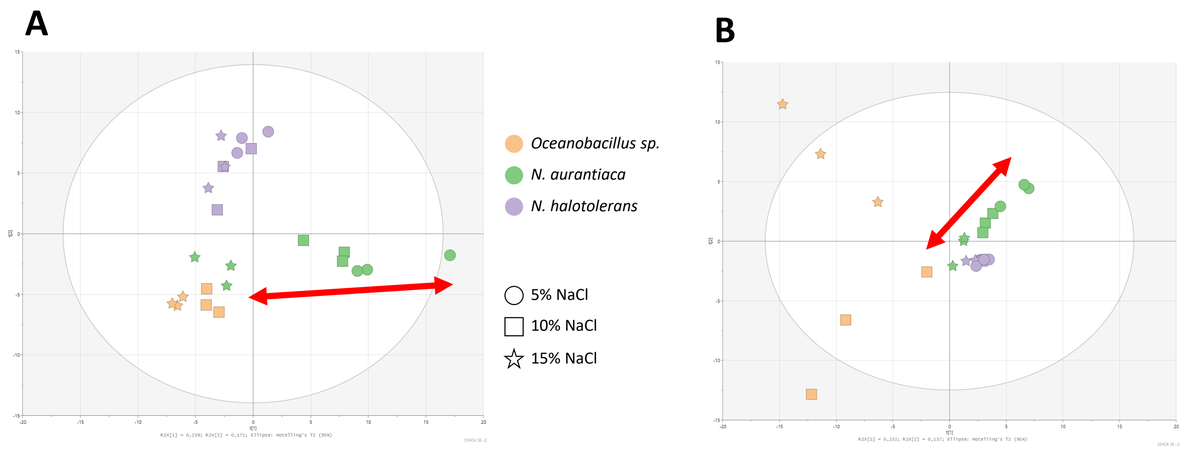


Figure 5. Principal Component Analysis of the absolute VOC emissions of the samples (A), and the VOC blend of each sample (B). The different strains cluster together, and there is a gradient of NaCl in *N. aurantiaca* (red arrows), corroborating the similarity test results.

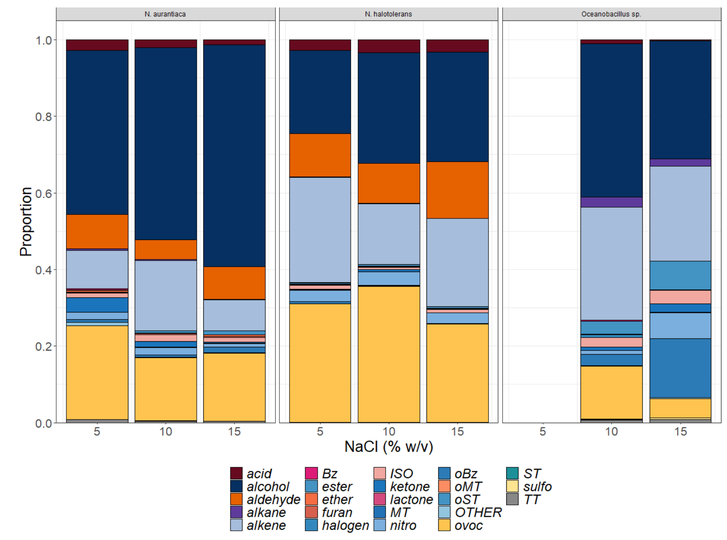
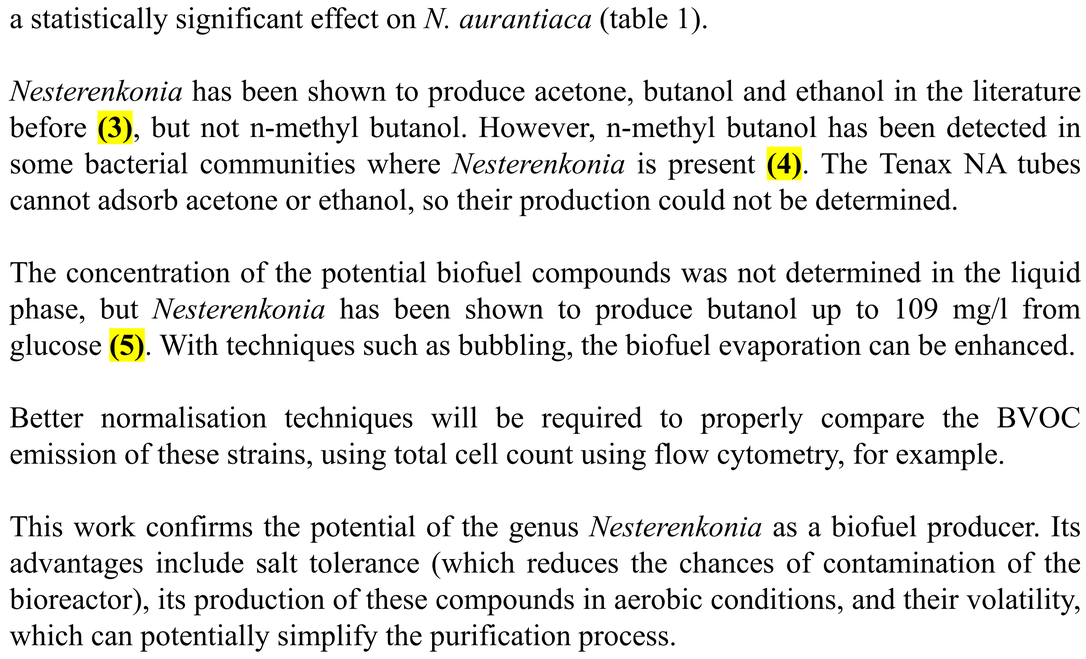


Figure 6. Average (n=3) blend of VOCs. Initials mean benzenoid (Bz), isoprene (ISO), monoterpene (MT), nitrogenated compound (nitro), oxygenated benzenoid (oBz), oxygenated monoterpene (oMT), oxygenated sesquiterpene (oST), non-identified oxygenated VOC (ovoc), sesquiterpene (ST), sulfur-containing VOC (sulfo), triterpene (TT).

Figure 7. Volatile compounds with potential biofuel applications produced by both *Nesterenkonia* strains. Average of 3 replicates.



DISCUSSION, CONCLUSSIONS AND FUTURE DIRECTIONS

- The three strains have distinct BVOC emissions, although NaCl concentration only has a statistically significant effect on *N. aurantiaca* (table 1).
- *Nesterenkonia* has been shown to produce acetone, butanol and ethanol in the literature before **(3)**, but not n-methyl butanol. However, n-methyl butanol has been detected in some bacterial communities where *Nesterenkonia* is present **(4)**. The Tenax NA tubes cannot adsorb acetone or ethanol, so their production could not be determined.
- The concentration of the potential biofuel compounds was not determined in the liquid phase, but *Nesterenkonia* has been shown to produce butanol up to 109 mg/l from glucose **(5)**. With techniques such as bubbling, the biofuel evaporation can be enhanced.
- Better normalisation techniques will be required to properly compare the BVOC emission of these strains, using total cell count using flow cytometry, for example.
- This work confirms the potential of the genus *Nesterenkonia* as a biofuel producer. Its advantages include salt tolerance (which reduces the chances of contamination of the bioreactor), its production of these compounds in aerobic conditions, and their volatility, which can potentially simplify the purification process.

FUNDING

This project is funded by the Novo Nordisk Foundation under the NNF Interdisciplinary Synergy Program grant no. NNF19OC0057374; Effects of bacteria on atmospheres of Earth, Mars, and exoplanets -- adapting and identifying life in extraterrestrial environments.

This project is also supported by The Danish National Research Foundation within the Center for Volatile Interactions (VOLT, DNRF168).

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