Application Note

MICROORGANISM SURFACE ANALYSIS

Using the TriVersa NanoMate for Liquid Extraction Surface Analysis of Bacteria and Fungi

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INTRODUCTION

Liquid Extraction Surface Analysis Mass Spectrometry (LESA-MS)[1] has been utilized for determination of small molecules, lipids and proteins from a variety of surfaces such as tissue sections, plant material, medical devices, TLC plates or DBS cards. It allows direct surface analysis without sample preparation from spot sizes of 1 mm diameter by aspiration of extraction solvent into a pipette tip, formation of a liquid junction at the intended target location and extraction of the analytes of interest. In the final step, analytes are ionized by static nano electrospray and detected in the mass spectrometer (Figure 1).

RESULTS

Recently, researchers from the University of Birmingham, United Kingdom, and the UK National Physical Laboratory found that a LESA-MS analysis workflow enabled them to directly characterize bacterial proteins from agar cultures either in contact or non-contact mode (Figure 2, 3), with or without further gas phase separation in an ion mobility cell [2,3].

Randall et al. found six proteins via top-down MS analysis with a protein coverage of 13-40%. The protein function ranged from stress related to DNA binding. They concluded that:

> *'The method has implications for microbiological research as it may be suitable for the study of bacterial growth, communication, and response to external factors such as pharmaceuticals and pH.'* [2]

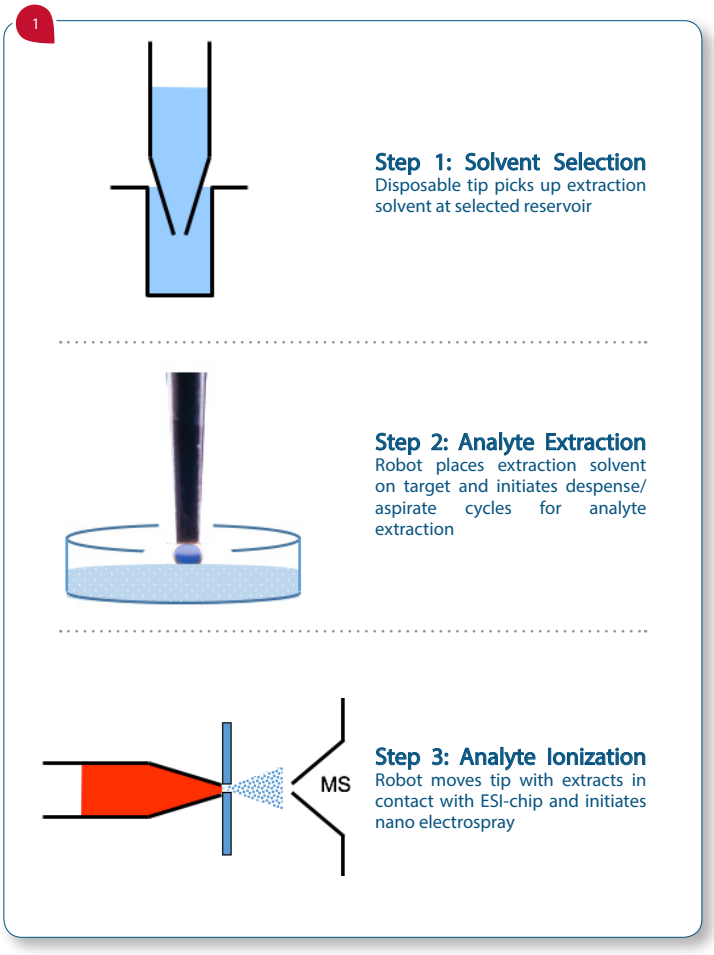

Figure 1: Schematic Workflow of a LESA-MS experiment utilizing bacteria or fungi cultures.

RESULTS CONTINUED

Researchers from the Max Planck Institute of Chemical Ecology in Jena utilized the LESA-MS analysis approach for direct screening of antibiotics released by five different strains of bacteria known to express thiazolyl peptides. After a 7-day cultivation, the grown colonies were sampled directly with LESA-MS and multhiomycin, thioplabin A and B, sulfomycin III and demethoxysulfomycin were detected respectively [4]. The authors concluded that LESA can detect antibiotics from bacterial surfaces and LESA being suitable to provide answers for signaling molecule production, occurrence and function.

Follow up work, also including a team from the University of Jena and the regional Leibnitz Institute, showed that they were able to study the interaction of different fungi when coming in contact with each other in a confrontation assay (Figure 4) [5].

Menezes[5] et al. found that the MS data from the sole fungi region differed significantly from the contact area, suggesting an interaction/ competition of the two fungi studied here. They conclude:

> *'Until now, we were not able to study in vivo chemical interactions of different colonies growing on the same plate..., even though the mycelia covered the agar surface, the sample could be directly measured since the ethyl acetate solvent dissolved the mycelium. ...LESA-HRMS was performed to analyze fungal interactions without sample preparations.'*[5]

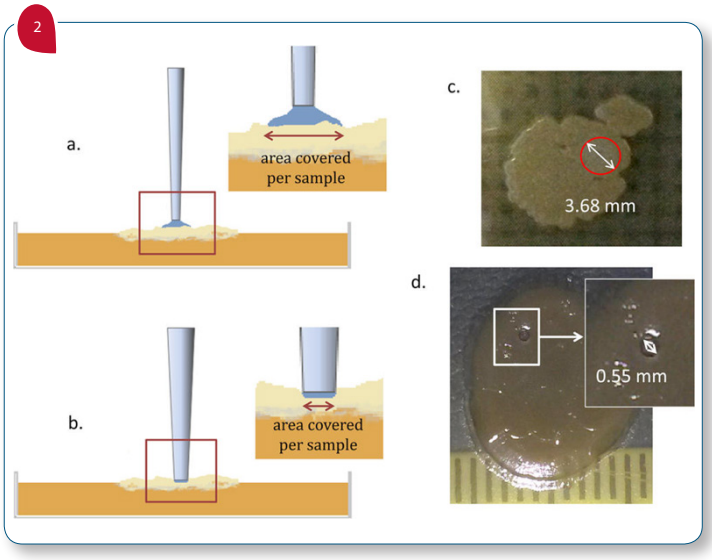

Figure 2: Schematic LESA analysis with a) non-contact mode liquid junction and b) contact mode. Post LESA analysis shows a surface area sampled of 3.68 mm for non-contact mode and 0.55 mm for contact mode. Reprinted under the creative commons licencing from (2).

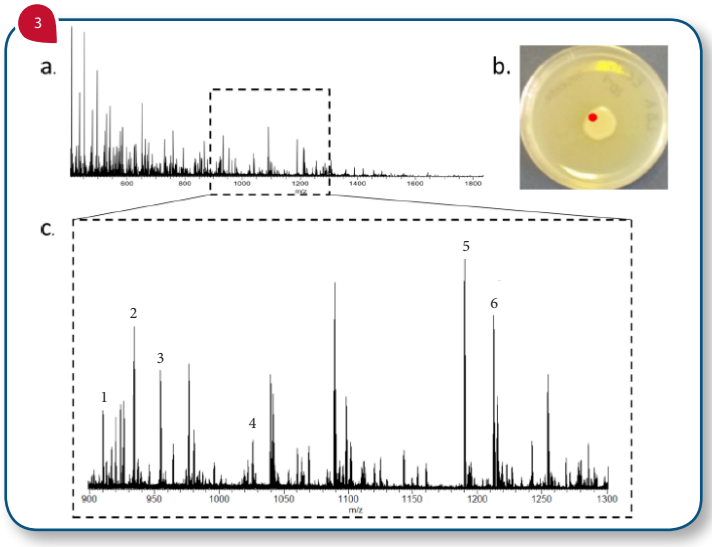

Figure 3: LESA-MS of *E. coli K-12* bacterial colony. (a) Full-scan mass spectrum; (b) photograph of bacterial colony growing on solid agar medium in a Petri dish (red spot marks the region sampled by LESA); (c) enlarged m/z 900-1300 region which contains the majority of peaks corresponding to protein ions. 1-6 indicate peaks subsequently selected for CID identification (data not shown). Reprinted under the creative commons licensing.[2]

RESULTS CONTINUED

A LESA-MS approach was also utilized to detect the toxin Roquefortine C from a molded section of a piece of wood retrieved from dog vomitus in a toxicological study to determine the cause of symptoms of tremor, shaking and sweating[6]. Here, the LESA-MS analysis confirmed Roquefortin C being present on the mold covered wood piece suggesting the fungi visible to be from the class of penicillins (Figure 4).

SUMMARY

The Advion TriVersa NanoMate automated ion source with LESA capability is uniquely suitable to analyze living and in-situ interacting bacterial colonies and fungi based on direct sample analysis with liquid extraction and ESI-MS. It can detect small molecule drugs, metabolites and messenger molecules as well as antibiotic peptides and proteins and provide new insight into the functionality and interaction of microorganisms.

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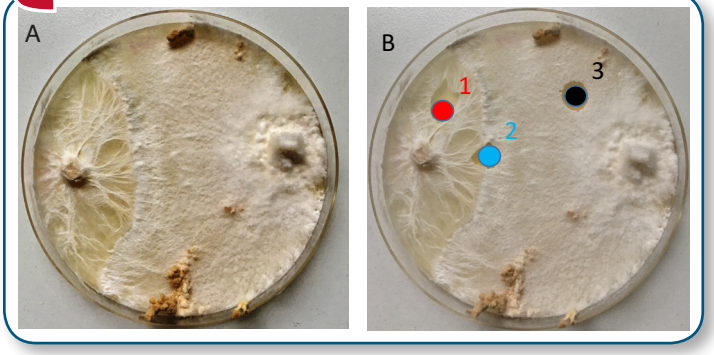

Figure 4: Co-cultivated strains of *S. commune* and *H. fasciculare*.

a. Picture of the confrontation assay before LESA-MS sampling with *S. commune* growing on the right and *H. fasciculare* growing on the right

b.Picture of the culture after LESA–MS analysis highlighting the investigation zones: (1-red) beneath *S. commune*, (2-blue) interaction zone between the fungi, and (3-black) beneath *H. fasciculare*.

Picture and data provided by Riya Menezes.

LESA-MS FOR MICROORGANISMS

- Surface analysis based on liquid extraction and ESI-MS
- Direct analysis without sample preparation
- Living and in-situ interacting bacterial colonies and fungi
- Suitable for small molecule drugs, metabolites and messenger molecules as well as antibiotic peptides and proteins

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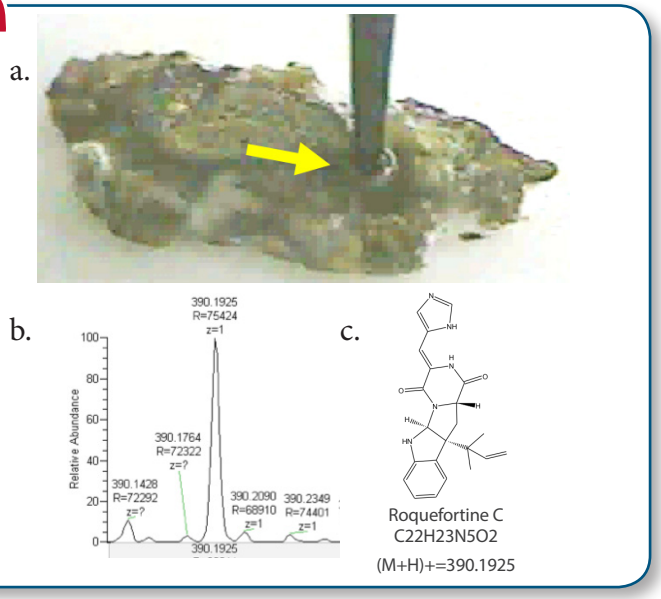

Figure 5: Various molds on a piece of wood retrieved from dog vomitus analyzed by LESA-MS [6]

a. Picture from on-board camera to visualize the liquid junction formed on the target (yellow arrow).

b. LESA-MS analysis on a Thermo Exactive system with 100,000 resolution setting, m/z 390.1925 indicates Roquefortine C with 0 ppm deviation from theoretical value (confirmation MS/MS from QQQ instrument not shown).

c. Chemical structure of Roquefortine C, a mycotoxin produced by penicillium fungi. Roquefortine C causes tremor and shaking in dog as was observed in this case.

REFERENCES

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