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Monitoring of Bacterial Growth and Rapid Evaluation of Antibiotic Susceptibility by Headspace Gas Analysis

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Abstract

Testing of antibiotic resistance by conventional microbiological techniques can be very time-consuming. Measuring the release of volatile organic compounds (VOCs) in the headspace of bacterial culture using mass spectrometry or other gas sensing principles might provide a novel approach in detection of antibiotic susceptibility. In the present study VOCs produced by different strains of Escherichia coli and Staphylococcus aureus were monitored by Ion-molecule Reaction-Mass Spectrometry (IMR-MS). Methanethiol was identified as a marker for bacterial growth. Subsequently, various metal-oxide gas sensors were screened for sensitivity to methanethiol in order to select appropriate cost-efficient sensors for future application in clinical routine.

Keywords: Bacteria; Susceptibility; Mass Spectrometry; Headspace Analysis; VOCs; Gas Sensors

1. Introduction

Worldwide increase in bacterial resistance to various antibiotics makes therapy more and more difficult. Unfortunately, the turnaround time of classical antibiotic susceptibility testing based on microbial growth inhibition is around 12 h. Faster detection and identification of antibiotic susceptibility would allow earlier, more accurate antibiotic targeting, leading to better patient outcomes and reduced costs. In recent years, there is a growing interest
in the detection and identification of bacteria by measuring their release of VOCs. Studies using mass spectrometry, often coupled with gas chromatography, have identified a large number of VOCs including alcohols, ketones and sulfur containing volatiles [1]. Measuring the release of VOCs in the headspace of bacterial culture might also provide a sensitive and fast method for detection of antibiotic susceptibility [2,3].

This study presents an investigation into marker VOCs for antibiotic susceptibility testing by IMR-MS and first tests of low-cost gas sensors for detection of these VOCs. The following bacterial strains were used for experiments: Escherichia coli ATCC 25922 (sensitive to ampicillin) and ATCC 700891 (resistant to ampicillin), Staphylococcus aureus ATCC 29213 (sensitive to oxacillin) and ATCC 43300 (resistant to oxacillin).

2. Material and Methods

Liquid cultures were prepared in Müller-Hinton medium. 10 ml of medium in 25 ml reagent tubes were inoculated with $5 \times 10^5$ colony forming units / ml of bacterial culture. The tubes were placed in a shaker-incubator system (ES-20, biosan) and continuously shaken at 200 rpm at 37°C. For monitoring the VOCs in the headspace an automated experimental set-up was built including a switching valve for sampling continuously each hour up to 8 samples in parallel and a CombiSense featuring an Electron Impact and an IMR-MS mass spectrometer in one instrument (V&F Analyse- und Messtechnik GmbH) (ref. Fig. 1). Experiments with the sensitive and the resistant strains were performed in comparison and as culture medium itself is a source of VOCs blank analyses had to be run, too.

3. Results and Discussion

In order to evaluate the VOC generation of bacterial strains during the growth phase two different strains of E. coli (one sensitive and one resistant to ampicillin) and two different strains of S. aureus (one sensitive and one resistant to oxacillin) were incubated without or with varying concentrations of the suitable antibiotic. Gas samples (~ 60 ml) from each headspace were taken every hour and analysed individually in the IMR-MS mass spectrometer. Background VOCs in the headspace resulting from the culture medium alone were regularly assessed also by analysing gas samples obtained from the headspace of medium alone in the IMR-MS mass spectrometer.
An increase of the amount of a molecule with the molecular mass 48 (later identified as methanethiol) proved to be directly correlated to bacterial growth of both bacterial strains (ref. Fig. 2 and 3). The concentration of methanethiol increased over the time when bacterial growth was visible at a later time point of the experiment (E. coli, resistant and sensitive to Ampicillin, incubated with 0 and 100 μg/ml Ampicillin, respectively; S. aureus, resistant and sensitive to Oxacillin, incubated with 0, 6, 30 μg/ml Oxacillin, respectively), whereas the concentration of methanethiol remained constant at a low level when bacterial growth was suppressed by antibiotics (E. coli, resistant to Ampicillin, incubated with 1000 μg/ml Ampicillin; E. coli, sensitive, incubated with 100 μg/ml Ampicillin; S. aureus, sensitive to Oxacillin, incubated with 6 μg/ml Oxacillin). Concentration of methanethiol in the headspace was found to be around 100 ppb at the beginning of the run and time to result for significant antibiotic susceptibility test was 6h. Methanethiol turned out to be a valid maker gas for bacterial growth for E. coli and S. aureus. Correlation of increase of methanethiol with growth of E.coli was also reported in the literature [4,5].

Fig. 2. Growth dependent increase of gaseous metabolite mass 48 (E. coli).
Absolute counts of mass 48 of each sample were taken. At 0 h absolute counts were set to 1.
Curves show the relative changes of mass 48 over the time.

Fig. 3. Growth dependent increase of gaseous metabolite mass 48 (S. aureus).
Absolute counts of mass 48 of each sample were taken. At 0 h absolute counts were set to 1.
Curves show the relative changes of mass 48 over the time.
First evaluation of the sensitivity of various gas sensors to the isolated marker methanethiol in relevant concentration ranges has been performed (Fig. 4). In order to make the response of different sensors comparable, the sensor signal has been normalized to 100 ppb methanethiol as 1. A distinct but small response to methanethiol in the range of 20 ppb to 100 ppb has been observed. Nevertheless the interference of ethanol has to be taken into account, since ethanol will occur in the headspace in essentially higher concentrations than methanethiol up to the higher ppm range.

![Fig. 4. Comparison of the response of 2 different metal oxide sensor types to ethanol and methanthiole](image)

In order to assess their eligibility in future clinical applications, the detection capabilities of the gas sensors in the matrix of headspace of the bacteria cultures will be validated in a next step. The improvement in selectivity needed will be addressed by applying transient operation techniques for the metal oxide gas sensors.

References