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Review

Use of volatile compounds as a diagnostic tool for the detection of pathogenic bacteria

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ABSTRACT

The analysis of volatile compounds (VCs) generated by bacteria has been proposed as a possible alternative method for the identification of pathogenic bacteria. Further investigations into the VCs generated by many different species and strains of the same species are required alongside the use of consistent growth conditions and procedures throughout VC analysis. Consequently, the true potential of the detection of bacterial VCs as a diagnostic tool in the identification of pathogenic bacteria in clinical and food samples can be determined.

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1. Introduction

Bacterial identification methods in clinical and food microbiology laboratories include more traditional methods, such as culturing and biochemical tests [1]. Culture methods are sensitive and selective, and they can often be invaluable for identification purposes, since the colonies of particular species often exhibit characteristic morphology, including colony size, color and shape. Fig. 1 shows the differentiation of *Escherichia coli*, *Proteus mirabilis* and *Enterococcus faecalis* using a commercially-available chromogenic agar plate; this type of culture medium incorporates enzyme substrates enabling differentiation of bacteria. A major disadvantage of traditional methods, particularly culturing, is that they can be incredibly laborious and time consuming with isolation of bacteria usually taking ~24–48 h, and, in some cases, identification can take much longer [2], so newer procedures are often developed for rapid bacterial identification and quicker diagnosis of disease. More modern methods used to

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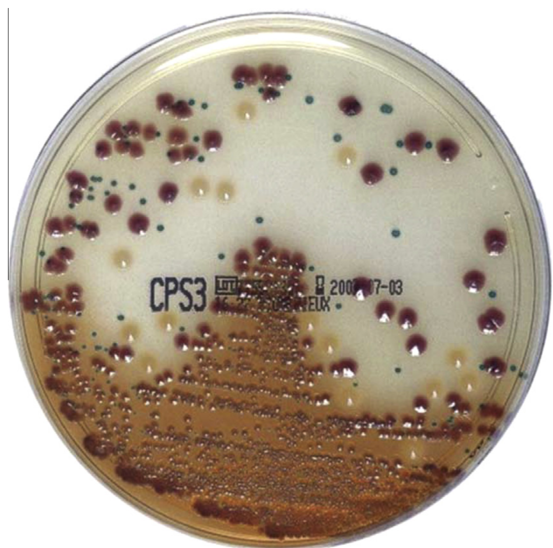

Fig. 1. Chromogenic identification of urinary tract pathogens using ChromID CPS medium (bioMérieux, Marcy-l'Étoile, France). Key: *E. coli*, red colonies indicating ß-glucuronidase activity; *P. mirabilis*, brown colonies indicating deaminase production; *E. faecalis*: green colonies indicating ß-glucosidase activity. (Photograph courtesy of John Perry).

identify bacteria include matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [3] and nucleic-acid-based methods [4]. An area of research involves utilizing bacterial volatile compounds (VCs) as an identification tool. VCs are low-molecular-weight compounds with high vapor pressures that are easily volatilized. They are a diverse range of compounds; here, the focus is on those associated with generation from pathogenic bacteria. VCs can account for the characteristic odors associated with particular bacteria; for example, the grape-like odor often indicative of *Pseudomonas aeruginosa* is 2-aminoacetophenone [5] and the distinctive odor associated with *E. coli* is that of indole [6]. The reasons that bacteria generate VCs are unclear, but it has been hypothesized that VCs are used as signaling mechanisms, defense mechanisms against competing organisms or as growth-promoting mechanisms [6]. The detection of characteristic bacterial VC profiles could potentially be a useful diagnostic tool in the identification of bacteria in clinical and food samples, with one or several VC markers being indicative of a particular species.

Attempts to identify bacteria using VC markers have been ongoing for over 20 years, with earlier reports using VC-collection methods, such as traps composed of a sorbent (e.g., Tenax) to which VCs are adsorbed [7,8], and more recent reports often using solid-phase microextraction (SPME) [9,10]. In both techniques, VCs are extracted from the headspace (HS) above a sample. Gas chromatography (GC), particularly GC coupled to mass spectrometry (MS) (GC-MS), has been widely used as the technique to separate and detect bacterial VCs [11,12] due to its high sensitivity and the ability to separate complicated mixtures of compounds rapidly.

A typical procedure for the analysis of bacterial VCs using HS-SPME coupled to GC-MS is outlined in Fig. 2. Other techniques include GC with a flame-ionization detector (GC-FID) [13], multi-capillary column-ion-mobility spectrometry (MCC-IMS) [14], selected ion-flow tube-MS (SIFT-MS) [15], secondary electrospray ionization-MS (SESI-MS) [16] and proton-transfer reaction-MS (PTR-MS) [17]. Samples prepared for VC analysis include bacteria inoculated into a liquid or solid culture medium, (i.e., a broth or agar), or samples that are simply the clinical or food samples themselves. The identification of food and clinical pathogens using VC markers could be achieved in a shorter time than current methods of bacterial identification allow. The rapid identification of contaminated food samples would enable sources of food-borne illness to be quickly determined and, in clinical cases, the rapid diagnosis of disease would allow rapid treatment with appropriate antibiotics. We review VCs frequently reported as generated by bacteria. Also, we evaluate the use of specific VCs as markers for bacterial identification and the use of VC profiles coupled with multivariate analysis as an identification method. We further consider variation in growth conditions and experimental procedures and their effects on VC generation.

2. VCs as markers for bacterial identification

Many compounds have been reported as bacterial VCs. Schulz and Dickschat [18] reviewed all 346 known VCs generated by bacteria, which have been detected using techniques such as GC-MS. VCs liberated by bacteria are diverse. Reported VCs include aromatic VCs, alcohols, ketones, fatty acids (FAs), hydrocarbons, sulfur compounds and nitrogen-containing VCs [18]. VC profiles can differ between species and it is on this basis that species can be differentiated. The VCs generated by the Gram-negative species *Morganella morganii* and *P. mirabilis* differ significantly (Fig. 3A and B).

Reports are often conflicting as to the identity of VCs evolving from particular species. For example, dimethyl disulfide, isoprene and 3-methyl-1-butanol were identified as the most abundant VCs liberated by *Enterobacter cloacae* by Scholler et al. [13]. However, Arnold et al. [19] detected long-chain alcohols in the VC profile of this species. Also, the range and the numbers of VCs reported vary massively. Scholler et al. [20] reported the liberation of over 120 VCs from *Streptomyces* species, including 3-methyl-1-butanol, dimethyl disulfide and 2-phenyl ethanol. Conversely, Kai et al. [21] reported that VCs were not generated by *Bacillus subtilis*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia*.

2.1. Alcohols, ketones, hydrocarbons and FAs

Long-chain aliphatic alcohols are commonly associated with bacteria in the Enterobacteriaceae family [19,22,23]. The reduction of FAs to their corresponding long-chain alcohols by *E. coli* was demonstrated by Hamilton-Kemp et al. [23]. Here, the culture medium was supplemented with the FAs octanoic, decanoic and dodecanoic acid. The concentration of the corresponding alcohols (i.e., 1-octanol, 1-decanol and 1-dodecanol), produced by *E. coli*,

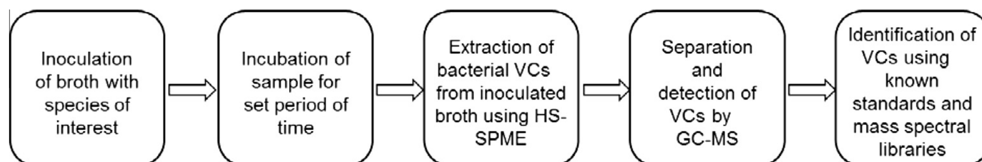

Fig. 2. Typical procedure for bacterial VC analysis using HS-SPME GC-MS.

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