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MINI-REVIEW

Terahertz spectroscopy for bacterial detection: opportunities and challenges

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Abstract The demand for advanced bacterial detection tools is continuously increasing, promoted by its significant benefits in various applications. For instance, in the medical field, these tools would facilitate decision making about more tailored therapies once the infection source has been identified. In the past few years, terahertz (THz = 10^{12} Hz) spectroscopy has also shown potential as a novel bacterial detection modality due to its unique advantages. Impressive breakthroughs have been achieved in this field related to bacterial component characterization, spore identification, and cell detection. However, some intrinsic limitations and technical bottlenecks have led to some debates about the practicability of its clinical adoption. In this review, we summarize the progress achieved in this field and discuss some challenges and strategies for future implementation of practical applications.

Keywords Bacterial detection · Biosensor · Rapid detection · Terahertz time-domain spectroscopy · Spore

Introduction

Fast and accurate detection of bacteria is vital for human health care, security inspection, and food safety control. Conventional culture-based methods are at the mercy of the microbial growth rate. It usually takes a few days or even weeks (for some slow-growing bacteria such as *Mycobacterium tuberculosis*) to unambiguously identify the target bacterium. Emerging technologies including molecular methods (nucleic acid based) and mass spectrometry (MS) have brought significant advances in detection speed by eliminating some time-consuming procedures. At the same time, various optical, piezoelectric, and electrochemical biosensors have also been developed to address the unmet needs for various applications, such as shorter turnaround time, enhanced detection sensitivity and applicability to minimally trained personnel (Lazcka et al. 2007). In addition to bacterial identification, the differentiation between living and dead bacteria under investigation is also of great importance. The detection of dead bacteria is of little significance in making a diagnosis of an infectious disease because the potential hazard is limited to the living portion of a mixed bacterial population (Buchan and Ledeboer 2014). Moreover, analyzing the living state of bacteria under investigation would be of great value for cell antimicrobial susceptibility testing, which is currently monitored by the visible changes in bacterial density (van Belkum et al. 2013). Routine differentiation between living and dead bacteria is mainly based on the assessment of membrane integrity through staining with DNA-binding dyes (Nocker et al. 2006). An alternative method for a label-free and non-invasive bacterial viability evaluation is urgently needed for many microbiological applications.

Terahertz (THz) radiation generally refers to 0.1 to 10 THz of the electromagnetic spectrum, 3.33 to 333 cm^{-1} in wavenumbers or 30 to 3000 μm in wavelength (Qin et al. 2013). Historically, this "THz gap" between photonics and electronics remains unexplored, until the advent of efficient THz sources and detectors in the 1980s (Auston and Nuss 1988; Pickwell and Wallace 2006). Currently, a variety of

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systems has been developed to explore this natural bridge between the quantum mechanical and classical descriptions of electromagnetic waves (Chan et al. 2007). THz radiation is now finding widespread applications in various fields such as biomedical research, security screening, communication, and chemistry (Williams 2007).

Due to its unique frequency range, THz radiation possesses some attractive properties that are well suited to bacterial detection (Table 1). When THz radiation propagates through bacterial cells, collective low-frequency vibrational modes can be excited synchronously, including weak interactions such as hydrogen bonds, van der Waals forces, and non-bonding (hydrophobic) interactions. Accordingly, THz spectroscopy enables the detection of bacterial cells based on their specific spectral characteristics as a result of the interactions between THz radiation and the low-frequency molecular motions of the cellular components of bacteria. Given the variance of cellular constituents, THz spectroscopy can be utilized as a rapid and label-free bacterial detection method. The underlying analytical principles of bacterial detection by THz spectroscopy are shown in Fig. 1. Some distinct spectral signatures (absorption peaks) have been detected for spores of *Bacillus* species by Fourier transform spectroscopy (FTS) (Brown et al. 2006), THz time-domain spectroscopy (THz-TDS) (Yu et al. 2005), and photomixing spectrometer (Zhang et al. 2014). In addition, vegetative cells of *Bacillus subtilis* and *Escherichia coli* exhibited different absorption spectra, although better spectral resolution is required for more obvious discrimination (Globus et al. 2012). Notably, the different absorption spectra of thermally treated and untreated *B. subtilis* cells clearly demonstrate the capability of THz spectroscopy for differentiating between living and dead bacteria under investigation. This review provides a brief introduction to the common THz systems for bacterial detection, followed by a summary of the progress to date of bacterial detection by THz spectroscopy; some challenges and strategies for future research will also be highlighted in the last section.

Instrumentation

FTS operating in the sub-THz range (10–25 cm^{-1}) has been utilized for bacterial detection due to its ability to reveal differences in spectral features between bacterial species (Globus et al. 2012). FTS provides the advantage of extremely wide spectral coverage in a single experiment, typically from 100 GHz to over 5 THz, despite its relatively poor signal-to-noise ratio (SNR). A detailed introduction to this mature instrument can be found in a previous review (Markovich and Pidgeon 1991) and will not be included here. In addition, THz-TDS and photomixing spectrometer are two commonly used THz systems for bacterial detection.

THz-TDS system

THz-TDS has been widely utilized in various biomedical applications since its invention by Grischkowsky et al. in the late 1980s (Fattinger and Grischkowsky 1988; Fattinger and Grischkowsky 1989; Grischkowsky et al. 1990). A THz-TDS system generally contains a femtosecond laser, which operates with a near 100-MHz repletion rate to produce a train of 100-fs laser pulses. The pulse train is split into a pump beam to generate THz radiation and a probe beam to gate the detector by a beam splitter

Table 1 Attractive properties of THz radiation for bacterial detection

Properties	Explanation	Benefits	References
Spectral fingerprint	THz spectroscopic patterns of different bacterial cells and metabolites are unique and can be utilized as their fingerprints	Collection of the unexplored "THz fingerprints" complement the knowledge of spectral data obtained with infrared and Raman spectroscopy for bacterial detection	Wang et al. (2010); Zhang et al. (2014)
Strong water absorption	Water molecules are significantly more absorptive than common macromolecules over the entire THz frequency range	THz spectroscopy can be used to rapidly assess the living state of bacteria (differentiating between living and dead bacteria) according to their different hydration levels	Globus et al. (2012); Yang et al. (2016)
Transparency of packaging materials	Materials composed of non-polar molecules such as paper and plastic, which are opaque to visible and near-infrared waves, are largely transparent in the THz range	This property facilitates easier security checks because THz radiation can penetrate packaging such as envelopes. Moreover, THz radiation should be an ideal light source for detection in smoke or dust	Qin et al. (2013); Wang et al. (2002)
Convenience of data analysis	Different from infrared spectroscopy, THz spectroscopy provides direct access to the electric field in both amplitude and phase	Spectral parameters can be obtained after Fourier transform of the electric field without Kramers-Kronig equations, including the absorption coefficient, refractive index, and complex dielectric constant.	El Haddad et al. (2013)

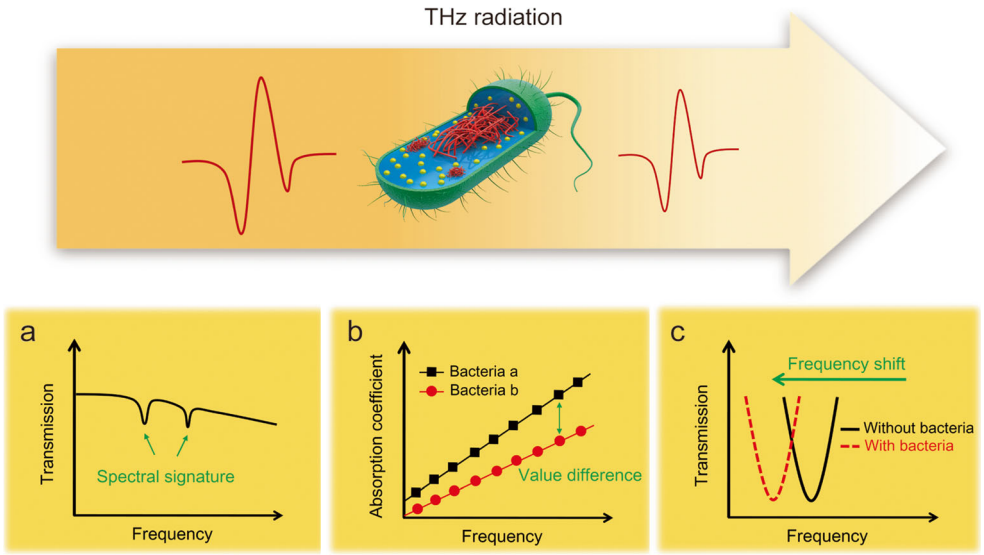

Fig. 1 Schematic representation of bacterial detection by THz spectroscopy. **a** Bacterial cell, spore, and intracellular metabolite can be characterized as distinct spectral signatures in the THz spectra. **b** Differences in THz optical constants enable the differentiation between bacterial species. **c** THz metamaterial biosensors empower highly sensitive bacterial detection by investigating the resonant frequency shifts

(Fig. 2a) (Ferguson and Zhang 2002). THz pulses can be generated when the pump beam is incident upon a THz emitter and subsequently collimated to the sample by a pair of parabolic mirrors. After transmission through the sample, the THz pulses carrying information about the sample are refocused on the THz detector by another pair of parabolic mirrors. Simultaneously, the probe beam used to gate the detector measures the THz electric field containing both the phase and amplitude information as a function of time. The transmitted THz electric field is measured coherently, which provides a time-resolved spectroscopic analysis of the sample and effectively rejects many common sources of noise. A typical THz-TDS system has a bandwidth between 2 and 5 THz with an SNR between 50 and 60 dB at an integration time of 1 s, a spectral resolution of 50 GHz and an acquisition time of under 1 min (Ferguson and Zhang 2002; Redo-Sanchez et al. 2013).

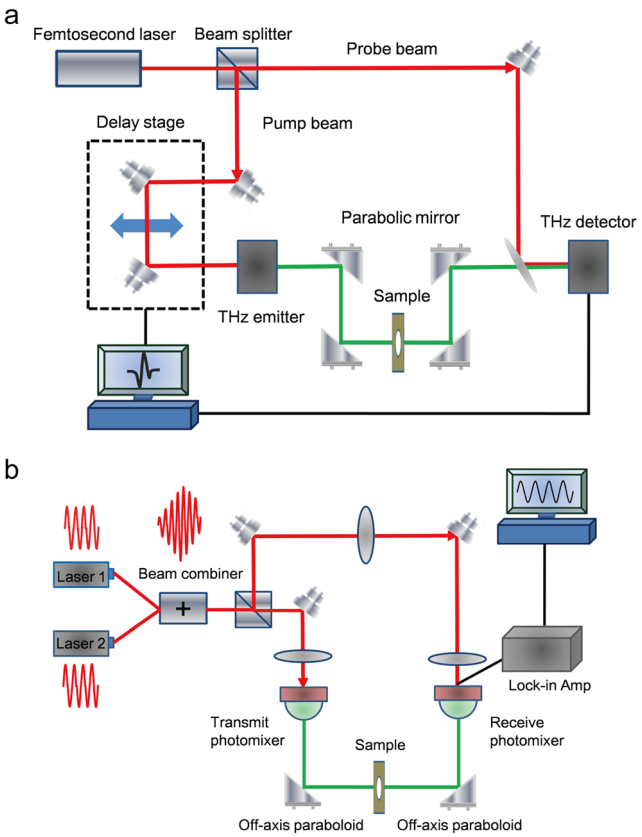

Fig. 2 Schematic diagrams of THz systems: **a** THz-TDS pump probe system. **b** Photomixing spectrometer

A reference spectrum is necessary to obtain the THz spectrum of the sample during measurement. When THz-TDS system operates in transmission mode, the reference pulse is usually measured in the absence of sample, and the sample pulse is obtained with the sample positioned in the focal point of THz radiation. In contrast, in reflection mode, the reference pulse is commonly obtained by replacing the sample with a material of known reflectance such as a mirror (Gowen et al. 2012). Transmission mode is frequently utilized for bacterial detection due to the relatively small cell size (1–3 μm) and sample thickness (typically <100 μm). In addition to the transmission spectra, various spectral parameters can be obtained in the frequency domain after a Fourier transform of the time-domain signals. The refractive index $n(f)$, absorption coefficient $\alpha(f)$, and complex dielectric constants $\varepsilon(f)$ can be calculated using the following algorithms (Fischer et al. 2005; Kindt and Schmuttenmaer 1996):

$$n(f) = \frac{|\varphi_s(f) - \varphi_{\mathrm{ref}}(f)|.c}{2\pi \mathrm{fd}} + 1 \quad (1)$$

$$\kappa(f) = ln\left[\frac{4n(f)}{\rho(f)[n(f)+1]^2}\right]\frac{c}{2\pi fd} \quad (2)$$

$$\alpha(f) = \frac{4\,\pi\, f\,\kappa(f)}{c} \quad (3)$$

$$\varepsilon(f) = [n(f) - i\kappa(f)]^2 = \varepsilon_1 - i\varepsilon_2 \quad (4)$$

where $\kappa(f)$ is the extinction coefficient of the sample, $\varphi_s(f)$ and $\varphi_{ref}(f)$ are the phase angles of Fourier transforms of the power transmissions of the sample and reference, $\rho(f)$ is the amplitude ratio of the sample and reference, ε_1 and ε_2 are the real and imaginary parts of the dielectric constants, c is the light speed in a vacuum, f is the frequency, and d is the optical path.

Photomixing spectrometer

Alternatively, a photomixing spectrometer is a high-resolution, coherent frequency-domain transceiver and is frequently used to detect spectral signatures of spores because of its excellent spectral resolution (Zhang et al. 2013). It generates continuous-wave THz signals by driving a GaAs photoconductor, on which interdigitated metal electrodes are patterned with two frequency-offset lasers. Two single-mode and temperature-tunable distributed-feedback (DFB) semiconductor lasers are at different frequencies $\nu_{\pm} = \nu_0 \pm \nu_{THz} / 2$. A schematic representation of a typical photomixing spectrometer is shown in Fig. 2b (Zhang et al. 2013). Two photomixers are used in the system as the transmitter and the receiver, respectively. The generated radiation is coupled from the planar square-spiral antenna to free space through silicon hyperhemispherical lens and then collimated by an off-axis paraboloid. The sample under investigation is mounted in the free-space path halfway between two photomixers. Subsequently, the transmitted signal is measured by a lock-in amplifier after a reverse process between the free space and the receive photomixer (Brown et al. 2010).

This spectrometer has an instrumental resolution of 500 MHz defined by the frequency step, as well as a high dynamic range of typically 70–80 dB around 0.1 THz and 40–50 dB at 1 THz (Zhang et al. 2014). Due to its excellent spectral resolution, distinct signatures can be directly observed in the transmission spectra without calculating the aforementioned spectral parameters. Although this technique requires a longer measurement time than THz-TDS, it is considered to be potentially more accurate, more inexpensive, and less complex than the conventional pulsed system due to its excellent frequency resolution and huge spectral density (Preu et al. 2011).

Bacterial component characterization by THz spectroscopy

All bacterial cellular components possess the potential to contribute to the overall THz spectra and might be essential for the detection of bacterial spores and cells (Bykhovski et al. 2007). In the past few years, there has been extensive research on the characterization of various cellular components, such as dipicolinic acid (DPA), DNA, and metabolites. DPA is a unique component of *Bacillus* spores, constituting 5 to 15 % of the entire dry weight of spores, and it does not exist in vegetative cells (Paidhungat et al. 2000). Therefore, the transmission spectrum of DPA shares common features with the spectra of *Bacillus thuringiensis* and *Bacillus globigii*, but evidently differs from that of *Aspergillus niger* (Fitch et al. 2004). Moreover, the distinct spectral signature at 1.54 THz of DPA powder coincides with the 1.538 THz peak observed in *B. subtilis* spores (Yu et al. 2005). These similarities indicate the possibility of detecting *Bacillus* spores through DPA detection. Another study demonstrated the different spectral signatures of DPA and its calcium complex Ca-DPA (Cook et al. 2004) to detect the spectral differences of components between *Bacillus* spores. *B. subtilis* contains DPA complexed with Ca in an amorphous form, whereas the Ca-DPA complex of *Bacillus cereus* is highly ordered and may be crystalline (Leuschner and Lillford 2001). However, it appears impossible to differentiate between *Bacillus* spores based on their spectral difference in DPA components because the signals can be largely masked by those of other components.

The spectral features of DNA have also been studied in detail. *B. subtilis* DNA and cells revealed common features were in the THz spectra, but a more pronounced correlation can be obtained between the spectra of DNA and spores (Bykhovski et al. 2005). This discrepancy is not surprising because cells contain more complicated components than spores. Specifically, the apparent similarity in the absorption spectra of *E. coli* DNA and cells clearly demonstrates the significant contribution of DNA to the overall spectra, which may be attributed to the strong absorption of hydrogen bonds in DNA (Globus et al. 2012). Furthermore, the spectra of *E. coli* DNA and *B. subtilis* DNA also displayed notable differences, although their base-pair compositions (the numbers of A = T and G = C) are not very distinguishable.

Remarkably, THz spectroscopy has shown potential in screening industrial strains due to its ability to detect intracellular metabolites. *B. subtilis* cells and the intracellular metabolite riboflavin have distinctive absorption features in the THz range (Fig. 3) (Wang et al. 2010). Specifically, riboflavin is highly absorptive of THz radiation and has several spectral signatures in its absorption spectrum; in contrast, *B. subtilis* cells exhibit a relatively small absorption and flatter absorption spectrum. This pronounced contrast makes it possible to distinguish riboflavin from *B. subtilis* cells, which further

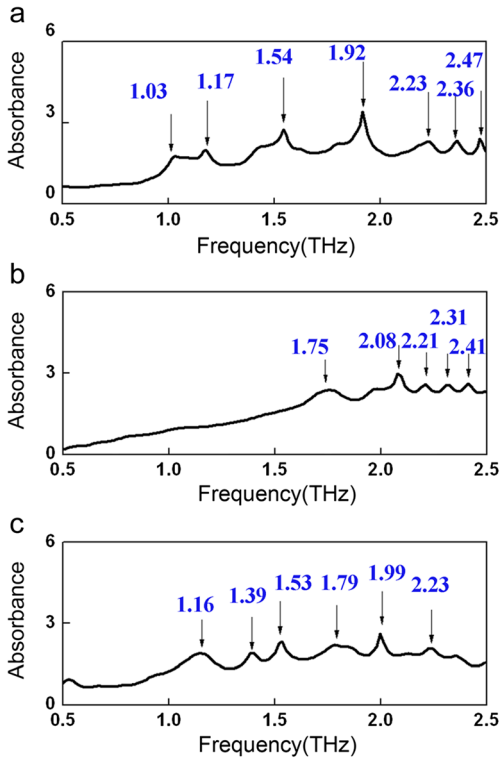

Fig. 3 THz absorbance spectra of pressed tablets of riboflavin and *B. subtilis*. **a** Riboflavin. **b** *B. subtilis* strains with poor riboflavin productivity. **c** *B. subtilis* strains with high riboflavin productivity (Wang et al. 2010). Permission obtained from Wiley

enables the differentiation between *B. subtilis* strains with poor riboflavin productivity and strains with high productivity. The feasibility of intracellular metabolite detection facilitates high-throughput industrial strain screening, which is a vital need for metabolic engineering (Tyo et al. 2007).

Motivated by an in-depth understanding of THz features, theoretical predictions of bacterial cellular components were made to simultaneously compare with experimental data. A series of elegant investigations in this field has been conducted by Globus group. Computational modeling techniques have been developed to understand and even to predict the spectral features of proteins and nucleic acids using the energy minimization, normal mode analysis, and molecular dynamics (MD) approaches. The reasonably good correlations clearly validate the experimental and theoretical results for tRNA (Bykhovski et al. 2007), DNA (Globus et al. 2013), and thioredoxin (Alijabbari et al. 2012) from *E. coli*. Moreover, the predicted THz signature of *Bacillus anthracis* DNA was quite distinguishable from its simulants, *B. subtilis* DNA or *E. coli* DNA (Bykhovski et al. 2005). However, MD simulations have only been performed for relatively small biomolecules and not the entire bacterial cell due to the limited level of current computational capabilities. Predictive capabilities of MD computational modeling can be further improved after acquiring greater clarity about the mechanism of intramolecular relaxation dynamics (Globus et al. 2012).

Due to its unprecedented ability to characterize weak interactions, THz spectroscopy is highly suited for monitoring the changes in cellular components. The subtle structural changes of microvessel endothelial cell monolayers after the vascular endothelial growth factor treatment can be characterized as the decrease of the THz differential signals, although these morphological differences cannot be observed with standard optical phase contrast microscopy (Liu et al. 2007). Therefore, THz spectroscopy possesses potential applications in monitoring biofilms for sterilization effect evaluation and dental plaque detection in hospital settings. Additionally, future studies can be envisioned, such as monitoring bacterial growth and morphology changes over time, that exploit this unique property. Moreover, cell membrane rupture, cytoplasm leakage, and DNA damage of bacterial cells after drug treatment can also be effectively probed, which could lead to the development of a novel tool for investigating small changes in bacterial cells during cell engineering in the future.

Bacterial spore identification by THz spectroscopy

Bacterial spores (i.e., endospores) are robust and dormant life forms that are resistant to extreme temperatures and environmental stresses. A typical spore is composed of a spore core containing nucleic acids and DPA, a spore cortex containing mainly peptidoglycan and a spore coat known as the protective shell (Russell 1990). The absorption spectra of *B. subtilis* spores and cells were closely correlated and also contained distinguishing features because spores have more compact structures than vegetative cells (Globus et al. 2012). *Bacillus* spores are generally a panel of standards used for evaluating the performance of new methods for bio-agent detection (Belgrader et al. 1999). The Brown group is well known for its extensive research on the THz signatures of bacterial spores. A summary of the measured samples and corresponding candidate signatures is provided in Table 2.

In the past decade, growing evidence has indicated that spectral signatures can be influenced by three main factors, with sample preparation (sample conformation) being first and foremost (Majewski et al. 2005). More specifically, the dilute *B. subtilis* powder mixed with polyethylene powder displays stronger absorption depths and narrower signatures than the concentrated samples without polyethylene powder, which is attributed to the smearing of single-bioparticle electromagnetic resonances (Brown et al. 2004). For concentrated samples, the electric field on a given bioparticle can be screened or distorted by neighboring bioparticles, which leads to the broad step-like feature. Thus, the different electromagnetic interactions owing to conformational differences between dilute and concentrated samples lead to their inconsistent spectral signatures. Despite the challenge of signature variations, reproducible signatures can still be compared in

Table 2 Candidate signatures of bacterial spores in the THz range

Bacteria	Candidate signatures	Specimen	Instrumentation	Temperature	References
B. subtilis	Dilute sample: 253, 418, 1037 GHz; concentrated sample: 250–290[a], 425 GHz	Powder	Photomixing spectrometer	Room temperature	Brown et al. (2004)
	Multiple resonance features	Bacterial suspension[b]	FTS	1.7 K	Globus et al. (2012)
	1538 GHz	Dry bacterial film	THz-TDS	Room temperature	Yu et al. (2005)
B. globigii	Dilute sample: 250, 415, 1035 GHz; concentrated sample: 275, 425 GHz; aerosol sample: 260, 420, 500 GHz	Powder	Photomixing spectrometer/FTS	Room temperature	Brown et al. (2006)
B. thuringiensis	917/955[c], 1015GHz	Bacterial colonies	Photomixing spectrometer	Room temperature	Zhang et al. (2014)

[a] Broad step-like feature

[b] Air-dried before measurement

[c] Depending on the hydration level

samples with identical preparation forms. For instance, *B. globigii* is phenotypically indistinguishable from *B. subtilis* except in terms of the pigment production on media containing an organic nitrogen source (Burke et al. 2004). However, the frequency shifts of the spectral signatures of their dilute samples may address the selectivity simply (Table 2). Second, the hydration level of bacterial spores may play a role in the THz signatures. Fresh (normal hydration) *B. thuringiensis* spores have a distinct signature at 917 GHz, whereas the corresponding signature for less hydrated *B. thuringiensis* spores increases by 38 to 955 GHz (Zhang et al. 2014). This difference between signatures can be reproducible in repeated measurements, and neither signature appears for completely dried samples, indicating that the source of THz absorption is likely from the polarized spore outer coat, which is sensitive to environmental changes and alters its hydration level accordingly. A detailed explanation of the physical origin of spectral signatures and the issue of signature reproducibility can be found in a previous study (Brown et al. 2006). Third, the experimental temperature also has an effect on the spectral signature. Absorption spectra of bacterial spores revealed multiple resonance features from FTS at a very low measurement temperature of 1.7 K (Globus et al. 2012). At room temperature, however, these resonance features can be washed out due to instantaneous conformational changes and a large density of overlapping states of absorption bands. Signatures obtained at room temperature should be more beneficial in practical applications (e.g., stand-off detection), for excluding the cryogenic cooling step.

Based on mathematically sound principles of detection and estimation theory, signal processing methods can extract the signatures from clutter, interferants, and noise (Majewski et al. 2005). As such, detection filters can be developed for bacterial spores using optimal algorithms after the collection of signature data. Preliminary analysis of a limited set of spectral data demonstrated that the *B. subtilis* detection filter is sensitive enough to discriminate against *B. thuringiensis* signatures, indicating that the proposed algorithms can effectively discriminate between nearby neighbors.

Remote sensing of biological warfare agents in environmental samples is still an unmet need (Irenge and Gala 2012), but THz spectroscopy appears to be of limited use in realistic atmospheric conditions due to the strong water vapor absorption and the current performance of THz systems (power and SNR). Detecting spore powders should be more practical for future applications considering the issue of signature stability. Therefore, a reliable database is urgently needed, which necessitates a baseline criterion for signature quality that considers sample preparation and experimental conditions. In addition, THz technology is an image-spectrum merging modality; therefore, its imaging ability should be investigated for bacterial spore identification. A previous study has demonstrated the feasibility of detecting 500 mg of *B. thuringiensis* spores inside envelopes by THz imaging (Wang et al. 2002). But single bacteria detection by THz imaging is too hard to be realized at present stage because ideal THz instruments with adequate spatial resolution are yet to be developed.

Bacterial cell detection by THz spectroscopy

Qualitative and quantitative analyses of bacteria have been realized using THz biosensors based on their different responses to THz radiation. The selective and enhanced detection of bacterial monolayers was realized by THz plasmonic antennas, on the basis of their different dielectric responses in the THz range (Berrier et al. 2012). The presence of a bacterial monolayer on a plasmonic chip can be characterized as a redshift of the resonance frequency in the extinction spectrum. Therefore, Gram-positive bacteria (*Staphylococcus epidermidis* and *B. subtilis*) can be effectively distinguished from Gram-negative bacteria (*E. coli*, *Moraxella catarrhalis*, and *Serratia marcescens*) by comparing their differences in

frequency shifts and extinction. The proposed method indicates that Gram-positive bacteria have smaller THz absorption than Gram-negative bacteria, and this trend has also been observed in a recent study (Yang et al. 2016). Additionally, *E. coli* was quantitatively detected through the interaction between the evanescent wave in a fiber and a thin layer of bacteria covering it (Mazhorova et al. 2012). T4 bacteriophages, which bound and eventually lysed the *E. coli* cells, were adsorbed to the fiber core. The transmission spectra of the fiber were compared during three steps: the fiber with adsorbed phages only, the fiber with bacterial cells bound to phages, and the fiber after bacterial lysis. The fiber transmission decreased first due to the binding of bacteria; it then increased due to bacterial lysis, but was still lower than the level of the first step as a result of fragments remaining bound to the fiber surface. From the correlation between the fiber absorption losses and bacterial concentration, a detection limit of 10^4 cfu/ml was obtained, which is comparable to existing commercial methods (Arya et al. 2011).

Moreover, the sensitive detection of bacteria in both ambient and aqueous environments is feasible via THz metamaterials (Park et al. 2014). The change in the effective dielectric constant of THz metamaterial patterns caused by bacterial coatings can lead to a shift in the resonant frequency, which has been investigated for bacterial detection. Alternatively, *E. coli* detection in an aqueous environment was also achieved utilizing a spacer with metamaterial patterns functionalized with *E. coli* anti-body. Because different bacteria have distinct dielectric responses in the THz region, THz metamaterial biosensors are potentially attractive for the highly sensitive on-site detection of different bacteria in various environments.

Another application of THz biosensing is to provide an insight into the spectral differences between living and dead bacteria. Distinguishing features in the absorption spectra were observed between thermally treated (100 °C, 60 min) and untreated cells of both *E. coli* and *B. subtilis* as a result of the irreversible transformation of cellular component structures (Globus et al. 2012). Essentially, the changes in collective low-frequency vibrational modes may account for the distinctive features in the transmission spectra. The spectral features of bacterial cells may have a significant contribution from DNA. However, the latest study on this topic revealed that intracellular water should be the dominant factor because the absorption coefficients of bacterial powder (intracellular water removed) were at least an order of magnitude lower than those of living bacteria (Fig. 4) (Yang et al. 2016). Thus, different species of bacteria could be distinguished by their different water contents. More importantly, living and dead bacteria of the same species also showed different absorption coefficients due to their different hydration levels. By comparing the absorption coefficient values at 1.0 THz, the absorption differences between living and dead bacteria were all statistically significant. In brief, THz spectroscopy can not only be used to detect different species of bacteria but also possesses the ability to differentiate between living and dead bacteria of the same species according to their different hydration levels.

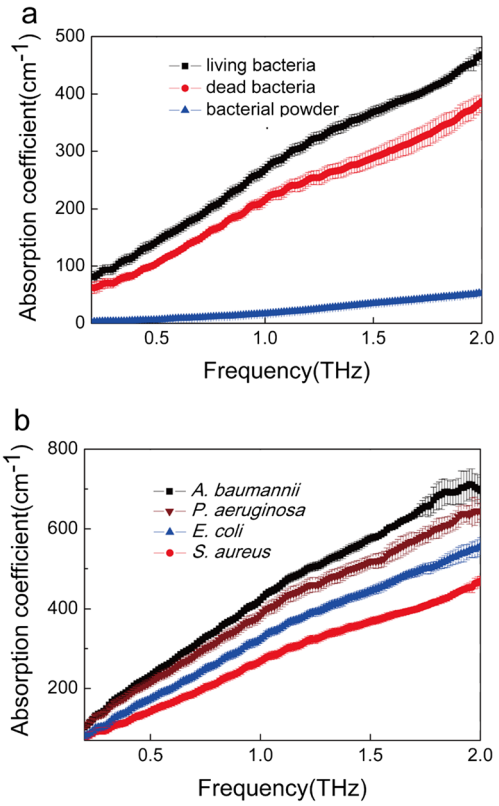

Fig. 4 THz absorption coefficients of bacteria. **a** Comparison of absorption coefficients for living bacteria, dead bacteria, and bacterial powder of *S. aureus* (living bacteria > dead bacteria > bacterial powder). **b** Distinctive absorption coefficients of different species of bacteria can be used for bacterial discrimination (*A. baumannii* > *P. aeruginosa* > *E. coli* > *S. aureus*). Absorption values for living and dead bacteria may be overestimated in calculation (Yang et al. 2016). Permission obtained from Wiley

Because the same species of bacteria have relatively constant water contents under identical culture conditions (standard deviation <1 % for living bacteria), a reference database composed of absorption coefficients can be built for bacteria of common interest. Akin to the spectral profiles delineated by mass-to-charge ratios for MS, THz spectroscopy enables the identification of an unknown bacterium based on its unique absorption coefficients delineated by its water content. Nevertheless, the absorption coefficient of a dead bacterium is likely to overlap with another living bacterium; therefore, such a method would serve only to supplement rather than replace existing methods. Moreover, the limited Gram-positive species investigated appear to have smaller THz absorption values than those of Gram-negative bacteria. It remains unclear whether this phenomenon was biased by the water contents of the limited number of species selected for investigation to date; therefore, further studies of additional species are needed to validate this trend.

Concluding remarks

A comparison of THz spectroscopy with the common existing tools for bacterial detection is provided in Table 3. The future prospects for THz spectroscopy should focus on developing rapid, label-free, and convenient biosensors for bacterial analysis instead on implementation in routine clinical laboratories. When the rapid identification of common pathogens is a priority to choose an appropriate therapy early enough, THz spectroscopy will be of great value because the spectra can be recorded within seconds to minutes. The simplicity of the portable THz system (Wilmink et al. 2011) will facilitate its future application in resource-limited settings such as remote villages or point-of-care tests on the battlefield. Alternatively, THz spectroscopy should also be invaluable for monitoring the small morphology alterations of bacterial cells. The non-invasive, in situ, and real-time investigation of cytoplasm leakage in epithelial cells has been realized by THz attenuated total reflection measurements (Grognot and Gallot 2015). The hydration state in human cancer cell monolayer, which is associated with various cellular activities, can also be effectively investigated by this novel technology (Shiraga et al. 2015). All these innovative findings pave the road for developing sensitive THz biosensors for analyzing structure changes of bacterial cells in the near future.

Despite these potential applications, some challenges in bacterial detection by THz spectroscopy still exist. First, the limited sensitivity is related to the size mismatch between the THz wavelength and bacterial cells. Specifically, the wavelength of THz radiation at 1 THz is 300 μm, whereas the size of a typical bacterial cell is 1–3 μm, on the order of ~λ/100. According to the Rayleigh scattering theory, the size mismatch can lead to a low scattering cross section, which can further hinder reliable data collection from small quantities of bacterial cells. Accordingly, the limited sensitivity should be addressed through THz metamaterials or antennas, which allow enhanced coupling between bacterial cells and THz radiation. By investigating the frequency shifts of the resonances resulting from the structures of metamaterials or antennas, small amounts of bacteria can be effectively detected. A previous study detailing THz antennas for the detection of molecules demonstrated that a sensitivity enhancement of at least three orders of magnitude can be achieved using antennas with a 5-μm-wide slot (Park et al. 2013). Thus, the sensitivity can be further improved by optimizing the antenna structure to be compatible with bacterial cells. Alternatively, metasurfaces (i.e., two-dimensional versions of metamaterials) are capable of extraordinary sensitivity enhancement due to strongly localized near-fields at the resonances (Xie et al. 2015), and these enhanced sensing methods should also be investigated for bacterial detection. Utilizing optimal THz metasurface structures, only a trace amount of bacterial cells or even single cells may be effectively detected.

Moreover, the absorption spectra of vegetative cells at room temperature exhibit only monotonic increases without any characteristic peaks. Thus, overlapping absorption spectra can be easily predicted when the database involves several bacteria with similar water contents. The issue of the specificity of THz spectroscopy should be carefully addressed before serving as an alternative tool for distinguishing between different bacteria. Hence, affinity-based assays using antibodies or aptamers can be utilized to specifically recognize the target bacterium. When combined with microfluidic cell

Table 3 Comparison of THz spectroscopy with common bacterial detection methods

Characteristics	THz spectroscopy	Conventional methods	Mass spectrometry	Molecular methods
Detection principle	Spectral features	Phenotypic and biochemical tests	Spectral profile based on mass-to-charge ratio	Specific nucleic acid amplification
Time required for identification	Minutes after culture	Days to weeks	Minutes after culture	One to several hours
Operational procedure	Simple	Complex	Simple	Complex
Reagent-free	Yes	No	Yes (Only matrix)	No
Limit of detection	10^4 cfu/ml[a]	10–100 cfu/ml[b]	10^5–10^6 cfu[c]	Several to tens of genome equivalents[c]
Ability to differentiate between living and dead bacteria	Yes	No	No	No[d]
Imaging ability	Yes[e]	No	No	No

[a] Mazhorova et al. (2012)

[b] Lazcka et al. (2007)

[c] Buchan and Ledeboer (2014)

[d] Except through optimized assays like reverse transcriptase PCR

[e] THz imaging

sorting, affinity-based identification methods could realize the rapid and selective detection of microbes of interest in aqueous solutions (Ishii et al. 2010). Notably, an innovative study has demonstrated the real-time detection of individual polystyrene-based microparticles (10 μm) flowing in water by THz spectroscopy after coupling the microfluidic platform with a plasmonic antenna (Masini et al. 2014). However, the signal-to-noise ratio of that lab-on-a-chip device needs further improvement for bacterial detection because the signal difference between the bacterial cells and water would be much smaller than that between polystyrene and water. Moreover, further developments including the adoption of sensitive room temperature detectors and replacing the polydimethylsiloxane fluidic layer with less absorptive materials such as quartz or silicon are desirable for practical implementations.

Strong absorption by water is another challenge that currently limits THz technology (Fan et al. 2014). On the one hand, subtle changes in the intracellular water content of bacteria can be accurately revealed by THz spectroscopy, hinting at the feasibility of assessing its living state. On the other hand, the strong attenuation of THz radiation by extracellular water affects precise detection. Although the microfluidic device can partially relieve this influence by reducing the water layer thickness when measuring in aqueous environments, significant effort is still needed to break through this intrinsic technical bottleneck.

Given these challenges, THz spectroscopy remains in its infancy and fails to exhibit obvious advantages over Raman spectroscopy (Pahlow et al. 2015). Future prospects for THz spectroscopy should be focused on developing convenient biosensors for bacterial analysis, taking full advantage of its good sensitivity for water contents and subtle structural changes. Moreover, chemometrics such as principal component analysis (El Haddad et al. 2013) and other statistical methods such as support vector machines (Xiang et al. 2016) should be combined to analyze the results automatically and unbiasedly. After utilizing compact THz systems with good operating performance, THz spectroscopy will be a complementary method used to acquire a more complete picture of bacterial detection.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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Conflict of interest The authors declare that they have no conflict of interest.

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