# Preliminary exploration on identification of probiotics in terahertz time-domain spectroscopy

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**Abstract:** Three kinds of probiotics in the biological food additives which are respectively Bacillus licheniformis, Bacillus subtilis and Bacillus coagulans have been tested and investigated with the terahertz time-domain spectroscopic (THz-TDS) technique at room temperature in nitrogen environment in the paper. The absorption coefficient and refraction index of three probiotics samples were calculated in the frequency of 0.2-1.6 THz. Test reveal that the absorption coefficients is increased as frequency increases and has considerable different absorption trend and characteristic absorption peaks among the samples, which means the vibration information made up of different molecular vibration and multi-molecular group is different in the THz spectrum of three samples. Furthermore, the average refractive indices were 1.71, 1.67 and 1.64 corresponding to Bacillus licheniformis, Bacillus subtilis and Bacillus coagulans, respectively. The refractive value indicates that abnormal dispersion, which meant three samples had the characters of strong selective absorption in this wave band. In contrast, the Fourier transform infrared spectroscopy (FTIR) in the 400-4 000 $cm^{-1}$ was utilized to measure the IR absorption spectra, and the results revealed that the probiotics is more active in the range of Terahertz than in the IR band and terahertz can be a good supplementary means of IR test. Meanwhile, some explanation on mechanism was put forward in this paper. The experiments showed that THz-TDS is a powerful tool to complement the conventional analytical approaches and can be applied to detect and identify the features of the constituent in probiotics or other biological food additives.

**Key words:** terahertz time-domain spectrum(THz-TDS); probiotics of the biological food additives; IR spectroscopy; Fourier transform

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# 太赫兹时域光谱技术在识别鉴定菌制剂中的初探

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**摘　要:** 使用太赫兹时域光谱技术对生物饲料添加剂中的地衣芽孢杆菌、枯草芽孢杆菌、凝结芽孢杆菌三种益生菌进行了室温、充氮环境下太赫兹时域光谱测试。得到了样品在 0.2~1.6 THz 波段的太赫兹吸收光谱和折射率谱。实验结果表明，随着频率增加三种益生菌的 THz 波段的吸收系数是增加的,并且有不同的吸收趋势及明显的特征吸收峰,这意味着三种样品的分子及官能团的构成和存在状态是不同的。三种样品明显不同的平均折射率(分别是 1.71,1.67,1.64)说明太赫兹光通过样品时所产生的色散和吸收是不同的,这更有力地揭示三种样品的确具有不同的生物成分组成和结构。为了更好地比较和说明太赫兹波识别鉴定益生菌的优势,还进行了同条件下的红外测试。通过对比发现菌制剂在太赫兹波段比在中红外波段更具有吸收活性,更能体现其结构差别，是红外光谱测试的有效辅助手段,同时,文中还依据益生菌的生物结构特征首次给出了机理上的解释和探讨。这项研究表明太赫兹时域光谱技术能够填补益生菌检测手段的匮乏，为益生菌的检测乃至为其它生物饲料添加剂检测提供了一种行之有效的方法。

**关键词:** 太赫兹时域光谱;　益生菌制剂;　红外光谱;　傅里叶变换

## 0 Introduction

Animal husbandry occupies a pivotal position in our country, in order to keep the animal health, some biological feed additives are always appended in the animals′ feed such as probiotic, vitamins and zymin, etc. These additives proved can be used to enhance animal immunity and increase the animal appetite, as a result, greatly promotes the growth of animals. What is more, these additives can also greatly decrease the influence caused by abuse of antibiotics, hormones and sulfonamide in the feed. Therefore, the biological feed additives are quite appropriate to prevent livestock disease by its unique advantages. Probiotics generated by microbial fermentation is an important biological feed additive in the field of bioengineering and fermentation engineering, and its main function is to stimulate immune cells directly, increase local immune antibody and enhance the body′s resistance[1].

Due to the demand of industrial production, detection and identification of all kinds of probiotics is necessary. At present, the microscopic observation and the biological detection are usually two main methods in the detection and recognition of the probiotics. The microscopic observation method is not suitable for precise detection because of limited accuracy grade, thus this method can′t be used to distinguish some probiotics with similar pattern. The biological detection method, namely polymerase chain reaction method [2] is very precise but cannot be used to detect in industry largely because of expensive price and long time testing period. Based on these reasons, continuous improvement on the basis of existing test methods have been made, and people have been trying to seek for new and more effective method.

In view of the fact that terahertz time-domain spectroscopy (THz-TDS) can indentify lots of organic molecules and biological macromolecules feature [3], people begin to apply THz-TDS in the research of organic compounds in order to get more comprehensive characteristics of organic matter. Terahertz wave refers to the electromagnetic radiation with frequency of 0.1-10 THz (wave length of 0.03-3 mm) between microwaves and infrared, which is particular transition scope from electronics to photonics. THz-TDS technology is sensitive to detect the micro differences in compound structures, the variations between isomer and geometric isomer, and strong response to weak interactions (hydrogen bond, van der Waals forces, dipole rotation and vibration transition) between organic molecule [4-6]. By recording the time-domain terahertz radiates field, high quality and resolution of low frequency vibration absorption spectrum can be obtained. The applications of THz-TDS technology can provide important information of vibration properties and structural level when organic molecules are in the low frequency band [7-8]. Moreover, terahertz wave is harmless for the biological tissue because of low power of THz photonic, as a result, the THz-TDS which is a nondestructive testing method can realize identifying probiotics composed of living cells.

In this paper, three kinds of probiotics with similar shape and color were tested by the transmission-type terahertz time-domain spectrometer at same room temperature and environment. Three samples are respectively the Bacillus licheniformis, the Bacillus subtilis and the Bacillus. As a result, time-domain spectroscopy of the samples in the frequency of 0.2-1.6 THz band were obtained, besides, the absorption coefficient and refraction index of three probiotics samples were calculated. The experiment show that the absorption coefficients is increased as frequency increases and has considerable difference absorption trend among the samples, the average refractive indices were 1.71, 1.67 and 1.64 corresponding to Bacillus licheniformis, Bacillus subtilis and Bacillus coagulans, respectively. In contrast, the Fourier transform infrared spectroscopy (FTIR) in the 400-4 000 $cm^{-1}$ was utilized to measure the IR absorption spectra, and the results revealed that the probiotics is more active in the range of terahertz than in the mid-IR band. The experiments showed that THz-TDS is a powerful tool to complement the conventional analytical approaches and can be applied to detect and identify the features of the constituent in probiotics or other biological food additives, and can provide a rich theoretical basis for researching and development of biological feed additive.

## 1 Experimental apparatus and samples

### 1.1 Experimental apparatus

The terahertz time-domain spectroscopy (THz-TDS) system [9] uses a self mode locked femtosecond laser which uses titanium sapphire, the femtosecond laser pulse (the center wavelength is 800 nm, the repetition frequency is 80 MHz, the pulse duration is 100 fs, the average output power is 960 mW) that laser produce is divided into the probe beam and the pump beam when it gets through the beam splitter mirror. After the pump beam is chopped by the 1.1 kHz chopper, it incident onto the GaAs crystal, and converted into THz pulse because of optical rectification effect, the pulse is focused on the sample by the off-axis parabolic mirror. The probe beam and terahertz pulse carrying sample information converge to same line and then focus on the detection element of electro-optic crystal made of ZnTe. To avoid the effect of water vapor in the air, the dry nitrogen is filled into the device. The experimental condition is that the relative humidity is less than 4% and the experiment temperature is about 20 ℃, the signal to noise ratio can reach 70 Db and the spectral resolution is better than 1 $cm^{-1}$. Figure 1 is the schematic diagram of terahertz time-domain spectroscopy measurement dlevice.

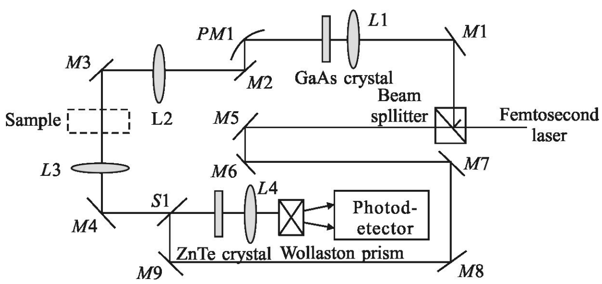


Fig.1 Schematic diagram of terahertz time-domain spectroscopy measurement device

### 1.2 Samples

The samples are collected from the HEMEIKESHENG Biological products co. LTD in Inner Mongolia and have similar color and shape. The probiotics samples were dried by drum for 5–6 h in order to reduce the influence of moisture on terahertz experiments. In addition, the sample in solid were pressed into a radius of 30 mm and thickness of 0.97–1.18 mm wafer by the powder pressing machine under the pressure of 20 MPa, as is shown in the Fig.2 (samples from left to right are respectively Bacillus bacillus, Bacillus subtilis, Bacillus coagulans). In this experiment, the polyethylene materials and the probiotics were mixed evenly according to 1:1 proportion, respectively. Because the polyethylene material has low dispersion and absorption to THz wave which has no influence on the spectral measurement.

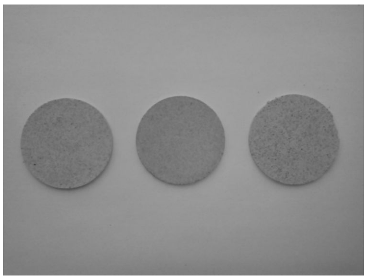

Fig.2 Diagram of tabletting of three kinds of samples in test

## 2 Theoretical analysis and results discussion

THz–TDS allows us to measure both phase and amplitude of the THz pulses propagating through the sample and reference, respectively. The frequency-dependent refractive index and absorption coefficient of SLO were extracted from the derivation of Fresnel law. By comparing reference and sample pulse, applying a numerical fast Fourier transform (FFT), the refractive index $n(\omega)$ and the absorption coefficient $\alpha(\omega)$ of SLO can be calculated:

$$T=\frac{4N}{(N+1)^2}e^{i2\pi(N-1)dv/c}=Ae^{i\varphi} \tag{1}$$

$$n(v)=\frac{c\varphi}{2\pi dv}+1 \tag{2}$$

$$\alpha(v)=\frac{4\pi vk(v)}{c} \tag{3}$$

where $A$ is amplitude, $\varphi$ is phase difference between sample field and reference field, $d$ is the sample thickness, $v$ is radiation frequency, $c$ is the light speed in vacuum.

Figure 3 shows the terahertz time-domain spectra of three types bacillus of the probiotic in the time range of 14–23 ps. Comparing with reference signal, the peak amplitude and peak moment change when THz passes the sample. The changes of peak amplitude are mainly due to reflection, scattering and absorption for the THz wave in the specimen surface. In addition, Fig.3 also reveals that there is a relative delay among samples. This phenomenon is mainly caused by the increase of the optical path when the pulse light passed through the sample. Specifically, the delay time of samples are respectively 2.05 ps (Bacillus coagulans), 2.3 ps (Bacillus subtilis), 2.35 ps (Bacillus licheniformis). In time-domain spectra, three samples correspond to different pulse strength. The THz energy loss results in amplitude fading which indicates that THz wave losses differently in different samples. The higher density of samples, the more energy lost is.

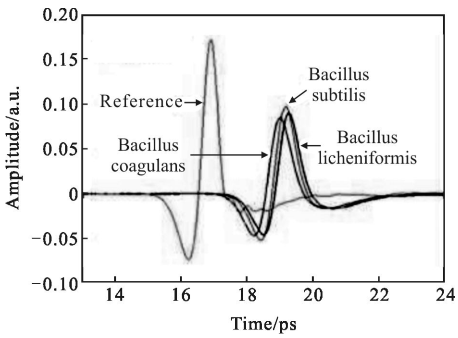


Fig.3 Terahertz time-domain spectra of three types of probiotic

Figure 4 is the frequency-domain spectrum in effective frequency range of 0.2–1.6 THz from fast

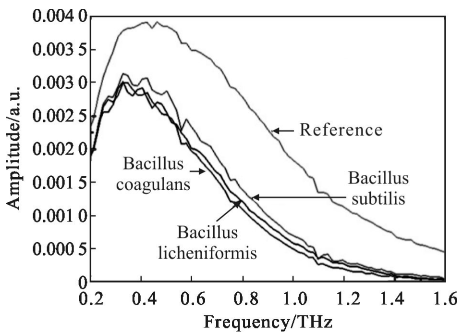


Fig.4 Frequency-domain spectra of three types of samples

Fourier transformation of time-domain spectrum. The absorption and surface reflections are different with different frequency. The amplitude decreases and spectrum waveform changes which indicate different refraction index, absorption coefficient and dielectric constant.

The absorption coefficients and the refractive index of samples were calculated in the frequency range of 0.2–1.6 THz by the formula (2)–(3). Figure 5 shows the absorption coefficients of samples in effective scope. Absorption peaks were found in Fig.5, and these characteristic absorption peaks are respectively in the frequency position of 1.417, 1.567 THz(Bacillus icheniformis), 1.097, 1.166 Thz (Bacillus subtilis), 1.350, 1.474 THz (Bacillus coagulans), which meant complexity of structure and composition from sample material, in detail, the same components and content are respectively presented in the same peak position and peak intensity and vice versa. The variation trend of three samples spectral lines are significantly different in the peak value, the peak width and the peak position, which meant the THz spectra contains different molecular vibration information and the combined vibration message of multi-molecular group from three samples. The results of the experiments showed that the obvious different absorption coefficient reflected the difference of structural components and content of various probiotic, for example, the protein content of bacillus can be seen clearly in Fig.5, that the bacillus coagulans has a much more stronger absorption of terahertz wave than other two kinds of probiotics, the absorption of the terahertz wave by bacillus licheniformis is a little weaker, the absorption of the terahertz wave by bacillus subtilis is the weakest. The result showed that the protein can be influenced easily by the electromagnetic field and behave as much more strong absorption to the THz wave band, in the result, bigger absorption coefficient correspond to higher protein content in sample[10].

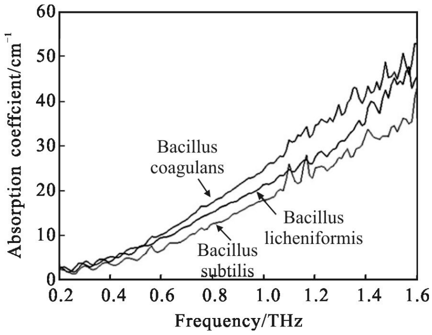


Fig.5 Absorption spectra of three types of samples

Figure 6 shows the refractive index of three kinds of probiotic samples in the frequency range of 0.2 –1.6 THz, the refraction index of the samples decreases between 1.50–1.77, average refractive index is 1.71, 1.67 and 1.64 corresponding to Bacillus licheniformis, Bacillus subtilis and Bacillus coagulans, respectively. Experimental data mentioned above indicate that the different component of three kinds of samples resulted in different dispersion of THz wave and different characteristic change of refractive index corresponding to different absorption feature, which means the sample of anomalous dispersion phenomenon maybe near the absorption peak. Therefore, good degree of differentiation of the refractive index curves and the absorption coefficients trend can be used to identify various probiotics.

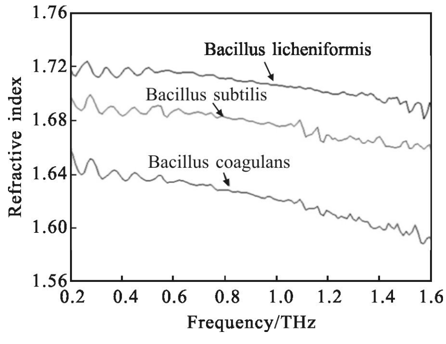


Fig.6 Refraction indices of three kinds of probiotics

The fact is based on that THz short-wave band is located a large portion of overlapping band with infrared long-wave band, three kinds of samples in the infrared spectrum were tested by the Fourier transform infrared spectrometer and were used to be compared with the THz absorption spectrum in order to confirm the function of THz-TDS in discerning and identifying probiotics. Obviously, Fig.7 reveals that most of the bands for the infrared absorption are the same, and absorption peaks are respectively located in 1 040, 1 238, 1 400, 1 658, 2854, 2 926, 3 160 $cm^{-1}$. According to the SADTLER norm infrared spectrogram analysis and infrared spectroscopy assistant analysis software, absorption peak position are respectively 3 160, 2 926, 2 854 and 1 400 $cm^{-1}$ from the rotation and vibration of carbon hydrogen bond and vest in lipid; 1 658 $cm^{-1}$ and 1 238 $cm^{-1}$ belong to the protein amide I band C=O stretching vibration and the amide three zones C-N scale vibration, respectively; 1 040 $cm^{-1}$ Vest in carbohydrate C-O and C-N key stretching vibration superposition. These characteristics mentioned above reflect that main components of the probiotics are protein, fat and carbohydrates. Although infrared spectrum can reveal the component but almost same absorption peaks and coefficients in the range of 400-4 000 $cm^{-1}$, but the purpose of identification of probiotics can not be achieved by infrared spectrum.

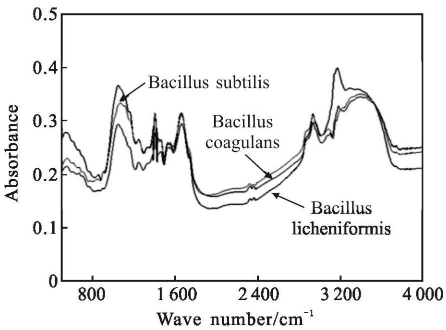


Fig.7 IR Fourier transformation absorption spectra of three probiotics

Comprehensively, the above several spectra and analysis reveal that THz-TDS can be a supplementary way of infrared spectrum because of greater different of the optical characteristic and parameters, such as different refractive index, absorption coefficients and absorption peak, etc. The differences will be explained from the following several aspects:

Firstly, theory show [11] that many large protein and deoxyribonucleic acid (DNA) molecules have collective vibrational and rotational modes in the THz range, which may provide characteristics fingerprints to identify the type of bio-molecules in biological tissues. As a result, in the paper, the vibration and rotation spectrum of the probiotics molecules maybe exactly located in the THz range and different absorption characteristics correspond to different tiny difference of structure and component of molecular bonds and functional groups in probiotics samples.

Secondly, the dispersion characteristic can not be tested by infrared spectrometer but the THz-TDS technology can test the dispersion of the probiotics, which had been verified in different time delay(in Fig.3) and different refractive index (Fig.6), etc. Therefore, the THz-TDS technology may become an efficient way in discerning and identifying various probiotics.

Thirdly, THz radiations possess coherence, and may obtain more information with respect to the phase.

In a word, the THz-TDS has a strong response to intermolecular weak interaction and can make sensitive reflection to small changes in the compound structure, and the sample has more absorption activity in THz band than in infrared absorption band. The application study of THz-TDS in probiotics field reveals that THz-TDS can complement the infrared spectrum technology to study the probiotics property and subtle structural differences in low frequency. Therefore, THz-TDS can provide more detailed basis for probiotics production and research and will be a powerful tool in identifying probiotics.

## 3 Conclusions

In conclusion, three types of bacillus with similar color and shape were studied by terahertz time-domain spectroscopy and infrared spectrum technology. Based on qualitative analysis by two methods, the samples show different characteristic spectrum which reveals that the sample has more absorption activity in THz band than in infrared absorption band, and obvious absorption and refraction differences in the THz band.

Therefore, as a complement method, THz -TDS technology can be used to study and identify probiotics property. By identifying absorption spectrum and refraction spectrum in THz band, it can qualitatively analyze and identify different types of probiotics, which provides basis for qualitative analysis of the sample parameters.

As a result, THz-TDS can not only effectively identify three kinds of probiotics but also can get the contents of nutrients based on the spectra with its good temporal and spatial coherence, and THz-TDS can bring a breakthrough in the field of detecting and identifying the biological feed additives.

## References:

[1] Pan Xiaodong, Wu Tianxing. The mechanism of probiotics on modulating mucosal immunity of the animal gastrointestinal tract [J]. ***Chinese Journal of Veterinary Medicine***, 2005, 39 (8): 32-35. (in Chinese)

[2] He Qixia. Quality inspection and quality control of feed and feed additive [J]. ***Feed Industry***, 2012, 33 (9): 60-64. (in Chinese)

[3] Lu Meihong. Application of terahertz technology in identification of drug and corn seed [D]. Beijing: Capital Normal University, 2006. (in Chinese)

[4] Ma Chunqian, Xu Xiangdong. Study on characteristics of terahertz spectra of organic functional groups [J]. ***Spectroscopy and Spectral Analysis***, 2015, 35(4): 870-874. (in Chinese)

[5] Yang Limin. THz absorption spectra of several carbohydrate derivatives[J]. ***Spectroscopy and Spectral Analysis***, 2008, 28 (5): 961-965. (in Chinese)

[6] Yu Bin, Huang Zhen. Study on THz spectra of nicotinic acid, nicotinamide and nicotine [J]. ***Spectroscopy and Spectral Analysis***, 2009, 29(9): 2334-2337. (in Chinese)

[7] Dai Hao, Xu Kaijun. Terahertz spectroscopy of $\beta$-lactam antibiotics[J]. ***Infrared and Laser Engineering***, 2013, 42(1): 90-95. (in Chinese)

[8] Zhang Tongjun, Cai Jinhui. Terahertz spectroscopic investigation of crystalline $\beta$-D-Galactopyranose [J]. ***Spectroscopy and Spectral Analysis***, 2008, 28(4): 721-725. (in Chinese)

[9] Bao Rima, Zhao Kun, Tian Lu, et al. Analysis of THz time-domain spectroscopy in crude oil ultrasound treatment [J]. ***Modern Scientific Instruments***, 2013, 2: 126-129. (in Chinese)

[10] Teng Xueming, Tian Lu, Zhao Kun. Investigation of protein content in nutriment by terahertz spectroscopy [J]. ***Modern Scientific Instruments***, 2012, 1: 91-94. (in Chinese)

[11] Du S Q, Li H, Xie L, et al. Vibrational frequencies of anti-diabetic drug studied by terahertz time-domain spectroscopy [J]. ***Appl Phys Lett***, 2012, 100: 143702.