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Abstract： Bacillus cereus is a kind of common food-borne pathogen that can cause vomiting and diarrhea shortly after ingestion. The spectroscopy properties of *Bacillus cereus* were measured using the infrared reflectance spectroscopy, which was a nondestructive technology. The influence of *Bacillus cereus* concentration on the spectroscopy was explored based on the two-dimensional (2D) correlation spectroscopy method. The results showed that some functional groups of capsule in pure Bacillus cereus culture, such as carboxyl、protein amide I and amide II、C-H methyl and methylene could induced some self-correlation peaks near 1592 cm^{-1} 、1652 cm^{-1}、 1512 cm^{-1} and 1412 cm^{-1} respectively. Some functional groups of spore, such as COO- group and C-H bond could induced some self-correlation peaks near 1348 cm^{-1} 、1616 cm^{-1} and 1592 cm^{-1} respectively. In milk sample，the functional groups of capsule and spore could induced some self-correlation peaks too. The infrared spectroscopy combined with 2D correlation spectroscopy analysis method could be a effective method for the Bacillus cereus detetion.

Keywords: Bacillus cereus, Infrared spectroscopy,Two-dimensional correlation spectroscopy, Milk.

1 Introduction

Bacillus cereus is a common aerobic spore-forming rod-shaped bacteria that can cause food poisoning, which was Widely distributed in dust, sewage, soybean products, flour rice, and dairy products. Traditional methods for detection of Bacillus cereu such as PCR technology, immunological techniques, and enzyme reaction,have high accuracy. While they need pre-treatment sample and were not suitable for real time detection. Hence development of methods for the real-time detection of Bacillus cereus has attracted the considerable interest [1].

Bacillus cereus cell structure contains a special cell structure such as capsule, spores, and other energy storage material, analysis by IR (known as "molecular fingerprint" [2]. These peaks can be quickly used to find structural features contained in the chemical composition, which determine the presence of these special structural form. It will provide important reference information for molecular microbial, cytology and taxonomy. At the same time it does not destruct the cells and add any chemicals, which greatly improves the authenticity and reduce the cost of analysis [3-5] in the analysis of the constitution.

In this work, the infrared spectroscopy technology combined with the two-dimensional (2D) correlation spectroscopy were used to detect of Bacillus cereus The relationship between spectroscopy and Bacillus cereus concentrations was explored.

2 Experiments and Methods

2.1 Experimental material and instruments

Equipment:The spectroscopy properties measurements were performed with Spectrum 100 FTIR (USA PerkinElmer Inc).
Microbial Sample：The Bacillus cereus is provided by Beijing North Carolina Chuanglian Biotechnology Research Institute. Potassium bromide, tryptone, yeast extract, sodium chloride and distilled water are provided by the plant protection laboratory of Tianjin Agriculture University. A Yili nonfat pasteurized milk sample was purchased from a local supermarket.

2.2 Experimental Methods

The activated cells were incubated at 30℃for 24h. The stock cultures were serially diluted with distilled water. A conventional spread plating method was used for bacterial counts. The plate was showed in Fig. 1. In analysis of real-life samples, the milk samples were spiked with different concentrations of *Bacillus cereus* cells.

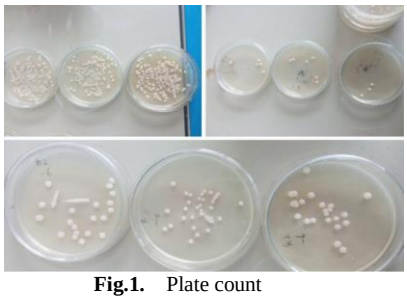


Fig.1. Plate count

For spectral measurements, settings were made to provide 16 measurements by the FTIR spectrometer Spectrum （100 type, USA PerkinElmer Inc.) with a scale from 4000 to 650 cm^{-1} and a resolution of 4 cm^{-1}. All experiments were carried out at room temperature. The sterile water was used as back. The infrared spectrum analysis software OriginPro7.5 and 2Dshige two-dimensional correlation spectra processing were used in analysis.

3 Results and Discussion

3.1. One-dimensional infrared spectroscopy of Bacillus cereus

The one-dimensional spectrum of different concentrations of pure *Bacillus cereus* and *Bacillus cereus* mixed with milk were shown in Fig. 2 and Fig. 3 respectively. It can be found that a direct one-dimensional spectrum was can not discriminate the concentrations of *Bacillus cereus*, since there were some spectral overlaps.

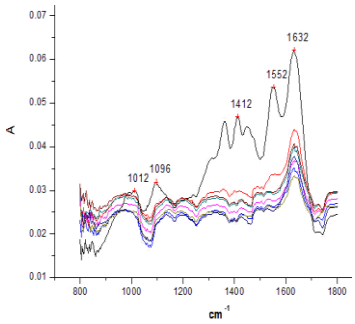


Fig. 2. The one-dimensional spectra of pure *Bacillus cereus*

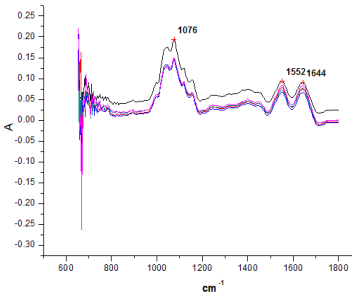


Fig.3. The one-dimensional spectra of *Bacillus cereus* mixed in milk

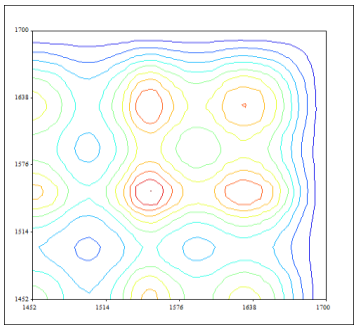
3.2 The analysis of two-dimensional correlation spectroscopy

3.2.1 The 2D correlation spectroscopy analysis of Bacillus cereus

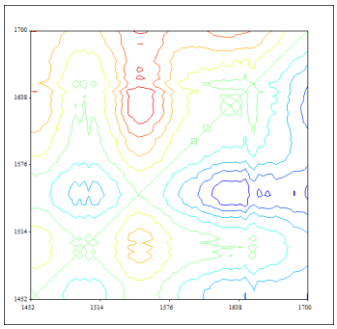
Experiments have found that, according to the autocorrelation peak of the two-dimensional correlation diagram can clearly distinguish the diagonal position *Bacillus cereus* spores and capsule in the corresponding group vibration peaks.

The two-dimensional spectral signal can be expanded to the second dimension, which has a higher resolution, and can be distinguished on the one-dimensional spectrum covered with small peaks and weak peak. Experiments have proved that, the diagonal position *Bacillus cereus* spores and capsule in the corresponding group vibration peaks can clearly be distinguished according to the autocorrelation peak of the two-dimensional correlation diagram[6-7].

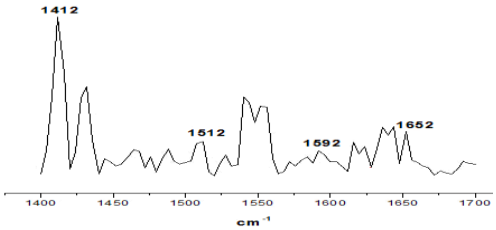
Therefore the two-dimensional correlation spectroscopy of *Bacillus cereus* was analysis in this study. Synchronous and asynchronous 2D correlation spectra of pure Bacillus cereus capsule from 1450 cm^{-1} to 1700cm^{-1} were shown in Fig. 4(a) and Fig. 4(b) respectively. The self-correlation spectroscopy was showed in Fig 4.(c).



(a)The synchronous correlation diagram



(b)The asynchronous correlation spectroscopy

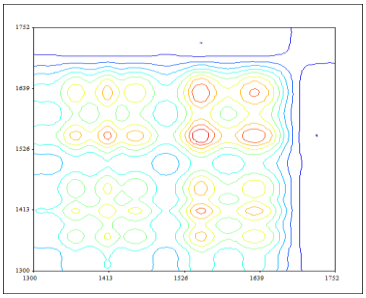


(c) The self-correlation spectroscopy

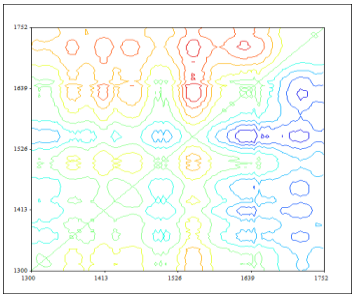
Fig. 4. 2D correlation diagrams of Bacillus cereus capsule after the second derivative

It can be seen clearly from Fig. 4(c) that there were two self-correlation peaks near 1652 cm^{-1} and 1512 cm^{-1}, which indicated that the 2D correlation spectroscopy was sensitive to the changes of Bacillus cereus on the concentration. That was induced by the protein amide I band and amide II band contained in capsule. The self-correlation peak near 1592 cm^{-1} was induced by the carboxyl stretching vibration in capsule. And the self-correlation peak near 1412 cm^{-1} was produced by the bending vibration of C-H methyl and methylene. These self-correlation peaks changes of characteristic functional groups can be clearly proved that the capsule existed in the pure *Bacillus cereus* culture.

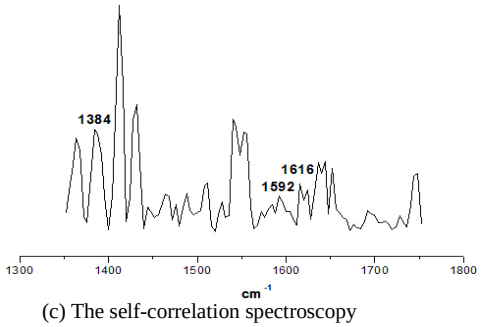
The synchronous and asynchronous 2D correlation spectra of Bacillus cereus spore from 1300 cm^{-1} to 1750 cm^{-1} were shown in Fig. 5(a) and Fig. 5(b) respectively. The self-correlation spectroscopy was showed in Fig. 5(c).



(a) The synchronous correlation diagram



(b)The asynchronous correlation spectroscopy



(c) The self-correlation spectroscopy

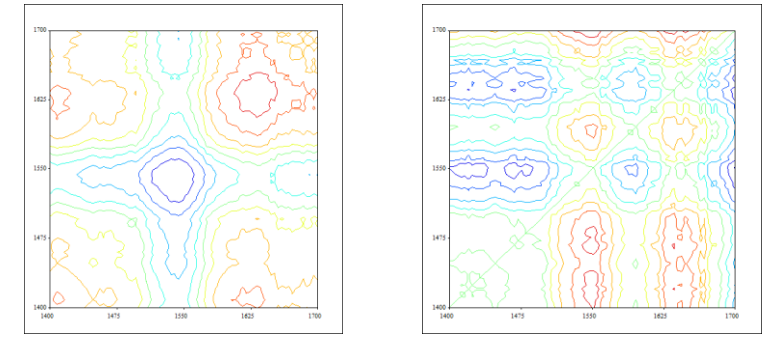
Fig.5 2D correlation diagram of Bacillus cereus bacteria's spore after the second derivative

It can be seen clearly from Fig. 5(c) that there were two self-correlation peaks near 1348 cm^{-1} and 1616 cm^{-1}, which indicated that the 2D correlation spectroscopy was sensitive to the changes of Bacillus cereus on the concentration. That was induced by the COO- group stretching vibration in Bacillus cereus spore. And the self-correlation peak near 1592 cm^{-1} was produced by the C-H bond contained in spore. These self-correlation peaks changes of characteristic functional groups can be further proved that the spore existed in the pure *Bacillus cereus* culture.

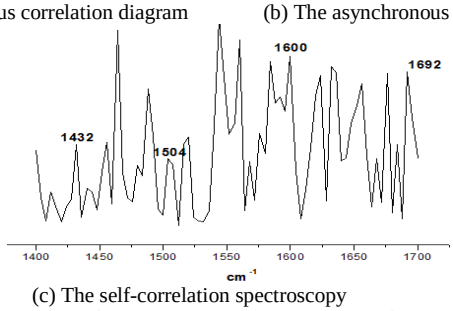
3.2.3 The 2D correlation spectra analysis of Bacillus cereus in Milk

Bacillus cereus is often foodborne, and unpasteurized milk and dairy products are common vehicles of transmission. In order to test the sensitivity of the method, different concentrations of *Bacillus cereus* cells were spiked with milk to simulate the real milk sample. The synchronous and asynchronous 2D correlation spectra of Bacillus cereus capsule mixed in milk from 1400 cm^{-1} to 1700 cm^{-1} were shown in Fig. 6(a) and Fig. 6(b) respectively. The self-correlation spectroscopy was showed in Fig 6.(c).

It can be seen clearly from Fig. 6(c) that there were four self-correlation peaks near 1692 cm^{-1}、1504 cm^{-1}、1600 cm^{-1} and 1432 cm^{-1}, which was maybe indicated that there was some capsule in milk sample. In order to improve it, the asynchronous 2D correlation spectra were explored at this wavelength range. According to Fig. 6(b), the self-correlation peaks near 1692 cm^{-1} and 1504 cm^{-1} was induced by the protein amide I band and amide II band contained in capsule. The self-correlation peaks near 1600 cm^{-1} was induced by the carboxyl stretching vibration in capsule. And the self-correlation peak near 1432 cm^{-1} was produced by the bending vibration of C-H methyl and methylene.



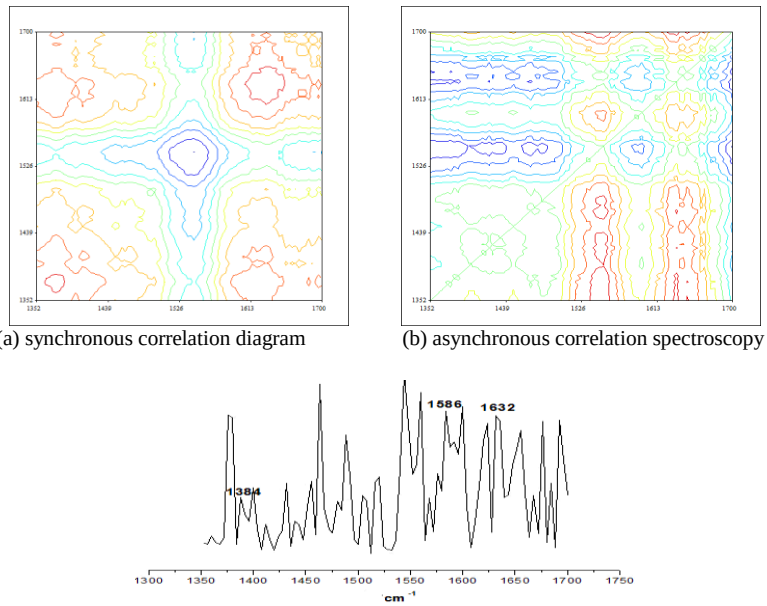
(a) The synchronous correlation diagram (b) The asynchronous correlation spectroscopy



(c) The self-correlation spectroscopy

Fig. 6. 2D correlation diagrams of Bacillus cereus capsule in milk after the second derivative

The synchronous and asynchronous 2D correlation spectra of Bacillus cereus spore mixed in milk from 1350 cm^{-1} to 1700 cm^{-1} were shown in Fig. 7(a) and Fig. 7(b) respectively. The self-correlation spectroscopy was showed in Fig. 7(c).



(a) synchronous correlation diagram (b) asynchronous correlation spectroscopy

(c) a self-correlation spectroscopy

Fig.7. 1350-1700cm^{-1} 2Dshige of Spores of Bacillus cereus in milk

It can be seen clearly from Fig. 7(c) that there were four self-correlation peaks near 1632 cm^{-1}、1384 cm^{-1} and 1586 cm^{-1}, which was maybe indicated that there was some spore in milk sample. In order to improve it, the asynchronous 2D correlation spectra was studied at this wavelength range. According to Fig. 7(b), the self-correlation peaks near 1632 cm^{-1} and 1384 cm^{-1} was induced by the COO- group stretching vibration in Bacillus cereus spore. The self-correlation peaks near 1586 cm^{-1} was produced by the C-H bond contained in spore. These self-correlation peaks changes of characteristic functional groups can be further proved that the spore existed in the milk sample.

4. Conclusion

The spectroscopy properties of capsule and spore in different concentrations of Bacillus cereus were studied using infrared spectroscopy and 2D correlation spectroscopy analysis method. The conclusions are as follows:

（1）In pure Bacillus cereus culture，some functional groups of capsule, such as carboxyl、protein amide I and amide II、C-H methyl and methylene could induced some self-correlation peaks. There was a carboxyl stretching vibration self-correlation peak near 1592 cm^{-1} and it was gradually weakened with the concentration of Bacillus

cereus increased. The self-correlation peak of protein amide I and amide II in capsule was presented near 1652 cm^{-1} and 1512 cm^{-1} respectively. And the self-correlation peak near 1412 cm^{-1} was produced by the bending vibration of C-H methyl and methylene. Some functional groups of spore, such as COO- group and C-H bond could induced some self-correlation peaks. Two self-correlation peaks near 1348 cm^{-1} and 1616 cm^{-1} was induced by the COO- group stretching vibration in Bacillus cereus spore. And the self-correlation peak near 1592 cm-1 was produced by the C-H bond contained in spore.

（2）In milk sample，the functional groups of capsule could induced some self-correlation peaks too. There was a carboxyl stretching vibration self-correlation peak near 1600 cm^{-1} and it was gradually weakened with the concentration of Bacillus cereus increased. The self-correlation peak of protein amide I and amide II in capsule was presented near 1692 cm^{-1} and 1504 cm^{-1} respectively. And the self-correlation peak near 1432 cm^{-1} was produced by the bending vibration of C-H methyl and methylene. Some functional groups of spore, such as COO- group and C-H bond could induced some self-correlation peaks. Two self-correlation peaks near 1632 cm^{-1} and 1384 cm^{-1} was induced by the COO- group stretching vibration in Bacillus cereus spore. And the self-correlation peak near 1586 cm-1 was produced by the C-H bond contained in spore.

These self-correlation peaks changes of characteristic functional groups can be further proved that the capsule and spore existed in the pure Bacillus cereus culture and milk sample. Thus the infrared spectroscopy combined with 2D correlation spectroscopy analysis method could be use for the Bacillus cereus detection.

Acknowledgments

This research was performed with financial support from National Natural Science Foundation of China under the project No.31171892, Tianjin Science and technology project13JCYBJC25700, and the Innovation and Entrepreneurship Training Plan for Undergraduate in Tianjin of China through grant number No. 201410061075.

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