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Abstract. Combing the one-dimensional infrared spectroscopy (FTIR) technology with two order derivative spectrum technology, the growth change rule of bacillus cereus, the common food borne pathogenic bacteria, are analyzed without destruction, It is found that capsule、spore and other structures of bacillus cereus can be identified, based on the two order derivative spectra characteristic absorption peak. Observing shows the symmetric &anti symmetric carboxyl group stretching vibration absorption peaks near 1604.48cm^{-1} and 1396.21cm^{-1} gradually weaken from lag phase to the stable phase. With protein amide absorption peak near 1654.63cm^{-1} tending to be stable in the three stages, the structural changes of cell capsule can be acknowledged. The DPA absorption peak near 1617.98cm^{-1}, 1384.64cm^{-1}, and 1560.13cm^{-1} indicates the presence of bacillus, changing in three stages from lag phase to stable phase. Experiments show that FTIR can distinguish cells' material structure, which lays a theoretical foundation for the related devices of fast detection for bacillus cereus.

Keywords: bacillus cereus; FTIR; capsule; spore

1 Introduction

Widely existing in nature such as in the air and the soil, bacillus cereus is a kind of gram-positive bacilli which can cause food poisoning[1]. Food poisoning is the result of fast growth and reproduction of bacillus cereus owing to improper insulation and long time placement, which gives rise to vomit enterotoxin and diarrhea enterotoxin, causing human gastrointestinal disfunction. Yet, bacillus cereus can produce antimicrobial substances, which inhibit the propagation of harmful microorganisms and degrade nutrient constituent in soil, thereby improving the ecological environment.

The traditional classification and identification of microorganisms is the application of microscopy, biochemical, physiological and the combination.Although these methods are reliable and effective, operation of them is complex, time-consuming, sometimes the result is not as accurate as expected, and furthermore, these methods

are difficult to realize the automation and computerization[2]. In the middle of 20th Century, infrared spectroscopy (FTIR) began to be used to distinguish different species of microbe[3].In 1991,Naum Ann[4]began to apply the Fourier transformation infrared spectroscopy for the discrimation, classification and identification of microorganism. Since then more and more researchers have begun to employ FTIR technology to study the characteristics of various microbe. With high resolution, Fourier transformation infrared spectroscopy (FTIR) can reflect the whole cell component molecular vibration characteristics, such as the characteristics of proteins, nucleic acids and other substances, as well as can quickly select, identify, and classify a large scale of microorganism in the subspecies level. Usually naked eyes can not identify different microorganism in terms of the spectrum which can only be distinguished by extracting special information with statistics, and the combination of different chemical metrology methods[3,5]. Instead of the traditional dyeing methods, this experiment employs infrared spectroscopy to observe the cell structure of waxy bacillus cereus, and study the growth regularity, which lays a theoretical foundation for the related devices of fast detection for bacillus cereus.

2 Experiments and Methods

2.1 Experimental material and instruments

The devices are IR200 Fourier transformation infrared spectrometer, and TCFW-4 type powder pressing machine; what is also needed in the experiments is test tube, Petri dishes, flask, coater, and gun head; the software is infrared spectrum analysis software OMNIC 8.2

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 laboratory of Tianjin University of Agriculture, and is a pure analysis; experimental water is the water distilled twice.

2.2 Experimental Methods

2.2.1 LB culturing medium configuration

After thawed, bacillus cereus in this experiment is cultured in LB medium whose ratio is 1L solid medium with 10g tryptone, 5g yeast extract,10g sodium chloride, 15g agar, and 1000ml distilled water. LB medium is stirred until all solid dissolve; if the PH value is not between 7.0-7.4, the addition of a small amount of sodium hydroxide is allocated to assure of the PH value. Then LB medium is sterilized with high temperature and shaken well; finally the configured LB medium is batched into the triangular flask.

2.2.2 Measurement of the reproduction number

As the individual number is required to be calculated to determine the number of microbial breeding, this experiment adopts plate counting method of indirect measurement method, specifically, a definite volume of diluted broth is mixed with appropriate solid medium before the solidification, or is coated on the solidified medium plate. After insulation culture, when colony number on (in) the plate is multiplied by broth dilution degree, the number of bacteria in the original bacteria liquid could be calculated. Usually on a Petri dish of 9cm diameter, 50~500 colony is appropriate[6].

2.2.3 Growth regularity of microbe

Bacterial cells are extremely small and in the whole growth process, complex biochemical and cytological changes occur in different stages. But, the study of this kind of change of a single bacterium is technically difficult. Methods currently available are: (1) ultrathin section of electron microscopy for the observation of bacterial cells; (2) synchronous culture techniques, which tries to make all the cell population be in the same cell growth and cell division cycle, and then analyze various biochemical characteristics of this group to understand the changes of single cells.

Microbial growth curves (growth curve) are groups of regular curves, which appear by inoculating pure microbe with single cell in a small amount of liquid medium with a constant volume in the appropriate temperature and ventilation (no ventilation for anaerobe). According to the growth rate constant of microorganism, the typical growth curves can be divided into lag phase, exponential phase, stationary phase and decline phase. After tracking and detecting for 28 hours in this experiment, according to the generating curve, there are three phases: lag phase, exponential phase and stable phase. Specific curves are as shown in figure 1.

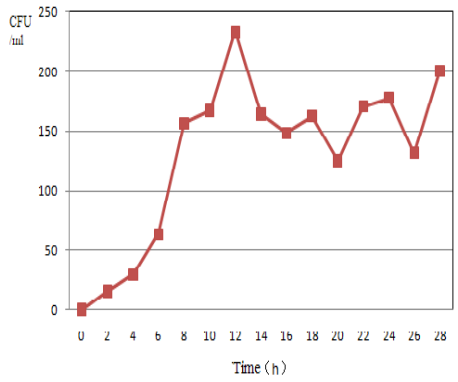

Fig.1. Bacillus cereus growth curve

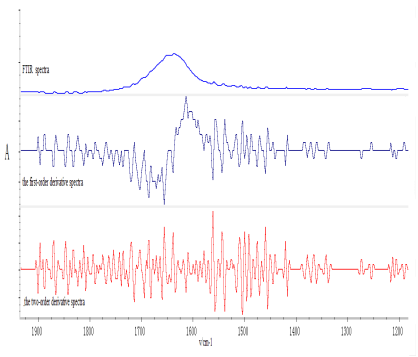

Fig.2. comparison chart of Infrared spectra, first derivative spectra, and the two order derivative spectra

From Figure 1, from 0-6 h, the growth curve of bacillus cereus is relatively slow as the lag phase; from 6-12 h, the growth curve is changing rapidly as the exponential phase; from 14-28 h, it tends to be smooth as the stable period.

2.2.4 Detection of FTIR

With doping small amount of cell into potassium bromide tabletting, infrared spectroscope of bacillus cereus is determined by the IR200 Fu Liye transformation infrared spectrometer with deuterated three glycine detector. With Spectral measurement range being $4000cm^{-1}$~$400cm^{-1}$, the spectral resolution being $4cm^{-1}$, and signal scan accumulating 48 times, the infrared spectroscope of bacillus cereus is obtained.

3 Results and Discussion

3.1 FTIR analysis of Bacillus cereus

Infrared spectroscopy is very valuable in detecting the cell structure of bacillus cereus in the whole growth period. This experiment is to find the changing law of spectral curve for bacillus cereus by collecting one-dimensional infrared spectra and second-order derivation spectra of bacillus cereus. As shown in figure 2, with the information extracted from one-dimensional infrared spectra being limited, the second-order derivative spectra are adopted, as it can improve the resolution, increase the amount of information, enhance the information quality, and highlight the spectrum characteristics. Not only can it distinguish peaks overlap, but it can easily distinguish the shoulder peak in the strong ones, and make the implicit information in the infrared spectra outstanding[7].

3.2. The two order derivative spectrum analysis of Bacillus cereus

3.2.1 Composition of Bacillus cereus

Bacillus cereus is composed of capsule, spore flagellum and other kinds of material structure, among which capsule and spore are the most important.

The capsule is transparent jelly-like material with a certain thickness wrapped with a fixed level over a single cell wall. Its chemical composition includes peptides, proteins, polysaccharides, lipids, lipoproteins, and lipopolysaccharide. The capsule can protect cells from drying and enhance certain pathogen pathogenicity[6].

Bacillus is a round or oval strong resistance dormant structure[6] which is formed in the cells during the later growth of bacillus cereus. In the generation process of bacillus cereus spores, cells producing spores can absorb a large amount of calcium ions and synthesize pyridine carboxylic acid (DPA) two. Therefore, the presence of DPA is the key to determine if there is the spore.

3.2.2 The second order derivative spectrum analysis of bacillus cereus in different periods

In Fig.2, Bacillus cereus growth curve can be divided into the lag phase, exponential and stationary phases. Concrete analysis is as follows:

（1）Lag phase

Also known as the adaptation period or period of adjustment, the lag phase is the beginning and culturing stage during which the cell number increase slowly after bacillus cereus is inoculated into a new culture liquid. At this stage, it has the following characteristics: (1) the growth rate constant equals to zero; (2) the morphology of the cells increase or become longer, which make many bacteria grow filamentous; (3) Active synthesis of metabolism, accelerate the synthesis of ribosome, enzyme and ATP, which makes enzyme easily inducible; (4) Reaction to the adverse external conditions is sensitive[6].

In the experiment, 0-6 hours is the lag phase, second order derivative spectra of 2h, 4H and 6h are as shown in Figure 3, 4.

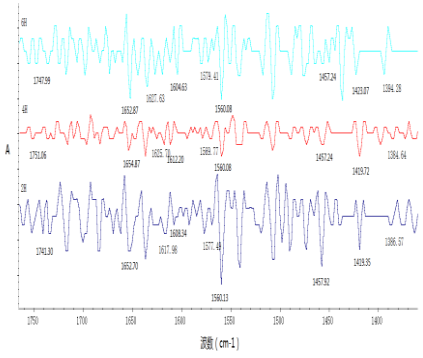

Fig.3. The second-order derivative infrared spectroscope during in lag phase(1400cm-1-1750cm-1）

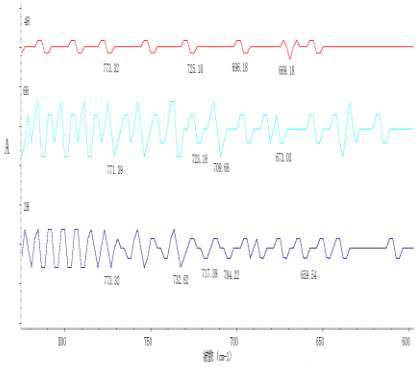

Fig.4. The second-order derivative infrared spectroscope in the lag phase(600 cm-1-800 cm-1)

Figure3,figure4 shows that for Bacillus cereus nearby 1604.48cm^{-1} and 1394.28cm^{-1} there appear two strong symmetry and anti stretching vibration absorption peaks of carboxyl, and from the second to the sixth hour, with the passage of time, the absorption peak tends to weaken; while the spectral peak near 1652.70cm^{-1} and 1560.13cm^{-1} , is mainly from protein amide belt and protein amide II band, and at this stage the absorption peak is increasing; As the bending vibration of C-H methyl as well as Methylene, the absorption peaks near the 1465.64cm^{-1} at this time are increasing; Near 771.39cm^{-1}, 723.18cm^{-1}, 709.68cm^{-1}, 673.03cm^{-1} there exist a series of related vibration absorption peaks which are in stable state; There appear very obvious ester absorption peak in the vicinity of 1735.62cm^{-1}, the main characteristic absorption peak of a poly beta hydroxybutyrate granules (PHB)[7], and at this stage it is weakening; These characteristics of peak change show the presence of capsule.

The absorption peaks near1627.63cm^{-1}and1394.28cm^{-1} are stretching vibration absorption peaks of two COO- groups produced by DPA in the spore, and the absorption peak near 1579.41cm^{-1} is caused by C-N bond in the DPA ring. In the lag

phase, three absorption peaks are gradually increasing, and the appearance of the three peaks indicates that this phase has generated spores.

（2）Exponential phase

Exponential phase is also known as logarithmic phase following a lag phase, during which cells divide by geometric rate. It has as the following characteristics: ① with growth rate constant R being maximum, generation time G for cell division each time or the time required for plasma doubling is the shortest; ② cells were balancedly growing and cell elements are well distributed; (3) Enzyme is in activity and metabolism is fast[6]. Figure 5 and 6 show the two derivative spectra collected during the sixth to the fourteenth hour.

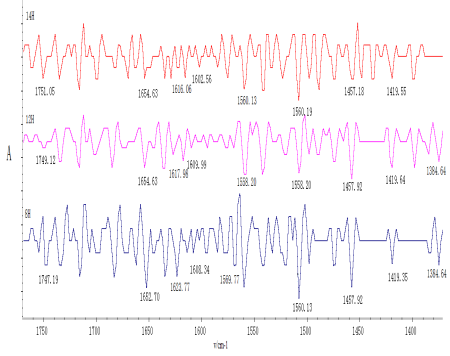

Fig.5. The second-order derivative infrared spectroscopy in exponential phase (1400cm^{-1}-1750cm^{-1})

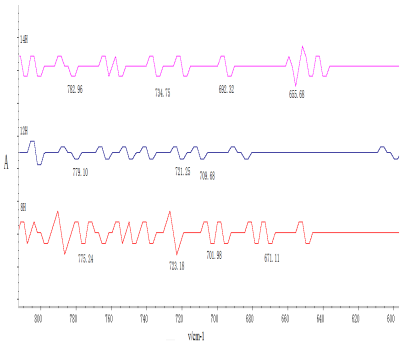

Fig.6. The second-order derivative infrared spectroscopy in exponential phase (600cm^{-1}-800cm^{-1})

Figure 5 and Figure 6 show in the exponential phase, symmetric and anti symmetric carboxyl stretching vibration absorption peak appear near 1602.56cm^{-1} and at this stage the absorption peak is weakening; while in the vicinity of 1400.07cm^{-1}, the absorption peak is gradually increasing; The protein amide I band absorption peak in the vicinity of 1654.63cm^{-1} tends to increase, in contrast, the amide II band absorption peak around 1560.13cm^{-1} does not change; Absorption peak at 1457.92cm^{-1}, generated by the bending vibration of C-H methyl and Methylene, basically hints no change; the absorption peak near 1751.95cm^{-1} in the ester group has increased; Among a series of stretching vibration absorption peaks, while absorption peak near 734.75cm^{-1}, 782.96 cm^{-1} is increasing, 655.68cm^{-1}, absorption peak near 692.32cm^{-1} is decreasing.

In addition, at this stage, absorption peak of pyridine two carboxylic acid is decreasing near 1560.13cm^{-1} and 1616.06cm^{-1}, but shows enhancement tendency in the vicinity of the 1385.56cm^{-1}, which means at this stage the spores are still rapidly and unstably growing.

（3）stable phase

Stable phase is also known as the constant regular or higher growth period. Its characteristic is the growth rate constant R is equal to 0, specifically, the number of newly breeding cells and the number of declining cells is the same, or positive growth and negative growth are in the dynamic balance[6].

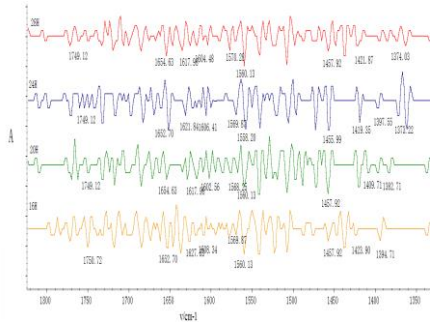

Fig.7. The second-order derivative infrared spectroscopy in the stable phase (1400cm^{-1}-1750 cm^{-1})

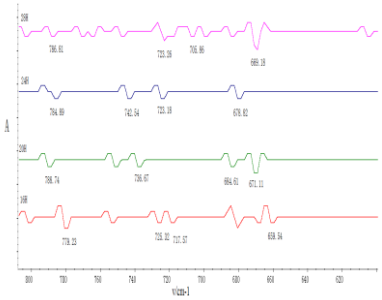

Fig.8. The second-order derivative infrared spectroscopy in the stable phase (600cm^{-1}-800cm^{-1})

Figure 7 and figure 8 state that in the stable period symmetric and anti symmetric carboxyl stretching vibration absorption peaks appear in the vicinity of 1396.21cm^{-1} and 1604.48cm^{-1}, and decrease in 16-28h; At this stage, the protein amide I band absorption peak near 1654.63cm^{-1} shows increasing trend; No change happens for amide II band absorption peak of 1560.13cm^{-1}; The absorption peak produced by the bending vibration of C-H methyl and Methylene near 1457.92cm^{-1} has increased; The ester absorption peak near 1749.12cm^{-1} basically shows no change; In a series of stretching vibration absorption peaks, while absorption peaks near 669.18cm^{-1}, 723.18cm^{-1} , and 771.39 cm^{-1} decrease, absorption peak near 707.75cm^{-1} increases. Figure 7 shows second order derivative spectra of the pyridine carboxylic acid in the stable period, during which absorption peak near the stage of 1617.98cm^{-1} weakens and there is no significant change for the absorption peak near 1384.64cm^{-1} and 1560.13cm^{-1}.

4. Conclusion

By calibration experiment and analysis of collected corresponding spectra information, it could be found:

(1) By Fourier transformation infrared spectrometer, the characterization of the main substance of Bacillus cereus spores (characteristic spectrum of capsule and other related substances) could be observed.

(2) By tracking and detecting the growth process of bacillus cereus, collecting the relevant spectral data in different periods, and analyzing growth curve of bacillus cereus, it infers that there is the law of its change in the lag phase, exponential phase and stable phase.

① In the whole growth change interval, for bacillus cereus symmetric and anti stretching vibration absorption peaks of carboxyl group appear nearby 1604.48cm^{-1} and 1396.21cm^{-1}, and are gradually weakening; The spectral peaks near 1654.63cm^{-1} and 1560.13cm^{-1} are from protein amide I band and protein amide II band, and tend to be stable; In the range of 1457.92cm^{-1}, bending vibration absorption peaks of the

methyl and Methylene also tend to be stable; And an ester absorption peak near 1749.12cm^{-1}shows a tendency of decrease. According to the analysis of absorption peaks of bacillus cereus, the presence of capsule can be determined in the growth process of bacillus cereus

② Bacillus cereus has absorption peaks of stretching vibration of the two COO- groups generated by DPA, which appear nearby 1617.98cm^{-1} and 1384.64cm^{-1}.The former has increased in the whole process of change, and the latter tends to be stable; Absorption peak near 1560.13cm^{-1} caused by C-N bond in the DPA ring, tends to be gradually weakening; Absorption peaks caused by DPA appear in the whole growth process of change, and DPA is the evidence determining whether there is the spore for bacillus cereus, it can therefore be concluded that the spore exists and tends to be stable in the stable phase.

In summary, Fourier transformation infrared spectroscopy equipment could quickly identify bacillus cereus spectral changes in different periods, which lays theoretical basis for develop portable devices for rapid detection of bacillus cereus.

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References

1. Morris J G.Bacillus cereus food poisoning[J].Archives of internal medicine,1981, 141(6):711-711.
2. CI YunXiang, ZANG KaiSai, GAO TiYu,FTIR Study of Microbes,CHEMICAL JOURN AL OF CHIN ESE UNIV ERSITIES,2002,Vol. 23 No. 6 1047-1049
3. Lu Feng,Lu Wei,Xiao zhenyu.A preliminary study of infrared spectrum discriminant method of drug-resistant candida albicans, Chinese Journal of Analytical Chemistry,2003,31(12):1532
4. Naumann D,Helm D, Labischinski H. Microbiological characterizations by FT-IR spectroscopy[J].Nature,1991,351(6321):81-82.
5. Caifei,Lufeng,The application of Fourier transform infrared spectroscopy combined with chemometrics in the discriminant, classification and identification of microbial, [J].The journal of pharm aceutical practice,2002,20(4):238-240.
6. Zhou deqing. Microbiology tutorial[M].Beijing: Higher education press, 1992.183-190
7. CHENG Cun-gui,YING Tao-kai.Spectroscopy and Spectral Analysis,2005,25(1):36.