Establishment of Molecular Biological Method for Identification of Bacteria by 16S rDNA and *gyrB* Gene

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Abstract [**Objectives**] The paper was to establish a molecular biological method for identification of bacterial strains. [**Methods**] The thalli of standard bacterial strains existing in the laboratory were collected and genomic DNA was extracted for amplification of 16S rDNA and *gyrB* gene. The 16S rDNA and *gyrB* gene sequences were obtained after sequencing. Sequences were aligned and analyzed via EzBioCloud and NCBI database, and phylogenetic trees were constructed to determine the species relationship of strains. Meantime, they were compared with known strains. [**Results**] This method could identify 5 standard strains accurately to the species level. The 16S rDNA and *gyrB* gene sequences were aligned and analyzed in EzBioCloud database and NCBI database. The strain with the max score was consistent with the known strain. And the query cover and ident were both above 99%. [**Conclusions**] The established molecular biological method for identification of bacterial strains by 16S rDNA and *gyrB* gene has good accuracy, which effectively solves the problem that the laboratory identification of bacteria relies on traditional methods and the accuracy can not be guaranteed, and further improves the identification ability of laboratory bacterial strains.

Key words 16S rDNA, *gyrB*, Bacterial identification, Molecular biological method

1 Introduction

Bacterial identification is mainly composed of phenotype and genotype identification[1-2]. Phenotype identification is the identification of bacteria through morphological observation, physiology and biochemistry using traditional culture methods. There are shortcomings such as long culture time, not obvious characteristic reaction, and easy to be affected by bacterial age, culture conditions and other external factors, so it is often difficult to achieve ideal identification effect for some difficult and rare bacteria[3-5]. Genotype identification is the application of molecular biological means in bacterial classification and identification, and strains tested are classified and identified at the molecular level through analysis of bacterial DNA. Common identification methods include gene sequencing[6-7], fingerprint technology[8-9], gene probe technology[10-11], polymerase chain reaction (PCR)[12-13], *etc.* Genotype identification is featured by high accuracy and good repeatability compared with traditional methods. At present, it is impossible to identify all bacterial strains with 100% accuracy through a single method, so it is necessary to combine morphological observation, biochemical identification, molecular biological identification and other means to accurately determine bacteria. Molecular biological identification is the "gold standard" for determination of bacteria[14].

Ribosomal DNA is the DNA sequence that encodes rRNA on biological chromosomes and exists in all biological chromosome genes. Ribosomal DNA-16S rDNA in prokaryotes is widely used in the identification of bacteria due to the existence of highly conserved regions and variable regions[15-16]. A highly conserved region refers to a specific region sequence that is the same in all bacteria, and a variable region refers to a specific region sequence that varies among different bacteria. The highly conserved region in 16S rDNA is used to design a universal primer for bacteria, further amplifies the variable region fragment in 16S rDNA of purified bacteria, and finally determines the species relationship of bacterial strains according to the sequencing and analysis of the variable region. In strain identification, the results of 16S sequence alignment are convincing at the genus level, so there are multiple results of the same genus but different species, which indicates that the experimental strain belongs to this genus. As for the species, it needs to be further identified through functional genes[17-18]. The functional gene *gyrB*[19-20], with a single copy and a sequence length of 1.2 -1.4 kb, is a common gene encoding gyrase B subunit in bacteria. This gene evolves quickly, with an average base replacement rate of 0.7% -0.8% per million years, which is faster than the 1% change in 16S rDNA per 50 million years, and can make up for the difficulty of accurately distinguishing bacteria sibling species by highly conserved 16S rDNA[21]. By establishing a molecular biological method for identification of bacteria by 16S rDNA and *gyrB*, this paper aimed to solve the problem that the laboratory bacteria identification relies on traditional methods and the accuracy can not be guaranteed, so as to further improve the identification ability of laboratory bacterial strains.

2 Materials and methods

2.1 Materials

2.1.1 Standard strains and reagents. The standard strains used

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in the test included *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* CICC 22956, *Cronobacter sakazakii* CICC 21560, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923.

The reagents used were universal primers for bacterial 16S rDNA, universal primers for bacterial functional gene *gyrB*, small extraction kit for bacterial genomic DNA, PCR Mix premix with sample loading buffer (2 ×), agarose, DNA Marker (DL2000, 50 × TAE buffer, 1 × TAE buffer), GelRed dye (10 000 ×).

2.1.2 Instrument and equipment. Biosafety cabinet, biochemical incubator, electric-heated thermostatic water bath, PCR meter, miniature centrifuge, electrophoresis apparatus, gel imager.

2.2 Methods

2.2.1 Extraction of bacterial genome. The standard strains stored in the laboratory were selected and the thalli to be tested were collected. The genomic DNA was extracted according to the instructions of small extraction kit for bacterial genomic DNA.

2.2.2 PCR amplification. Bacterial 16S rDNA sequence amplification was performed in a 50 μL reaction system containing 25 μL of PCR Mix premix, 1 μL of each 10 μmol/L primer 27 F and 1492 R, 22 μL of sterilized deionized water, and 1 μL of template. The sample template was the DNA of the strains extracted, the positive control template was the verified bacterial genome DNA, and the negative control template was sterilized deionized water. PCR amplification was conducted in the following procedures: pre-denaturating at 94 ℃ for 2 min; denaturating at 94 ℃ for 30 s, annealing at 55 ℃ for 90 s, extension at 72 ℃ for 1.5 min, 30 cycles; final extension at 72 ℃ for 10 min, preservation at 4 ℃. The amplification system of bacterial functional gene *gyrB* sequence was the same as that of bacterial 16S rDNA sequence except for primers $gyrB^{b}$-F and $gyrB^{b}$-R. The reaction procedure was as follows: pre-denaturating at 94 ℃ for 2 min; denaturating at 94 ℃ for 30 s, annealing at 55 ℃ for 90 s, extension at 72 ℃ for 1 min, 30 cycles; final extension at 72 ℃ for 10 min, preservation at 4 ℃.

2.2.3 Detection of PCR amplification products by electrophoresis. The amplified products were detected by electrophoresis with 1.5% agarose, with DL2000 DNA as the marker, and were imaged, observed and recorded by gel imager after electrophoresis.

2.2.4 Sequencing of PCR amplification products of samples to be identified. If the bacterial genome extraction and PCR reaction were normal, the PCR amplification products of the samples to be identified would be sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing.

2.2.5 Sequencing splicing and analysis of PCR amplification products. Observation of sequencing peak map: Professional software BioEdit was used to observe the sequencing peak chart to determine whether the sequencing results were normal. If the entire sequence peak chart had overlapping peaks, it indicated that the bacteria to be identified had not been completely isolated and purified, and there was interference from infectious bacteria, or pollution brought into the PCR process affected the sequencing result. At this time, the sequencing result was judged to be abnormal and discarded. Splicing and alignment analysis of sequencing results: Professional software DNAMAN was used to splice the two sequences, and the spliced sequences were analyzed via online analysis websites NCBI and EzBioCloud.

2.2.6 Construction of phylogenetic tree. Collection of strain 16S rDNA sequence: According to the analysis results in Section **2.2.5**, the 16S rDNA sequences of related species were downloaded from EzBioCloud website and sorted into a file, which was then saved in FASTA format. Construction of phylogenetic tree: Professional software MEGA was used to align and analyze the collated sequences, and Neighbor-Joining phylogenetic trees were constructed after removing the redundant sequences at both ends.

2.2.7 Result interpretation. The detection results of 16S rDNA sequence should be interpreted first. When the detection results of 16S rDNA sequence could not accurately identify the strain to the species level, the detection results of *gyrB* sequence should be interpreted. In the interpretation of 16S rDNA sequence results, EzBioCloud and NCBI alignment results and 16S rDNA phylogenetic tree results should be integrated.

3 Results and analysis

After DNA extraction, 16S rDNA sequence and *gyrB* sequence of strains tested were amplified. After detected by electrophoresis, obvious and bright amplification bands appeared at 1 500 bp and 1 200 bp, which could be judged that the genome extraction, 16S rDNA PCR assay and *gyrB* PCR assay of the bacteria to be identified were normal. Subsequently, the sequencing results of the strains tested were analyzed. MEGA7 software was used to build phylogenetic trees, and the evolutionary distance was calculated using Kimura 2-parameter method. The 16S rDNA sequence phylogenetic trees of various strains are shown in Fig. 1-5, and only values with probability greater than 70% are shown in the figures.

3.1 *P. aeruginosa* The 16S rDNA sequence of this strain was aligned and analyzed in EzBioCloud database, and the alignment result showed that only *P. aeruginosa* had the similarity greater than 98.7%, so the strain was identified to be *P. aeruginosa*. The 16S rDNA sequences were aligned and analyzed in NCBI database. The top 5 strains with the max scores were *P. aeruginosa*, and the query cover and ident were 100%. As can be seen from the phylogenetic tree in Fig. 1, the evolutionary distance between *P. aeruginosa* and the strain was closest. In summary, the strain was identified to be *P. aeruginosa*. The known standard strain was *P. aeruginosa*, indicating that the method was accurate and could identify *P. aeruginosa* to the species level.

3.2 *S. enterica* The 16S rDNA sequence of this strain was aligned and analyzed in EzBioCloud database, and the alignment result showed that only *S. enterica* had the similarity greater than 98.7%, so the strain was identified to be *S. enterica*. The 16S rDNA sequences were aligned and analyzed in NCBI database. The top 5 strains with the max scores were all *S. enterica*, and the

query cover and ident were 100%. As can be seen from the phylogenetic tree in Fig. 2, the strain had the closest evolutionary distance with *S. enterica*. In summary, the strain was identified to be *S. enterica*. The known standard strain was *S. enterica*, indicating that the method was accurate and could identify *S. enterica* to the species level.

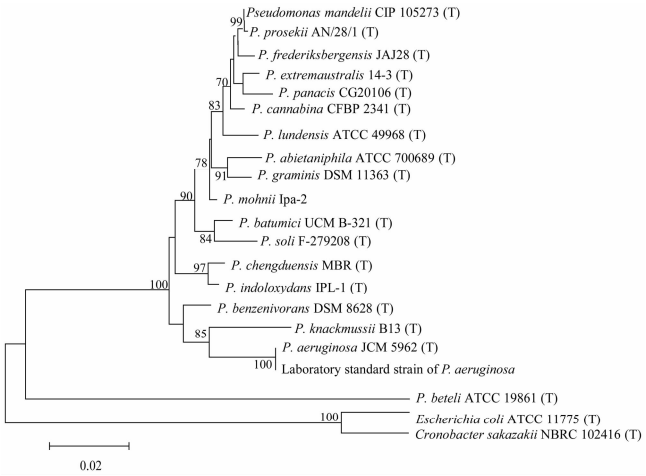

Fig. 1 16S rDNA phylogenetic tree of *Pseudomonas aeruginosa*

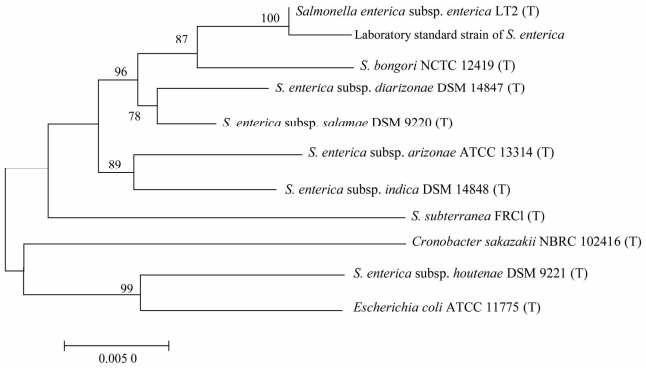

Fig. 2 16S rDNA phylogenetic tree of *Salmonella enterica*

3.3 *C. sakazakii* The 16S rDNA sequences were aligned and analyzed in EzBioCloud database, and there were three species of the genus *Cronobacter* with the similarity greater than 98.7%. Therefore, the strain was identified to be *Cronobacter*. The 16S rDNA sequences were aligned and analyzed in NCBI database. The top 5 strains with the max scores were *C. sakazakii*, and the query cover and ident were above 99%. According to the phylogenetic tree in Fig. 3, it can be seen that the strain had the closest evolutionary distance with *C. sakazakii* and *C. malonaticus*. Partial sequences of *gyrB* gene of the strain were aligned with *gyrB* gene of *Cronobacter* strains in NCBI database. Compared with *C. sakazakii*, the query cover was 100% and the ident was more than 99%, and the alignment score was 2 126. Compared with other *Cronobacter* species, the ident was as high as 96%, so the identification result of *gyrB* gene confirmed that the strain was *C. sakazakii*. In summary, the strain was identified to be *C. sakazakii*. The known standard strain was *C. sakazakii*, indicating that the method was accurate and could accurately identify *C. sakazakii* to the species level.

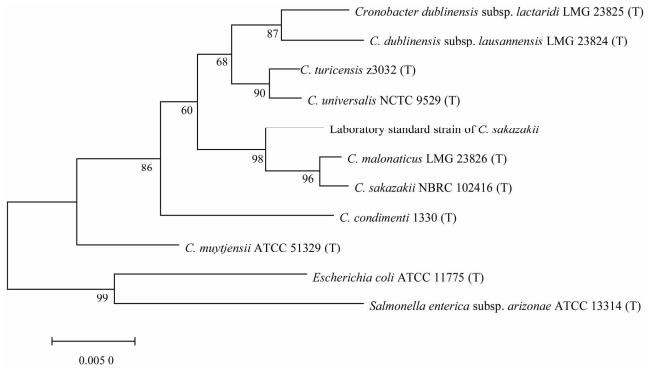

Fig. 3 16S rDNA phylogenetic tree of *Cronobacter sakazakii*

3.4 *E. coli* The 16S rDNA sequences were aligned and analyzed in EzBioCloud database. The alignment results showed that 4 strains of *Shigella* and 4 strains of *Escherichia* had the similarity greater than 98.7%, which were identified to be Enterobacteriaceae. The 16S rDNA sequences were aligned and analyzed in NCBI database. The top 5 strains with the max scores were *E. coli*, and the query cover and ident were more than 99%. As can be seen from the phylogenetic tree in Fig. 4, the strain had the closest evolutionary distance with *E. coli*. Partial sequences of *gyrB* gene of this strain were aligned with those of *Shigella* and *Escherichia* strains in NCBI database. The highest query cover and ident were more than 99%, but the score of *E. coli* was higher than that of *Shigella*. In summary, the strain was identified to be *E. coli*. The known standard strain belonged to *E. coli*, indicating that the method was accurate and could accurately identify *E. coli* to the species level.

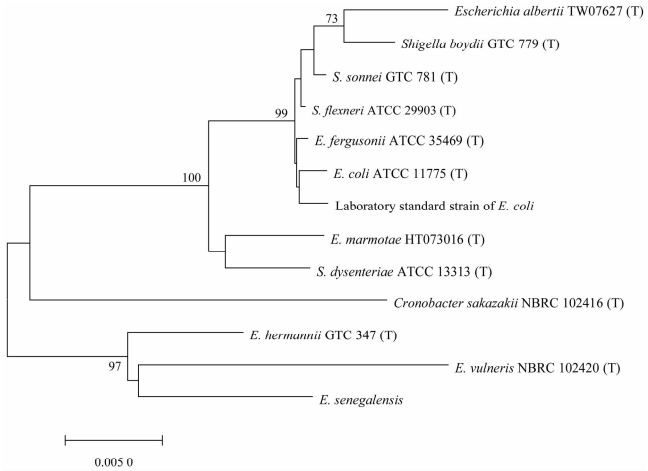

Fig. 4 16S rDNA phylogenetic tree of *Escherichia coli*

3.5 *S. aureus* The 16S rDNA sequences were aligned and analyzed in EzBioCloud database, and there were 4 strains of *Staphylococcus* with the similarity greater than 98.7%, so the strain was identified to be *Staphylococcus*. The 16S rDNA sequences were aligned and analyzed in NCBI database. The top 5 strains with the max scores were all *S. aureus*, and the query cover and ident were 100%. As can be seen from the phylogenetic tree in Fig. 5, the strain had the closest evolutionary distance with *S. aureus*. Partial sequences of *gyrB* gene of this strain were aligned with those of

Staphylococcus strains in NCBI database. Compared with *S. aureus*, the query cover was 100%, the ident was more than 99%, and the alignment score was 2 162. Compared with other *Staphylococcus* strains, the highest ident was 95%, so the identification result of *gyrB* gene confirmed that the strain was *S. aureus*. In conclusion, the strain was identified to be *S. aureus*. The known standard strain was *S. aureus*, indicating that the method was accurate and could accurately identify *S. aureus* to the species level.

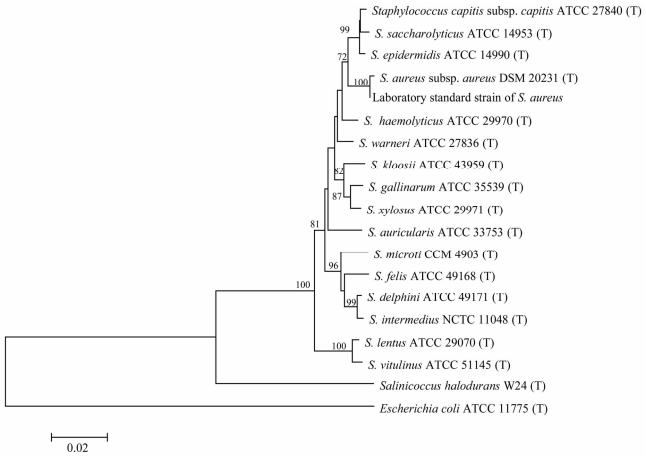

Fig. 5 16S rDNA phylogenetic tree of *Staphylococcus aureus*

4 Discussion

Molecular biological methods to some extent make up for the shortcomings of traditional methods such as long identification cycle, easy to be affected by culture conditions, poor repeatability and low accuracy, and have fast identification and high accuracy when exploring the taxonomic status and species relationship of bacteria from the gene level. In the classification of species, if the homology of 16S rDNA sequence between two taxonomic units is higher than 97.5%, the two taxonomic units can be classified as the same species[22]. However, for some closely related strains, there may be more than one strain with high homology after alignment of 16S rDNA gene sequence in database, so it can not be differentiated at the species level, and other methods should be combined to supplement identification.

Recent studies have found that that *gyrA* and *gyrB* genes are widely used because they can make up for the shortcomings of 16S rDNA gene method. The combined identification method of 16S rDNA and *gyrB* gene sequence is a scientific and internationally recognized method for identification of newly isolated species[23]. At present, most studies have applied the combination method of 16S rDNA and *gyrB* gene in the identification of closely related bacterial populations[24-26], and received good results. Compared with traditional methods and single use of 16S rDNA sequence for identification, the method can identify strains quickly and accurately to the species level. Fu Qi *et al.*[27-28] used a phylogenetic tree based on 16S and *gyrB* gene sequences to accurately and rapidly identify a strain of *Bacillus licheniformis* isolated from seawater and a strain of *B. amyloliquefaciens* isolated from soil. Lu Jiaqi *et al.*[29] used combined 16S rDNA and *gyrB* gene sequence to identify a strain of *B. cereus*.

5 Conclusions

In this study, a molecular biological method of combined 16S rDNA and *gyrB* identification of bacterial strains was established. Five standard strains, which were the most representative in the laboratory, were selected for experiments. Proper amount of thalli were collected to extract genomic DNA, and 16S rDNA and *gyrB* gene sequences were obtained through 16S rDNA amplification and *gyrB* gene sequencing. The 16S rDNA sequences were aligned and analyzed in EzBioCloud database and NCBI database, and the phylogenetic trees were constructed. *P. aeruginosa* and *S. typhimurium* could be accurately identified to the species level by 16S rDNA sequence analysis. But *C. sakazakii*, *E. coli* and *S. aureus* could only be identified to the genus level through 16S rDNA sequence analysis. Therefore, continued interpretation of *gyrB* sequence detection results finally accurately identified the strain to the species level. The results were consistent with known standard strains, indicating that this method had good accuracy, and *gyrB* gene could make up for the deficiency of 16S rDNA molecular identification method.

References

[1] SCHLEIFER KH. Classification of bacteria and archaea: Past, present and future[J]. Systematic and Applied Microbiology, 2009, 32(8): 533-42.

[2] BUSSE HJ, DENNER EB, LUBITZ W. Classification and identification of bacteria: Current approaches to an old problem: Overview of methods used in bacterial systematics[J]. Journal of Biotechnology, 1996, 47(1): 3-38.

[3] HE XQ, YUAN XY, LIN J, *et al.* Application of 16S rDNA sequence analysis technique in clinical bacterial identification[J]. Medical Laboratory Science and Clinics, 2013(2): 51-52. (in Chinese).

[4] LI L, LI JN, YU WY. Brief introduction of the method of bacterial classification and identification[J]. Journal of Anhui Agricultural Sciences, 2004, 32(3): 549-551. (in Chinese).

[5] ZHU FZ, CHEN LY, CHEN HC. Identification of pathogenic microorganism by sequencing 16S rRNA gene[J]. Journal of Central South University, 2013, 38(10): 1035-1041. (in Chinese).

[6] GAO YJ, ZHAN FQ, CHEN C, *et al.* Screening, identification and optimization of fermentation conditions of antagonistic *Xenorhabdus bovienii* 445 against *Aspergillus niger*[J]. Xinjiang Agricultural Sciences, 2022, 59(5): 1203-1215. (in Chinese).

[7] PENG NN, NING HM, WANG W, *et al.* Isolation, identification and drug resistance analysis of *Salmonella* from Yueyang, Hunan[J]. Swine Production, 2022(3): 126-128. (in Chinese).

[8] ZHAO FJ, ZHAO ZG, XIA JB, *et al.* Sediment bacterial community structure diversity from coastal wetland of shell ridge island in Yellow River Delta[J]. Journal of Beihua University (Natural Science), 2022, 23(4): 530-537. (in Chinese).

[9] LIN TX, LI C, GONG MF. Genetic diversity of antagonistic endophytic bacteria isolated from *Sophora alopecuroide*[J]. Journal of Anhui Agricultural Sciences, 2022(24): 14673-14675. (in Chinese).

[10] SONG SQ. Application of gene probe technology in food hygiene detection[J]. Dietary Science: The Second Half of the Month, 2020(5): 1. (in Chinese).

[11] WU YB, BO XC, YAN LR, *et al.* Design of 16S rRNA-based oligonucleotide array using group-specific non-unique probes in large scale bacteria detection[J]. Progress in Biochemistry and Biophysics, 2009(8): 1025-1034. (in Chinese).

[12] GONG QL, WANG ZY, CHAI TJ, *et al.* PCR-16S rDNA identification

of aerobe and facultative anaerobe from rumen of cows and affected by the dietary[J]. Chinese Journal of Animal and Veterinary Sciences, 2008(10): 1367 – 1372. (in Chinese).
[13] SONG LP, YANG ZP, WANG B, *et al.* Analysis on the establishment of PCR sequencing method for rapid identification of bacteria based on 16S rDNA and its evolutionary relationship[J]. China Medicine and Pharmacy, 2020, 10(17): 86 – 91. (in Chinese).
[14] LI YQ, ZHU BL, WU J, *et al.* Molecular biological analysis of bacterial community in milk based on 16S rRNA[J]. Food Science, 2013, 34(20): 255 – 260. (in Chinese).
[15] TAN FX, CHAI Y. Molecular identification of four strains of *Pseudomonas* based on 16S rDNA sequence[J]. Jiangsu Agricultural Sciences, 2013(6): 31 – 35. (in Chinese).
[16] BAI ZP, CAO MN, ZHANG N, *et al.* Application of 16S rRNA gene sequence in the classification and identification of clinical atypical bacteria[J]. Chinese Journal of Laboratory Diagnosis, 2022, 26(6): 914 – 917. (in Chinese).
[17] AN R, YI TY, XIAO QM, *et al.* Application of *gyrB* gene in bacterial classification and detection[J]. Acta Agriculturae Jiangxi, 2010, 22(4): 18 – 20. (in Chinese).
[18] HAO YJ, HAN SZ. Application of *gyrB* gene in bacterial phylogenetic analysis[J]. Biotechnology Bulletin, 2008(2): 39 – 41. (in Chinese).
[19] WANG LT, LEE FL, TAI CJ, *et al.* Comparison of *gyrB* gene sequences, 16S rRNA gene sequences and DNA-DNA hybridization in the *Bacillus subtilis* group[J]. International Journal of Systematic and Evolutionary Microbiology, 2007, 57(8): 1846 – 1850.
[20] HOU XL, CHEN Z. A new marker *gyrB* gene for the classification and identification of bacteria[J]. Foreign Medicine (Epidemiology and Infectious Diseases Volume), 2005, 32(1): 38 – 41. (in Chinese).
[21] LI XM, WANG XF, YANG HY, *et al.* Application of *gyrB* in the identification of closely related bacteria: A review[J]. Acta Microbiologica Sinica, 2008, 48(5): 701 – 706. (in Chinese).
[22] IGUCHI A, ITO H, UENO M, *et al.* Molecular phylogeny of the deep-sea *Buccinum* species (Gastropoda: Buccinidae) around Japan: Inter and intraspecific relationships inferred from mitochondrial 16S rRNA sequences[J]. Molecular Phylogenetics & Evolution, 2007, 44(3): 1342 – 1345.
[23] BIE XM, LU ZX, FANG YW, *et al.* Identification of a microorganism strain producing the antimicrobial substance with 16S rDNA sequence analysis[J]. Food Science, 2006, 27(11): 466 – 470. (in Chinese).
[24] YU GH, NIU CY, CHEN YF, *et al.* Use of 16S rDNA, *gyrA* and *gyrB* gene sequence to identify a biocontrol *Bacillus* sp. strain R31[J]. Chinese Journal of Biological Control, 2010, 26(2): 160 – 166. (in Chinese).
[25] YAMAMOTO S, HARAYAMA S. PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains[J]. Applied and Environmental Microbiology, 1995, 61(3):1104 – 1109.
[26] NARAYANAN GS, VIJAYARAGHAVAN R, MENON T. Identification of *Corynebacterium jeikeium* from a CAPD patient by 16S rDNA PCR: A case report[J]. BMC Infectious Diseases, 2014, 14(s3): 34.
[27] FU Q, LIN JJ, GAN MY, *et al.* Identification of a *Bacillus licheniformis* strain from seawater using a phylogenetic tree based on 16S and *gyrB* gene sequences[J]. Marine Sciences, 2020, 44(4): 90 – 95. (in Chinese).
[28] FU Q, LIN JJ, FENG YD, *et al.* Identification of a *Bacillus amyloliquefaciens* strain using a phylogenetic tree based on 16S and *gyrB* gene sequences[J]. Food and Fermentation Industries, 2020, 46(12): 116 – 120. (in Chinese).
[29] LU JQ, LI SC, WANG DH, *et al.* Combined 16S rDNA and *gyrB* gene sequence to identify a strain of *Bacillus cereus*[J]. Journal of Henan University of Science and Technology (Natural Science Edition), 2018(1): 73 – 77, 83.

(From page 20)

nomenon, which conforms to the law of socialist development and is an exploration of the socialist road of poverty alleviation. It is inevitably full of unknowns and unpredictability. Third, the *Poverty Alleviation Act* should constantly change with the development of poverty alleviation. With the gradual deepening of poverty alleviation, the accumulated problems and contradictions will continue to increase. On the one hand, with the change of time and space of poverty alleviation, some provisions of the *Poverty Alleviation Act* are outdated and cannot explain the actual poverty alleviation, so some contents need to be revised again. On the other hand, the existing *Poverty Alleviation Act* can't explain a large number of new things, and it is necessary to add and enrich new contents. Obviously, the *Poverty Alleviation Act* is not fixed, and we should change the relevant contents and add new contents at any time according to the needs of the development of poverty alleviation, and constantly guide the development of poverty alleviation.

4.3 Purpose of *Poverty Alleviation Act* The purpose of *Poverty Alleviation Act* is to strictly regulate and restrain the behavior of all poverty alleviation resources in poverty alleviation. According to the types and quantities of poverty alleviation resources needed by poverty alleviation objects at different stages of development, scientific coordination and allocation are carried out, thus forming a comprehensive integration effect of poverty alleviation resources, and then promoting the process of poverty reduction.

Any kind of poverty alleviation resources can't exist alone in poverty alleviation, and can't play an independent role, because the existence and function of any kind of poverty alleviation resources are limited and influenced by other poverty alleviation resources. Therefore, only under the action of *Poverty Alleviation Act* can all poverty alleviation resources be led, and all poverty alleviation resources be organically combined, so that the cost of poverty alleviation can be reduced, and the goal of poverty alleviation can be realized as soon as possible.

China is a socialist country and shows an overall interest-oriented economic form. Covering all people on the road of getting rid of poverty is the purpose and principle of the cause of poverty alleviation. Only through the *Poverty Alleviation Act* can we maximize the role of all poverty alleviation resources and continuously promote the socialist poverty alleviation cause as a whole.

References

[1] MAO ZD. Selected Works of Mao Zedong, Volume IV (2nd Edition)[M]. Beijing: People's Publishing House, 1991, 1298. (in Chinese).
[2] MAO ZD. Selected Works of Mao Zedong, Volume II[M]. Beijing: People's Publishing House, 1991, 526. (in Chinese).