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A Quick DNA Extraction Method for High Throughput Screening in Gram-positive Bacteria

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Abstract

In this study, a sonication-based DNA extraction method was developed, in which the whole process can be finished within 10 min. This method is almost zero cost and time-saving, which is useful for high throughput screening, especially in the screening of mutants generated in random mutagenesis. This method is effective in genomic DNA extraction for PCR amplification in several Gram-positive bacteria, including *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus subtilis*, and *Listeria monocytogenes*.

Keywords: Gram-positive Bacteria, DNA Extraction, Ultrasound, High Throughput Screening, PCR

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Background

Colony PCR is commonly used to verify the expected genome DNA in *Escherichia coli*, but it fails in Gram-positive bacteria such as *Bacillus subtilis* even when treated with chemical reagents (Packeiser et al., 2013; Azevedo et al., 2017). The reason colony PCR fails in these cases is the thick cell wall that exists in most Gram-positive bacteria, which impedes cell lysis and subsequent DNA release. Enzymes such as lysozyme, proteinase K, and other chemical reagents are used to lyse the Gram-positive bacterial cell wall (Mertens et al., 2014); however, these methods are costly, time consuming, and not eco-friendly for the DNA extraction of a huge quantity of samples. For example, it is very difficult to achieve a fast screen towards thousands of mutants generated in random mutagenesis. It is therefore necessary to set up a convenient and economical method.
Sonication is a common physical method employed to disrupt cells by transmitting ultrasonic energy to the liquid region (Taylor et al., 2001). Sonication-based DNA extraction is considered as an efficient and cost-effective method. In the intestinal microflora and compost microbes of fish, the ultrasonic lysis method combined with lysozyme was used to extract DNA (Yang et al., 2006; Han et al., 2018). Moreover, direct extraction of DNA from sputum clinical sample (containing diverse microbes) for real-time PCR proved to be convenient and suitable (Bello et al., 2020). In this study, a sonication-based DNA extraction method was developed, which could screen large samples in just one day. Despite not being as pure as the commercial kit, this method can be used for high-throughput screening purposes, which requires extracting great amounts of DNA, in Gram-positive bacteria such as *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus subtilis*, and *Listeria monocytogenes*.

Materials and Reagents

Materials

1. 1.5 mL tube (Axygen, catalog number: MCT-150-C)
2. 0.2 mL PCR tube (Axygen, catalog number: PCR-02-C-P)
3. 10 and 100 μL pipettes (Eppendorf, catalog numbers: 3120000224 and 3120000828)
4. 10 and 100 μL micropipettes (Axygen, catalog numbers: TGL-10FT-17-R and TF-100)
5. Petri dishes (Guangdong Huankai, catalog number: 20221700)
6. Inoculation loop (Guangdong Huankai, catalog number: 00416571)

Strains

1. *Bacillus cereus* ATCC 14579 (ATCC, catalog number: ATCC 14579)
2. *Bacillus thuringiensis* ATCC 10792 (ATCC, catalog number: ATCC 10792)
3. *Bacillus subtilis* ATCC 6633 (ATCC, catalog number: ATCC 6633)
4. *Listeria monocytogenes* ATCC 19115 (ATCC, catalog number: ATCC 19115)

Reagents

1. Nutrient agar plate (Guangdong Huankai, catalog number: 1024089)
2. Taq DNA polymerases (Thermo Fisher Scientific, catalog number: 10966018)
3. Marker D, 100–2,000 bp (Sangon Biotech, catalog number: B600335-0050)
4. GoldView™ (Coolaber, catalog number: GD001-1mL*5)
5. 50 × TAE buffer (Sango Biotech, catalog number: B548101-0500)

Equipment

1. Incubator (SHANGHAI MINQUAN INSTRUMENT, model: LAB-0001-0004-SHMQ)
2. Benchtop centrifuge (Eppendorf, model: 5415D)
3. Ultrasonic cleaning machine (LANGEE, model: UC-9120)
4. Thermal cycler (Bio-Rad, model: C1000 Touch)
5. Electrophoresis imaging system (Bio-Rad, model: GelDoc XR Biorad)
6. Spectrophotometer (Thermo Fisher, model: NanoDrop™ One Microvolume UV-Vis)
7. Autoclave (ZEALWAY, model: GR60DA)

Software

1. Image Lab (Bio-Rad)

Procedure

1. Cultivate the strains (listed in materials) conserved in glycerol at 37 ℃, at 200 rpm overnight (for approximately 8–12 h).
2. Dip a small amount of the overnight culture using a 10 μL inoculation loop and streak lightly on the nutrient agar plate. Then, cultivate the plate at 37 ℃ overnight.
3. Suspend a single colony into 30 μL of ddH_2O in a 1.5 mL tube.
   Note: The size of the colonies of B. cereus ATCC 14579, B. thuringiensis ATCC 10792, B. subtilis ATCC 6633, and L. monocytogenes ATCC 19115 is recommended in Figure 1 and Table 1, and the quality of DNA is listed in Table 2.

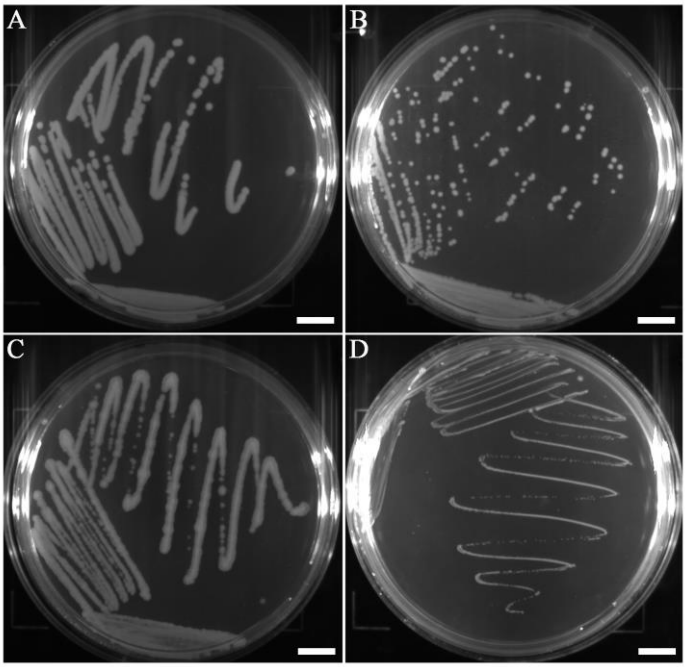

Figure 1. *B. cereus* (A), *B. thuringiensis* (B), *B. subtilis* (C), and *L. monocytogenes* (D) colonies formed on Petri dishes. Scale bars represent 10 mm.

Table 1. Colony size of *B. cereus*, *B. thuringiensis*, *B. subtilis*, and *L. monocytogenes*

Microorganism	Colony size
B. cereus ATCC 14579	2–4 mm
B. thuringiensis ATCC 10792	1–2 mm
B. subtilis ATCC 6633	1–3 mm
L. monocytogenes ATCC 19115	0.2–0.5 mm

4. Treat the bacterial suspension by sonication at 40 kHz and 120 W at room temperature for 5 min and then centrifuge at 10,000 ×*g* for 1 min at room temperature.
   Note: No need to set pauses between the pulses.
5. Carefully transfer the upper aqueous phase to a new 1.5 mL tube without disturbing the pellet.
6. The DNA samples are tested using 1 μL of supernatant as DNA template by PCR. Primers for PCR are listed in Table 3. Taq DNA polymerases are added and parameters for PCR are as follows: initial denaturation: 95 ℃, 3 min; denaturation: 95 ℃, 30 s; annealing: 55 ℃, 30 s; extension: 72 ℃, 1 min; final extension: 72 ℃, 10 min; number of cycles: 25×. After electrophoresis on 1.2% agarose gels (in 1× TAE diluent), all amplicons are visualized under UV light with GoldView™ staining. The results are shown in Figure 2.
   Note: The bands are brighter if you increase the PCR reaction cycles.

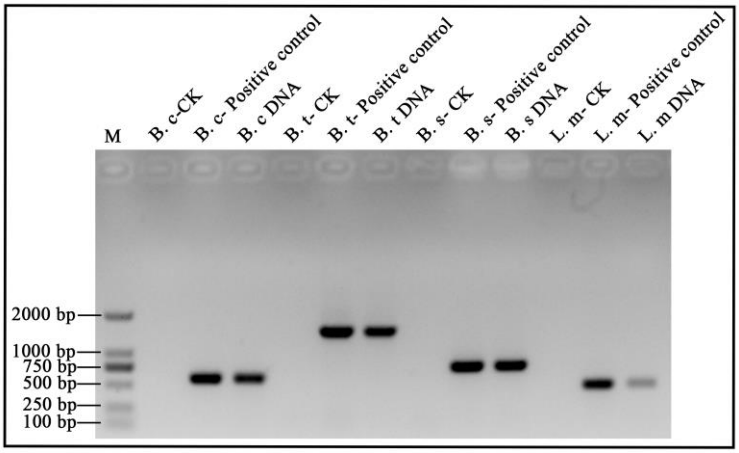

Figure 2. *B. cereus*, *B. thuringiensis*, *B. subtilis*, and *L. monocytogenes* DNA analyzed by electrophoresis. Lane M: 100–2,000 bp DNA ladder (Marker D). B. c: *B. cereus*; B. t: *B. thuringiensis*; B. s: *B. subtilis*; L. m: *L. monocytogenes*. DNA: DNA extracts by ultrasonic method for 5 min. Positive control: DNA extracted with a commercial kit (1 μL DNA template). CK: Check control (no template).

Table 2. The quality of DNA sample by spectrophotometry analysis

Microorganism	Nucleic Acid (ng/μL)	A_{260}/A_{280}	A_{260}/A_{230}
B. cereus ATCC 14579	20.343	1.517	0.641
B. thuringiensis ATCC 10792	19.43	1.5	0.605
B. subtilis ATCC 6633	21.935	1.303	0.634
L. monocytogenes ATCC 19115	6.321	1.808	1.018

Table 3. Primer sequences used in *B. cereus*, *B. thuringiensis*, *L. monocytogenes*, and *B. subtilis*, respectively

Microorganism	Target gene	Primer sequence (5′ to 3′)
B. cereus ATCC 14579	*gmk*	F-TTAAGTGAGGAAGGGTAGG
		R-AATGTTCACCAACCACAA
B. thuringiensis ATCC 10792	*pycA*	F-GTGAAAGCAAGAACACAAGC
		R-ATAGTTTTTGTATCCAACTGCG
B. subtilis ATCC 6633	*SigB*	F-ATGACACAACCATCAAAAACTACGA
		R-TTACATTAACTCCATCGAGGGATCT
L. monocytogenes ATCC 19115	*prfA*	F-GTCAAAACATACGCTCTTATC
		R-ACATAATCAGTCCAAAGTAGATGC

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Competing interests

There are no conflicts of interest or competing interests.

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