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# Gene-based identification of bacterial colonies with an electric chip

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**Abstract**

A method for the identification of bacterial colonies based on their content of specific genes is presented. This method does not depend on DNA separation or DNA amplification. *Bacillus cereus* carrying one of the genes (*hblC*) coding for the enterotoxin hemolysin was identified with this method. It is based on target DNA hybridization to a capturing probe immobilized on magnetic beads, followed by enzymatic labeling and measurement of the enzyme product with a silicon-based chip. An *hblC*-positive colony containing $10^7$ cells could be assayed in 30 min after ultrasonication and centrifugation. The importance of optimizing the ultrasonication is illustrated by analysis of cell disruption kinetics and DNA fragmentation. An early endpoint PCR analysis was used to characterize the DNA fragmentation as a function of ultrasonication time. The first minutes of sonication increased the signal due to both increased DNA release and increased DNA fragmentation. The latter is assumed to increase the signal due to improved diffusion and faster hybridization of the target DNA. Too long sonication decreased the signal, presumably due to loss of hybridization sites on the targets caused by extensive DNA fragmentation. The results form a basis for rational design of an ultrasound cell disruption system integrated with analysis on chip that will move nucleic acid-based detection through real-time analysis closer to reality.


*Keywords:* *Bacillus cereus*; Enterotoxin; Hemolysin; DNA fragmentation; Ultrasonication

Increasing concerns regarding food contamination by microorganisms have made more critical the importance of developing fast, reliable, and sensitive analytical methods for use in the monitoring of pathogens [1–5]. Traditional methods to detect food-borne bacteria rely on time-consuming growth in culture media followed by isolation, biochemical identification, and sometimes serological determination [6,7]. In many cases, it is not enough to identify a contaminating bacterium only at the species level. For instance, only approximately 50% of isolated *Bacillus cereus* was classified as pathogenic [8], and *Escherichia coli* is mostly not pathogenic, but some strains are harboring genes for toxins (e.g., shiga toxin [9]) that may cause fatal diseases. Furthermore, the antibiotics resistance of bacteria can mostly be genetically defined, and it is strain dependent rather than species dependent. These problems increase the demand for genetically based diagnostic assays.

The developments in bioinformatics have widened the basis for organism identification to also include nucleic acid analysis. Thus, new analytical instruments, monitoring devices, and rapid test kits have been created to detect and quantify bacteria [10–13]. Among them, DNA-sensing systems have become a powerful tool for the detection of various pathogenic microorganisms [2,14].

We recently reported on rapid detection of specific nucleic acid sequences by means of electric chips [15]. This method permits DNA analysis of microorganisms without prior nucleic acid purification or amplification

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Table 1
Characteristics of oligonucleotide primers and probes used in this study

| Name | 5′ position | Function | Nucleotide sequence[a] (5′–3′) |
|---|---|---|---|
| HblC U-ABCDEF | 270 | PCR upper primer ABCDE for *hblC* gene | TAATGTTTTAATGAACAACATAACT |
| HblC L-A | 1180 | PCR lower primer A for *hblC* gene | GATAGAGTTCCGATGACCATTCCTT |
| HblC L-B | 1016 | PCR lower primer B for *hblC* gene | ATATCCATGCCTTCCTGTTGAGTTT |
| HblC L-C | 751 | PCR lower primer C for *hblC* gene | TACTTACCTCTCACTTCGATACTCT |
| HblC L-D | 624 | PCR lower primer D for *hblC* gene | ACAGAACCGCGAGAATCAATAAACC |
| HblC L-E | 456 | PCR lower primer E for *hblC* gene | CACTTTTGTTATGCAGASAACTTAGA |
| HblC L-F | 343 | PCR lower primer F for *hblC* gene | CACTATAATTCCTATTAGCGTAACC |
| HblC C | 270 | Capture probe for *hblC* gene | xTCAGTAATGTTTTAATGAACAACATAACT |
| HblC D | 296 | Detection probe for *hblC* gene | GTATGACCAGACAGAAAGGATAAGGACTAy |

[a] x and y are for amino group and biotin in that order.

by PCR [16]. One application is determination of the presence of pathogenic strains of *B. cereus*. This organism is widely distributed in nature and commonly occurs in a variety of foods where it may produce different toxins [8,17]. The detection of this bacterium by classical methods often requires selective enrichments of up to 48 h followed by selective plating for 24–48 h. Thus, the rapidity and simplicity of *B. cereus* DNA analysis using electrochemical detection on a chip is a promising alternative. The method detects the selected pathogenicity-encoding nucleic acid sequence of *B. cereus* when it simultaneously hybridizes with a single-stranded DNA capture molecule immobilized on a solid surface of magnetic microbeads and a DNA detection probe molecule from a solution labeled with an enzyme. A miniaturized amperometric biosensor device enables evaluation of biomolecular interactions by measuring the redox recycling of enzymatic reaction products [18]. When applied to analysis of bacterial colonies, the main sample preparation includes only suspension of the colony in a buffer, ultrasonication, and centrifugation [16].

We report here on the optimization of the ultrasonication with the purpose of fragmenting the DNA and thereby improving the hybridization rate. We also explore an early endpoint semiquantitative PCR as a simple and inexpensive method for evaluating the DNA fragmentation without requiring expensive equipment or sophisticated probe preparation.

## Materials and methods

### *Reagents*

ExtrAvidin alkaline phosphatase conjugates (Ext-ALP),[1] bovine serum albumin (BSA), 100 mg/ml carbodiimide (EDC), 0.1 M ethanolamine/deoxynucleotide mix (each dNTP 10 mM), and *Taq* DNA polymerase (5 U/μl) and PCR buffer were purchased from Sigma (Steinheim, Germany). *p*-Aminophenyl phosphate (pAPP) was purchased from ICN Biomedicals (Aurora, OH, USA). Paramagnetic beads (Dynabeads M-270 carboxylic acid) were obtained from Dynal (Oslo, Norway). 2-[*N*-morfolino]ethonesulfonic acid (MES, 0.4 M) was adjusted to pH 5.0. Tris-buffered saline (TBS) was prepared by dissolving 30 mM tris(hydroxymethyl)aminomethane and 100 mM sodium chloride in water and adjusting to pH 8.0 by adding hydrochloric acid. Phosphate-buffered saline (PBS, pH 7.4) contained 2 mM sodium dihydrogen phosphate monohydrate, 8 mM disodium hydrogen phosphate dihydrate, and 150 mM sodium chloride. Dulbecco's buffered saline (DBS, pH 7.3) was prepared by dissolving 160 mM sodium chloride, 3 mM potassium chloride, 8 mM disodium hydrogen phosphate dihydrate, and 1 mM potassium hydrogen phosphate dihydrate.

### *Oligonucleotides*

Purified oligodeoxynucleotides (with 5′ amino group or 3′ biotin modification) were purchased from Thermo Hybaid (Ulm, Germany). The designed primer pairs for PCR and probes for chip analyses are listed in Table 1. The oligonucleotide design is based on sequence complementarity to the selected toxin gene. The amino groups or biotin were linked to the probes with a spacer sequence of a few bases in length, each of which was selected noncomplementary to the target strand. HblC U-ABCDEF (upper primer) and HblC L-A, HblC L-B, HblC L-C, HblC L-D, HblC L-E, and HblC L-F (lower primer-A, -B, -C, -D, -E, and -F, respectively) were primer pairs designed from *hblC* sequence by computer analysis using the Oligo primer analysis software (MedProbe, Oslo, Norway). With the exception of the linker, the capture probe (C) was identical in sequence to the upper PCR primer HblC U-ABCDEF. The detection probe (D) was chosen to hybridize with only a 1-bp space directly next to the capturing probe [19]. In this way, the probe names HblC C and HblC D are abbreviated from *hblC* capture and *hblC* detection, respectively (Fig. 1).

[1] *Abbreviations used:* Ext-ALP, ExtrAvidin alkaline phosphatase; BSA, bovine serum albumin; EDC, carbodiimide; pAPP, *p*-aminophenyl phosphate; MES, 2-[*N*-morfolino]ethonesulfonic acid; TBS, Tris-buffered saline; PBS, phosphate-buffered saline; DBS, Dulbecco's buffered saline; pAP, *p*-aminophenol.