Label-free detection of multiple bacterial pathogens using light-scattering sensor

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ABSTRACT

Technologies for rapid detection and classification of bacterial pathogens are crucial for securing the food supply. This report describes a light-scattering sensor capable of real-time detection and identification of colonies of multiple pathogens without the need for a labeling reagent or biochemical processing. Bacterial colonies consisting of the progeny of a single parent cell scatter light at 635 nm to produce unique forward-scatter signatures. Zernike moment invariants and Haralick descriptors aid in feature extraction and construction of the scatter-signature image library. The method is able to distinguish bacterial cultures at the genus and species level for *Listeria, Staphylococcus, Salmonella, Vibrio,* and *Escherichia* with an accuracy of 90–99% for samples derived from food or experimentally infected animal. Varied amounts of exopolysaccharide produced by the bacteria causes changes in phase modulation distributions, resulting in strikingly different scatter signatures. With the aid of a robust database the method can potentially detect and identify any bacteria colony essentially instantaneously. Unlike other methods, it does not destroy the sample, but leaves it intact for other confirmatory testing, if needed, for forensic or outbreak investigations.

1. Introduction

Outbreaks of methicillin-resistant *Staphylococcus aureus* in communities (Klein et al., 2007), *Escherichia coli* O157:H7 in spinach and ground beef (Heaton and Jones, 2008; Jay et al., 2007), *Salmonella* in peanut butter (Gerner-Smidt and Whichard, 2007), *Listeria monocytogenes* in ready-to-eat meats (Swaminathan and Gerner-Smidt, 2007), and *Clostridium botulinum* in canned chili sauce (CDC, 2007) are examples of recent public-health threats. In addition, concerns about intentional administration of pathogens to food or agricultural commodities (Manning et al., 2005; Relman et al., 2006) call for improvement in diagnosis and detection (Ligler et al., 2003; Lim et al., 2005).

Currently employed detection methods based on antibodies or DNA involve multiple steps and are labor intensive, time consum-

ing, and often unable to detect low numbers of cells. Moreover, samples are terminally destroyed by the test and are thus unavailable for further confirmatory assessment. Though biosensor tools are able to improve sensitivity they must rely on the use of specific reporter molecules such as antibodies or nucleic acid probes coupled with fluorophores or enzymes, thus limiting their broad application for multipathogen detection. Additionally, direct detection of analytes from real-world samples and overall cost per analysis has not yet matched expectations (Bhunia, 2008).

The conventional culture-based detection method is highly reliable and is still considered to be the "gold standard" for microbiological analysis. For high-profile investigations such as those related to biosecurity (Kiratisin et al., 2002; Bhunia, 2006) or foodborne outbreaks (Jay et al., 2007), results obtained by rapid methods must always be confirmed by culture-based techniques (CDC, 2006; Lim et al., 2005). Therefore, sensors that incorporate a traditional culture-based approach as a part of the detection procedure are highly desirable. In culture methods, clinical or food specimens are diluted in buffer and distributed onto appropriate solid agar plates

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to allow individual bacterial cells to grow and form a colony. A colony consisting of progeny (millions to billions) of a single parent cell is considered pure and can be subsequently tested by metabolic or genetic fingerprinting, immunoassays, or polymerase chain reaction (PCR) assays. However, all the aforementioned techniques require extensive sample handling and 3–24 h before a definitive result can be obtained (Bhunia, 2008). These cumbersome multistep processes require specific reagents, such as a panel of carbohydrates or proteins as substrates for bacterial metabolism to produce metabolic fingerprint patterns, or nucleic acid restriction enzymes, primers, and labeled DNA probes for genetic fingerprinting or PCR. Owing to the nature of the detection modality, immunoassays require specific enzyme- or fluorescence-labeled antibodies. An ideal sensor devoid of these problems and capable of direct, nondestructive, and label-free identification would revolutionize routine microbiological analysis of food, agricultural, environmental, and clinical samples.

Light-scattering technology has been used before to interrogate bacterial cells in suspension (Wyatt, 1969; Wyatt and Phillips, 1972) and characteristic angular distribution was exploited to provide information on the metal toxicity on bacteria, size distribution, shape, and refractive index (Bronk et al., 2001; Perkins and Squirrell, 2000; Wyatt, 1969). The scope of this approach is very narrow and only a limited number of bacterial species could be detected successfully. In a recent work, we found that interrogation of individual bacterium on surface of agar in a semi-solid state could provide a possible differentiation (Nebeker et al., 2001) and this work was extended to analyzing bacterial colonies of different species which provided distinctive forward-scattering pattern although their visual morphology looked similar (Bae et al., 2007; Banada et al., 2007; Bayraktar et al., 2006). Here, we report the design and construction of a laser light-scattering analyzer that detects and identifies bacterial colonies growing on a Petri dish in seconds without destroying the colony. The system was tested for its ability to distinguish bacterial species and strains from five different genera; *Listeria, Staphylococcus, Salmonella, Vibrio,* and *Escherichia* in pure form and from inoculated food matrices and experimentally infected animal.

2. Materials and methods

2.1. Light-scattering instrumentation

Initial light-scattering instrument setup was on an optical bread board (Banada et al., 2007). In this study, we designed an automated BARDOT (Bacterial Rapid Detection using Optical scattering Technology) system and a local start up manufacturing company (En'Urga Inc., W. Lafayette, IN) worked together with our team to build two prototype units (Fig. 1). The system uses a laser (635 nm) to illuminate individual colonies and create a forward-scatter signature that is collected and subsequently analyzed. The working prototype of a fully automated system consists of a sample locator (laser line scanner), a forward-scatter measurement system (an interrogating source and a CCD sensor), and a sample control (*x*–*y* motorized stage). The additional system components are described in Supporting information.

2.2. Bacterial cultures and growth condition

Representative species and strains of *Listeria, Escherichia, Salmonella, Staphylococcus* and *Vibrio* used in this study and their culture conditions are listed in Tables S1–S6. Preparation of these bacterial cultures for light-scattering experiments was done following the protocol described before (Banada et al., 2007). Each
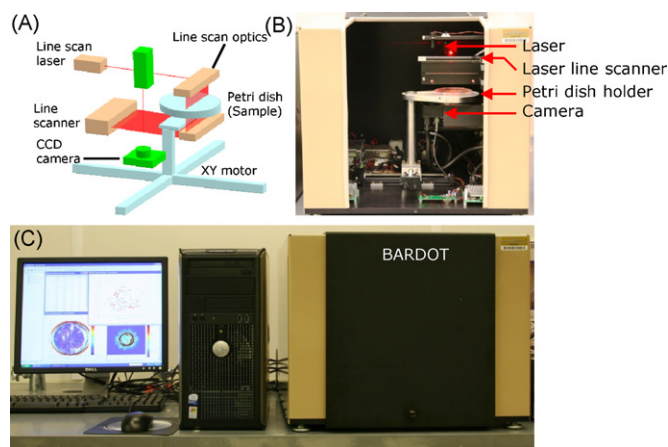

Fig. 1. (A) Components and schematics of the automatic light scatterometer, BARDOT (Bacterial Rapid Detection using Optical light-scattering Technology). The system integrates 3 major components: scatterometer, colony counter/locator, and motion control. (B) The photo shows a Petri dish on the BARDOT system (dimension: 24 in. × 20 in. × 17 in.; weight: 75 lbs) ready to capture the forward-scattering patterns. The laser line scanner acquires the transmission characteristics of the bacterial colonies (40 s per plate) and the colony center coordinates is computed via quadrant balancing of the scattering pattern. A laser diode (635 nm) is incident on top of the single bacterial colony and the forward-scattering signature is captured by a CCD image sensor (requires 5 s/colony). (C) The complete BARDOT setup.

bacterial culture, stored in −80 °C in BHI (brain-heart-infusion) broth-glycerol stock were sub-cultured twice in BHI (Difco, Sparks, MD) broth for 18–24 h at 30 or 37 °C depending on the culture and were surface plated onto BHI agar (1.5% w/w) plates. The plates were incubated at 30 or 37 °C until the colony size reached 1.3 ± 0.2 mm in diameter. BHI agar plates were prepared with 15 mL/plate of sterile tempered to 45 °C media (original volume before solidification).

2.3. Effect of bacteriological growth media on scattering patterns

Samples of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* were prepared as described above. In addition to surface plating on BHI agar, cultures were plated on Tryptic soy agar (TSA) and a selective differential media, xylose-lysine deoxycholate (XLD) for *Salmonella*, Modified Oxford media (MOX) for *Listeria* and Cefixime-Tellurite-sorbitol MacConkey agar (CT-SMAC) for *E. coli*. Plates were incubated until colonies reached appropriate size (1.3 ± 0.2 mm). Incubation times were identical for cultures on BHI or TSA. Longer times were necessary for cultures on selective media (16 h for *E. coli* on CT-SMAC, 16 h for *Salmonella* on XLD, 42 h for *Listeria* on MOX) to achieve colony diameters of 1.3 ± 0.2 mm.

2.4. Effect of pre-exposing bacteria to different stressors on scattering patterns

Cultures of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were exposed to several stress inducing conditions such as temperature (42 °C), pH 4.0 and osmotic stress (5% NaCl) for 3 h (Hahm and Bhunia, 2006). Stress-exposed cultures were plated on BHI agar plates and incubated at 30 or 37 °C and scatter images of at least 20 colonies were taken using BARDOT. Control cultures did not receive any stress (see Supporting information).

2.5. Food sample testing

Food samples (3–4 each) tested in this study included ready-to-eat hotdog and shredded beef, raw ground beef and chicken, frozen and fresh spinach, and fresh tomato. Several 25 g portions