We present a new platform for visual and spectroscopic detection of bacteria. The detection scheme is based on the interaction of membrane-active compounds secreted by bacteria with agar-embedded nanoparticles comprising phospholipids and the chromatic polymer polydiacetylene (PDA). We demonstrate that PDA undergoes dramatic visible blue-to-red transformations together with an intense fluorescence emission that are induced by molecules released by multiplying bacteria. The chromatic transitions are easily identified by the naked eye and can also be recorded by conventional high-throughput screening instruments. Furthermore, the color and fluorescence changes generally occur in shorter times than the visual appearance of bacterial colonies on the agar. The chromatic technology is generic and simple, does not require identification a priori of specific bacterial recognition elements, and can be applied for detection of both gram-negative and gram-positive bacteria. We demonstrate applications of the new platform for reporting on bacterial contaminations in foods and for screening for bacterial antibiotic resistance.

The emerging global risks of bioterrorism, the recurring incidents of bacterial food contaminations, and the need to monitor sterile environments in health care applications and other industries have pushed the development of methods for detection of pathogens to the forefront of technological and scientific research. Numerous technologies for reporting on bacterial presence have been developed (3, 5, 9, 25, 26). There are, however, limitations to existing bacterial detection techniques as rapid and generic approaches. Specifically, many bioanalytical techniques employed for pathogen detection (such as culture-based methods) provide results after relatively long time spans (several hours to days) (10). Other currently employed technologies often involve complex detection mechanisms that require specialized instrumentation, application by trained personnel, and the need for active operation (addition of reagents, initiation of chemical reactions, etc.), which overall do not lend their use in settings other than laboratory environments (3, 6). Furthermore, a prerequisite for many detection methods is the detailed understanding of the biochemical and structural properties of the bacterial species sought, limiting applications in the case of unknown pathogens or variants (9, 31).

Various membrane-active compounds are released by bacteria to their environments (2, 30), a process that often has an essential functional role as a means for overcoming host defense mechanisms, allowing colony proliferation, and facilitating bacterial communication (17). Membrane-active peptides and toxins, in particular, are produced by bacteria, for example, pneumolysins secreted by streptococci (27) and α-toxin, which is the major cytolysin emitted by Staphylococcus aureus (1). Secretion of pore-forming exotoxins by bacteria is abundant, and endotoxins, such as lipopolysaccharides, which are often released by gram-negative bacteria, strongly interact with membrane components of the host cell (21). Here, we describe a new bacterial detection platform generating dramatic visible color changes accompanied by intense fluorescence emission that are induced by membrane-active molecules secreted by proliferating bacteria.

MATERIALS AND METHODS

Materials and bacterial strains. Dimyristoylphosphatidylcholine (DMPC), Tris (Tris base buffer), kanamycin, and streptomycin were purchased from Sigma. The diacytelenic monomer 10,12-tricosaosydonic acid was purchased from GFS Chemicals (Powell, OH), dissolved in chloroform, and passed through a 0.45-μm nylon filter before use. Luria-Bertani (LB) agar was purchased from Pronadisa (Spain).

The bacteria used in the studies were Salmonella enterica serovar Typhimurium 1a (strain CS093 [22]), Bacillus cereus, Escherichia coli K-12 strains C600, C600 pMRInv, and MC4100 (provided by Ehud Gazit, Tel Aviv University), and E. coli BL (provided by Dudy Bar-Zvi, Ben Gurion University).

Bacterial growth. Salmonella enterica serovar Typhimurium 1a, E. coli BL, and E. coli K-12 C600 were grown to saturation at 37°C and streaked. E. coli K-12 C600 pMRInv was grown in LB medium supplemented with kanamycin (10 μg/ml). Bacterial concentrations were determined from absorption at 600 nm on a Jasco V-550 spectrophotometer.

Vesicle preparation. Vesicles containing DMPC and 10,12-tricosaosydonic acid (2.3 molar ratio) were prepared at a concentration of 1 mM. The lipids were dried together in vacuo. Following evaporation, distilled water was added and the suspension was probe sonicated at 70°C. The resultant vesicle solution was cooled at 4°C overnight and then polymerized by irradiation at 254 nm for 0.5 min.

Visible spectroscopy. Samples were prepared by adding 30 μl bacterial samples (at the different concentrations tested in the experiments) to 30 μl of 1 mM vesicle solutions, followed by the addition of 30 μl Tris base at 50 mM, pH 8, and dilution in water to 1 ml prior to spectral acquisition. All measurements were carried out at room temperature on a Jasco V-550 UV-VIS spectrophotometer.

Preparation of chromatic lipid-PDA agar matrix. Unpolymerized DMPC-polydiacetylene (PDA) vesicles at a concentration of 5 mM were added right after the sonication stage to hot LB agar. The mixture was then cooled to room temperature and poured into a six-well plate (Cellstar, Greiner Bio-one). After solidification of the agar, the plate was kept at 4°C for 2 days and

Rapid Chromatic Detection of Bacteria by Use of a New Biomimetic Polymer Sensor

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Polymerized by irradiation (254 nm, 40 s) in a UV cross-linker (UV-8000; Stratagene, California).

**Multiwell fluorescence spectroscopy.** On the surface of the chromatic DMPC-PDA agar (in each well), we spotted 10^7/H9262 diluted bacterial samples (10^7 particles), placed the plate in a multiwell fluorescence plate reader (Fluoroscan Ascent; Thermo, Finland), and kept it at 26°C or 29°C. All measurements were carried out using 485-nm excitation and 555-nm emission, using LP filters with normal slits. Acquisition of data was automatically performed every 30 min.

**Color scanning.** Scanned images were recorded on an Epson Perfection 4990 photo scanner.

All experiments were repeated at least three times.

**RESULTS**

**The chromatic PDA sensor.** The new bacterial detection approach utilizes biomimetic chromatic membranes that undergo colorimetric and fluorescence transformations through interactions with bacterially secreted substances. A schematic description of the sensing scheme is shown in Fig. 1: vesicular nanoparticles comprising phospholipids (forming a biomimetic membrane bilayer) and PDA (blue), (Right) Bacterial proliferation (green oval) on the agar surface causes blue-to-red transformation of embedded vesicles due to bacterially secreted compounds that diffuse through the agar.

PDA aggregates and films have previously been shown to undergo dramatic blue-to-red changes due to conformational transitions in the conjugated polymer backbone that are induced by external structural perturbations, such as binding of positively charged ions and molecules or amphiphilic molecules (4, 19). The colorimetric transformations of PDA go hand in hand with induction of fluorescence; no fluorescence is emitted by the initially polymerized blue-phase PDA, while the red-phase PDA strongly fluoresces at 560 nm and at 640 nm (29). The chromatic transitions of PDA also occur in biological contexts; recent studies have demonstrated that blue-to-red and fluorescence transformations could be induced in vesicle assemblies comprising phospholipid bilayers interspersed within the PDA matrices by membrane-active compounds (12, 13, 18). Other reports have demonstrated that PDA “nanopatches” attached to surfaces of living cells could report on local membrane events through the chromatic changes of the polymer (20). In all such systems, the mechanism for the chromatic transformations corresponds to surface perturbations and fluidity changes within the lipid domains, which induce the structural/chromatic transformations of the adjacent PDA matrix through the molecular interface between the two components (13).

The new bacterial sensor exploits the chromatic response induced upon interaction of lipid-PDA assemblies with membrane-active substances as the underlying mechanism for bacterial detection. Previous studies have employed chemically derivatized PDA for bacterial sensing; however, those systems relied mostly on the covalently displaying specific recognition units on the PDA surface, producing signals only through actual binding of bacterial proteins to the PDA matrix. Such assemblies require chemical modifications of the PDA units, and their detection levels are determined by the number of bacterial particles attaching to the PDA-displayed receptors (15, 23). A previous study employing PDA for bacterial sensing exhibited a detection limit of 10^8 bacterial particles (16). The concept we describe here is simpler and more generic and features higher sensitivity. Essentially, we show that bacterially released substances diffuse through the agar matrix and interact with the incorporated phospholipid-PDA nanoparticles, consequently inducing the colorimetric/fluorescence transitions that result from bacterial presence.

**Color detection of bacteria.** Fig. 2 depicts a representative experiment showing the color transitions induced by bacteria in DMPC-PDA agar. Specifically, Fig. 2B and C show scanned

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**FIG. 1.** Chromatic sensor concept. (Left) Agar scaffold containing vesicular nanoparticles composed of phospholipids (black) and PDA (blue). (Right) Bacterial proliferation (green oval) on the agar surface causes blue-to-red transformation of embedded vesicles due to bacterially secreted compounds that diffuse through the agar.

**FIG. 2.** Color transitions induced by bacterial colonies. Scanned images of DMPC-PDA agar plates prior to bacterial growth (A), 18 h after three colonies of *Bacillus cereus* were transferred (B), and 18 h after three colonies of *E. coli* BL were transferred (C). The plates were incubated at 26°C.
images of DMPC-PDA agar plates onto which we transferred colonies of the gram-positive Bacillus cereus and the gram-negative Escherichia coli BL strains, respectively, and incubated them at 26°C. The pictures in Fig. 2B and C clearly show that red hallows form around the bacterial colonies following incubation (note that the apparent “doublets” in Fig. 2B are due to the reflection of the scanner light). The blue-to-red transformation of the matrix was directly related to bacterial proliferation; each colony was surrounded by an area in which the blue agar matrix changed color to red, while the remaining blue agar matrix stayed blue (Fig. 2B and C). The dispersion of red regions under and around the bacterial colonies indicates that the color transitions were due to diffusion of substances released by the bacteria into the surrounding matrix.

To corroborate the relationship between the color transitions of the PDA assemblies and bacterially secreted substances, we investigated the reaction between blue DMPC-PDA vesicles in aqueous solutions and either the pellet or the supernatant extracted after growth of bacteria to saturation (Fig. 3A). In the experiment whose results are depicted in Fig. 3A, we grew Salmonella enterica serovar Typhimurium 1a to saturation (20 h at 37°C, number of bacteria was approximately 10^9), separated the supernatant solution from the pellet, mixed each extraction with the lipid-PDA vesicles (the pellet was resuspended and thoroughly washed in water prior to addition to the vesicles), and incubated the mixtures for 5 min prior to color recording. The pictures shown in Fig. 3A clearly demonstrate that the bacterial particles themselves did not induce a noticeable change (matrix remained blue), while the addition of the supernatant gave rise to an intense purple-red color.

We further examined the time dependence of the colorimetric transformation of the lipid-PDA vesicles during the bacterial growth curve (Fig. 3B). The data depicted in Fig. 3B show that several hours after the initiation of bacterial growth, the lipid-PDA vesicle solution appeared increasingly redder (i.e., ratio between 500 nm and 640 nm is higher; see Materials and Methods) as the number of bacteria in the suspension increased. Figure 3B also indicates that the relative intensity of
the 500-nm signal (i.e., extent of “redness” of the solution) increased significantly only after the bacteria reached the plateau region in the growth curve (i.e., reached saturation). The time lag between the increase in bacterial concentration and the occurrence of the blue-to-red colorimetric transition confirms that bacterially released substances, but not the bacterial particles themselves, are the primary factor responsible for the color transformations. Importantly, the contribution of the small pH change in the bacterium-vesicle solutions to the color change was found to be insignificant, indicating that the color transformations are indeed related to bacterially produced compounds rather than a pH effect.

**Bacterial detection through fluorescence.** In addition to color transformations, the fluorescence properties of PDA can be exploited as well for bacterial detection using the new assay. Figure 4 shows three-dimensional fluorescence “topographic maps” recorded in a conventional bacterial growth agar plates used as a control (A and B) and DMPC-PDA agar plates (C and D) are shown. The z axes depict the fluorescence intensity (arbitrary units), while the x and y planes correspond to the well surface area. (A and C) Initial measurements (time zero). (B and D) Measurements 6 h after *Salmonella enterica* serovar Typhimurium 1a colonies were streaked on the agar surface. Growth was carried out at 26°C.

**FIG. 4.** Fluorescence induced by bacterial colonies. Three-dimensional fluorescence intensity distributions, recorded using a multiwell fluorescence reader, in conventional bacterial growth agar plates used as a control (A and B) and DMPC-PDA agar plates (C and D) are shown. The z axes depict the fluorescence intensity (arbitrary units), while the x and y planes correspond to the well surface area. (A and C) Initial measurements (time zero). (B and D) Measurements 6 h after *Salmonella enterica* serovar Typhimurium 1a colonies were streaked on the agar surface. Growth was carried out at 26°C.

The formed red-phase PDA, appeared exactly where the bacterial colonies were placed on the agar surface. Significantly, the fluorescence signals could be detected in a few hours (initial signals appeared in less than 6 h) and several hours before actual bacterial colonies were visible to the eye on the agar surface. Kinetic profiles of the fluorescence emissions induced by bacterial suspensions at different concentrations are shown in the supplemental material.

**Practical applications.** Fig. 5 and 6 depict the results of experiments in which the new assay was used for evaluation of bacterial resistance to antibiotics (Fig. 5) and for detection of bacteria in foods (Fig. 6). In the experiment whose results are shown in Fig. 5, we streaked *E. coli* strains exhibiting different antibiotic resistances onto plates containing the lipid-PDA agar matrix that further incorporated antibiotic compounds and incubated the plates at 29°C. Figure 5B features fluorescence distribution maps of the plates recorded 8 hours after streaking that clearly reveal which of the bacteria were resistant to the antibiotic compound present in the agar matrix. For example, fluorescence emission appeared in a plate containing kanamycin-DMPC-PDA agar that further incorporated antibiotic compounds and incubated the plates at 29°C. Figure 5B features fluorescence distribution maps of the plates recorded 8 hours after streaking that clearly reveal which of the bacteria were resistant to the antibiotic compound present in the agar matrix. For example, fluorescence emission appeared in a plate containing kanamycin-DMPC-PDA agar that further incorporated antibiotic compounds and incubated the plates at 29°C. Figure 5B features fluorescence distribution maps of the plates recorded 8 hours after streaking that clearly reveal which of the bacteria were resistant to the antibiotic compound present in the agar matrix. For example, fluorescence emission appeared in a plate containing kanamycin-DMPC-PDA agar that further incorporated antibiotic compounds and incubated the plates at 29°C. Figure 5B features fluorescence distribution maps of the plates recorded 8 hours after streaking that clearly reveal which of the bacteria were resistant to the antibiotic compound present in the agar matrix. 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induction was recorded only in a streptomycin-DMPC-PDA agar plate onto which the streptomycin-resistant E. coli K-12 MC4100 strain was streaked (Fig. 5B, bottom left). Importantly, the fluorescence transitions could be detected earlier than the bacterial colonies, highlighting the usefulness of the new assembly as a bacterial screening tool. The striking color transitions induced by proliferation of the antibiotic-resistant strains (Fig. 5C), which appear exactly in the regions where fluorescence was recorded, further emphasize the practicality of the new assay for visual screening of bacterial antibiotic resistance.

Figure 6 demonstrates the potential of the new colorimetric assay as a tool for visualizing bacterial contamination in foods. In the experiment whose results are depicted in Fig. 6, we placed a meat sample (chicken liver purchased at a store) for 24 h under ambient conditions at room temperature, thus facilitating bacterial proliferation on the exposed meat sample. Following this treatment, we contacted the meat sample for 2 to 3 seconds with the surface of the blue DMPC-PDA agar matrix and incubated the plate overnight at 29°C. As depicted in Fig. 6B, after incubation the blue matrix was completely transformed to red. To confirm that the blue-to-red change was indeed induced by bacteria that were present on the surface of the meat sample and consequently grew on the chromatic agar plate, we carried out a control experiment in which part of the same liver specimen was boiled for a few minutes, then placed in contact with the chromatic agar surface, and incubated overnight (Fig. 6C). As apparent in the scanned image in Fig. 6C, the boiled liver sample did not give rise to any color change within the lipid-PDA agar matrix after incubation, most likely due to sterilization of the meat sample through boiling.

**DISCUSSION**

This work presents a new generic bacterial detection scheme based on colorimetric and fluorescence transformations induced within lipid-polymer assemblies by bacterially released substances. The technology does not require a priori knowledge of the composition or exact identities of the bacterially secreted compounds. Indeed, because the chromatic response of the agar-embedded vesicles is due to the general affinity of the lipid-PDA assemblies to membrane-active, lipophilic, or positively charged compounds released by bacteria, the new platform should be able to report, in principle, on the presence of any bacterial species. An additional important facet of the new bacterial sensor is the amplification capabilities inherent in the agar scaffolding, which facilitates bacterial growth and thereby lowers the detection threshold. The semipermeable agar additionally functions as an effective filter, reducing background signals by preventing nonbacterially secreted substances, such as ions and components of the growth media, from interacting with the embedded vesicles.

The sensitivity of the new assay is essentially determined by the number of bacteria and the time of detection; even a single bacterium can be detected following its proliferation on the agar and reaction with PDA. This feature distinguishes this sensor from existing bacterial sensing approaches and PDA-based assays that rely mostly on specific recognition elements displayed by the bacteria.

The reported experiments demonstrate that bacterially secreted molecules accumulate and diffuse within the porous agar scaffolding, subsequently inducing the color and fluorescence transformations of lipid-PDA. The chromatic sensing capabilities of lipid-PDA assemblies have previously been demonstrated in various applications; in particular, the vesicles were previously shown to constitute a useful platform for detection of membrane-active and amphiphilic molecules (28). A recent study demonstrated the induction of colorimetric transitions in lipid-PDA vesicles by a pore-forming bacterial toxin (14). In the context of this work, the lipid-PDA nanoparticles constitute the reporter element within the sensing assembly, emitting signals in response to bacterial presence.

Utilization of either visual color changes observed by the naked eye or fluorescence emission as a viable detection method is an important advantage of the new bacterial sensing system. While the pronounced color changes should facilitate bacterial detection by nonexperts in diverse settings, the fluorescence properties of the chromatic matrix could additionally open the way for employing the platform for high-throughput screening applications requiring high sensitivity, large sample quantities, or automation. Indeed, the fluorescence images in Fig. 3 and 4 underlie the enhanced sensitivity of the platform to bacterial proliferation and the possibility of detecting bacteria in shorter times than with conventional microbiology methods.

Microbe selection was designed to demonstrate the general applicability of the assay, and the choice of bacterium was also related to the type of experiment presented. Specifically, the experiments aimed to show the fundamental features of the technique (Fig. 2 to 4) were carried out with widely used conventional bacterial species: E. coli (gram negative), B. cereus (gram positive), and S. enterica serovar Typhimurium (representing common pathogenic bacteria). In the experi-
ments depicting specific applications of the technique for antibiotic screening (Fig. 5), we have used _E. coli_ genotypes containing the desired antibiotic resistance.

The chromatic platform could be employed for diverse practical applications. Utilizing the assay for screening for antibiotic resistance of bacteria is feasible through incorporation of antibiotic compounds within the chromatic matrix. Figure 5, for example, demonstrates that evaluation of bacterial sensitivity to antibiotic substances can be carried out in such matrices by using a conventional fluorescence multwell reader. The experiment whose results are depicted in Fig. 5 also points to applications of the chromatic platform for rapid screening of putative antibacterial properties of compound libraries. The color/fluorescence sensitivity of the chromatic assembly and the amenability to conventional high-throughput screening formats are attractive features of the new system for diagnostic and industrial utilization. The facile blue-to-red changes following bacterial proliferation could be useful in other applications, such as monitoring food freshness. Figure 6, for example, demonstrates that visible color transformations provide a direct alert on the presence of bacteria in a contaminated food specimen.

The new bacterial detection assay is not capable, at this stage, of differentiating among bacteria. However, the generality of the detection concept and the nonspecificity of the chromatic platform can, in fact, be advantages in applications in which reporting on the presence of any type of bacteria is required, for example, monitoring sterile environments, evaluating food freshness, screening for bacterial resistance to existing and new antibiotic compounds, and others. Research currently being carried out in our laboratory is exploring avenues for facilitating specificity of detection among bacterial species by varying the lipid composition within the chromatic platform.

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