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Plasmonic-based colorimetric and spectroscopic discrimination of acetic and butyric acids produced by different types of *Escherichia coli* through the different assembly structures formation of gold nanoparticles

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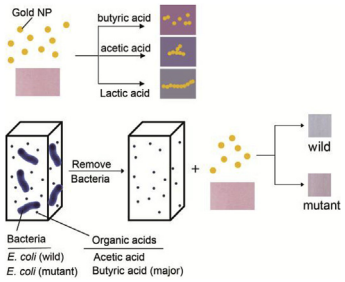
HIGHLIGHTS

- A plasmonic colorimetric differentiation of some organic acids produced by bacteria is presented.
- Different assembly structures and optical properties of Au NPs are formed for different organic acids.
- Acetic and butyric acids produced by different types of *Escherichia coli* are discriminated.
- Number of wild-type *E. coli* can be estimated from the colorimetric method.

GRAPHICAL ABSTRACT



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ABSTRACT

We present a plasmonic-based strategy for the colourimetric and spectroscopic differentiation of various organic acids produced by bacteria. The strategy is based on our discovery that particular concentrations of DL-lactic, acetic, and butyric acids induce different assembly structures, colours, and optical spectra of gold nanoparticles. We selected wild-type (K-12 W3110) and genetically-engineered (JHL61) *Escherichia coli* (*E. coli*) that are known to primarily produce acetic and butyric acid, respectively. Different assembly structures and optical properties of gold nanoparticles were observed when different organic acids, obtained after the removal of acid-producing bacteria, were mixed with gold nanoparticles. Moreover, at moderate cell concentrations of K-12 W3110 *E. coli*, which produce sufficient amounts of acetic acid to induce the assembly of gold nanoparticles, a direct estimate of the number of bacteria was possible based on time-course colour change observations of gold nanoparticle aqueous suspensions. The plasmonic-based colourimetric and spectroscopic methods described here may enable onsite testing for the identification of organic acids produced by bacteria and the estimation of bacterial numbers, which have applications in health and environmental sciences.

1. Introduction

Microbes and their chemical products greatly influence various aspects of human life and ecosystems [1–6]. For example, the presence of pathogenic bacteria and toxins in our bodies, food, and

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other substances could have harmful consequences to human health [1–3]. In addition, bacteria and the chemicals they produce in the soil and water could also influence ecosystems and animals [4–6]. However, some chemicals (e.g., lactic and butyric acid) produced by bacteria are beneficial to the health of human colons [7,8]. Accordingly, the detection of microbes and their products is becoming increasingly important. Numerous methods have been suggested to detect microbes or chemicals; they are based on various principles, such as polymerase chain reaction and immunoassays [9], bioluminescence/fluorescence [10,11], chromatography [12], infrared spectroscopy [13], mass spectroscopy [14], flow cytometry [15] electrophoresis [16], surface plasmon resonance [17] and electrochemistry [18,19]. Recently, these methods have been combined with the state-of-the-art nanodevices and microarrays [20,21].

Currently, nanoparticles are used as probes to detect bacteria by taking advantage of their physiochemical/optical characteristics [22–41]. Magnetic nanoparticles have been used to separate bacteria from an aqueous suspension [22–25] and to sense bacteria based on their relaxation behaviors [26–28]. Nanoparticles that exhibit various optical characteristics have also been used to detect bacteria. These optical nanoparticles include fluorescently-labelled nanoparticles [29–32], quantum dots [33–36] and gold nanoparticles [37–41]. Detection has relied on the decrease in optical signals of nanoparticles after binding/uptake onto/by the bacteria in specific or nonspecific ways. Although these nanoparticle-based methods have simplified the detection process and, in some instances, have lowered detection limits, the methods still require a careful spectroscopic analysis or the use of other instruments for successful detection.

In addition to the careful and accurate analysis of bacteria and their products, convenient methods for the prediction/discrimination of bacteria and their chemicals are sometimes necessary. Continual efforts have been made to identify bacteria based on changes in the colours of nanoparticle suspensions [42–47]. Polydiacetylene vesicles bearing antibodies have been designed to trigger colour changes via alterations of their molecular conformations in the presence of bacteria [42,43]. Gold nanoparticles themselves also display a colour change upon the formation of bacterial-gold nanoparticle aggregates through a specific interaction [44,45]. As a nonspecific method, the catalytic role of gold core-platinum shell nanoparticles attached to bacteria was used to induce colour changes of indicators after chemical reactions [46]. In another study, a fluorescent polymer that was quenched by gold nanoparticles exhibited a fluorescent colour after its release from the nanoparticles due to the nonspecific nanoparticle-bacteria interaction [47]. However, nanoparticle-based colourimetric and spectroscopic bacterial sensing systems have yet to be deeply explored for the identification and discrimination of chemicals produced by bacteria, and thus for the identification of bacteria.

Here, we suggest a novel plasmonic-based colourimetric and spectroscopic approach for the detection and discrimination of organic acids produced by bacteria. The strategy is based on our experimental results that gold nanoparticles displays different assembly structures, colours, and optical spectra when mixing respectively with DL-lactic, acetic, and butyric acids at particular concentrations. As a model bacteria, we select wild-type (K-12 W3110) and genetically-engineered (JHL61) *Escherichia coli* (*E. Coli*) that can primarily produce acetic and butyric acids as a major product, respectively (see experimental data in Table S1, Supplementary Data) [48,49]. When gold nanoparticles are mixed with acetic and butyric acids produced from the K-12 W3110 and JHL61 *E. Coli*, respectively, different assembly structures and optical signals of gold nanoparticles are also observed. Moreover, we also demonstrate a direct estimate of the number of bacteria is possible based on time-course colour change observations of gold nanoparticle aqueous suspensions mixed with K-12 W3110 *E. Coli*. Applicability and limitation of the present method are discussed.

2. Experimental

2.1. Materials

$HAuCl_4$ (99.9%), sodium citrate (99%), glycerol (≥99%), spectinomycin (≥98.0%), and H_3PO_4 (99.99%) were purchased from Aldrich (Yongin, Korea). DL-lactic (90%), Butyric (≥99%), and Acetic acid (99%) were purchased from Fluka, Aldrich, and Duksan Chemicals (Korea), respectively. Luria-Bertani (LB) broth and agar powder (extra pure) were purchased from BD Difco and Junsei Chemical Co., respectively. The preparation and composition of LB culture medium was described in the Supplementary Data.

2.2. Optical responses of gold nanoparticle aqueous suspension to various organic acids

We used gold nanoparticles with 15 ± 3 nm in diameter (Fig. S1; the synthetic method was also described in the Supplementary Data). To investigate the effects of organic acids on the colour changes of gold nanoparticles, 0.9 mL of deionized water containing gold nanoparticles was mixed with 0.1 mL organic acid aqueous solution. The organic acids we tested were reagent-grade DL-lactic acid, acetic acid, and butyric acid. After mixing, the concentration of gold nanoparticles was 0.4 nM. The concentration of nanoparticles was quantified using inductively-coupled mass spectroscopy (Perkin Elmer, USA). Colours and optical spectra of gold nanoparticles-organic acid aqueous mixtures were recorded by photography and UV–vis spectroscopy (Cary 50, Agilent, USA) for a certain period of time. The resulting assembly structures of gold nanoparticles were observed by TEM (JEM 2010, JEOL, Japan).

2.3. Colourimetric and spectroscopic detection of acetic and butyric acids produced from bacteria in gold nanoparticle suspension

All the experiments were conducted at room temperature (25–30 °C) unless otherwise noted. A protocol for the wild-type (K-12 W3110) and genetically-engineered (JHL61) *E. coli* culture was described in the Supplementary Data. JHL61 *E. coli* was kindly received from Prof. Gyoo Yeol Jung at Pohang University of Science and Technology, South Korea [49]. To investigate the optical changes of gold nanoparticles for the organic acids produced from the K-12 W3110 and JHL61 *E. coli*, we conducted the following experiments. First, 0.1 mL culture medium containing the bacteria was added to 0.9 mL deionized water. The mixture of deionized water and culture medium used for K-12 W3110 contained 17 mM of sodium chloride, 0.05 wt% yeast extract, and 0.1 wt% trypton. The mixture used for JHL61contained 5.5 mM glycerol, and 5 μg/L spectionmycin in addition to same concentration of sodium chloride, yeast extract, and trypton. The bacterial concentration in the mixture was 6.67×10^6 cell/mL for the K-12 W3110 and varied from 6.67×10^6 to 2.48×10^7 for the JHL61. After a certain bacterial storage time (4 h for the wild type and 8 h for the mutant) in the mixture, the mixture of culture medium and deionized water was separated from the bacteria by centrifugation. Analysis of chemicals produced from bacteria was conducted with gas chromatography (GC), which is well-described in the Supplementary Data (see also Table S1 for the results). And then, 0.95 mL of deionized water-medium mixture was mixed with 50 μL gold nanoparticle aqueous suspensions. After mixing, the concentration of gold nanoparticles was 0.4 nM, and the deionized water-medium mixtures were diluted by 5% (v/v). Colours and optical spectra of