Research Article

Chemotaxis Away from 4-Chloro-2-nitrophenol, 4-Nitrophenol, and 2,6-Dichloro-4-nitrophenol by *Bacillus subtilis* PA-2

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Bacterial strain PA-2 exhibits chemotaxis away from 4-chloro-2-nitrophenol, 4-nitrophenol, and 2,6-dichloro-4-nitrophenol. This strain was identified as *Bacillus subtilis* on the basis of the 16S rRNA gene sequencing. The drop plate assay and the chemical-in-plug method were used to demonstrate negative chemotactic behavior of strain PA-2. The growth studies showed that strain PA-2 did not utilize 4-chloro-2-nitrophenol, 4-nitrophenol, and 2,6-dichloro-4-nitrophenol as its sole sources of carbon and energy. This is the first report of negative chemotaxis of 4-chloro-2-nitrophenol, 4-nitrophenol, and 2,6-dichloro-4-nitrophenol by any bacterium.

1. Introduction

Chemotaxis is the movement of bacterial cells toward or away from chemicals [1]. If bacterial cells move toward a chemical compound, the process is known as positive chemotaxis, while movement away from a chemical compound is referred to as negative chemotaxis [1]. Nitrophenols (NPs) and chloronitrophenols (CNPs) comprise a group of toxic chemicals that are used to manufacture drugs, dyes, pesticides, and other useful products [2–4]. These compounds have been released into environment by various human activities, where they contaminate soil and water. Several bacteria have been isolated and applied to the degradation of NPs and CNPs, some of which are motile and show positive chemotaxis toward these compounds [2, 3]. *Burkholderia* sp. SJ98, which utilizes 4-nitrophenol, 2-chloro-4-nitrophenol, and 3-methyl-4-nitrophenol as the sole source of carbon and energy, showed positive chemotaxis toward these compounds [5–7]. The phenomenon of chemotaxis has also been shown to increase the capacity for bioremediation by the bacteria because they can move toward the chemicals and then degrade them [3].

NPs and CNPs are toxic not only to humans and animals but also to bacteria. Some motile bacteria that are unable to degrade toxic chemicals move away from them on exposure.

Earlier reports showed negative chemotaxis by a variety of bacteria to a number of chemical compounds [8–12]. Young and Mitchell [8] reported negative chemotaxis of marine bacteria to hydrocarbon and heavy metals. Tso and Adler [9] demonstrated negative chemotaxis of *Escherichia coli* to various chemical compounds, including indole, 3-methyl indole, and indole-3-acetic acid. Ohga et al. [10] showed negative chemotaxis of *Pseudomonas aeruginosa* to thiocyanic and isothiocyanic esters. Benov and Fridovich [11] demonstrated that *Escherichia coli* exhibited negative chemotaxis to various concentrations of hydrogen peroxide, hypochlorite, and N-chlorotaurine, which were the products of the respiratory bursts of phagocytic cells. Gliding bacteria have also been shown to exhibit negative chemotaxis. For example, Liu and Fridovich [12] demonstrated negative chemotaxis in a motile gliding bacterium, *Cytophaga johnsonae*. In this communication, we report negative chemotaxis of the cells of *Bacillus* sp. strain PA-2 to 4NP, 4C2NP, and DCNP.

2. Material and Methods

2.1. Chemicals. 4-Chloro-2-nitrophenol (4C2NP), 4-nitrophenol (4NP), and 2,6-dichloro-4-nitrophenol (DCNP) were purchased from Sigma-Aldrich (St. Louis, USA). All

FIGURE 1: Drop plate assay demonstrating negative chemotaxis of (a) 4-chloro-2-nitrophenol, (b) 4-nitrophenol, and (c) 2,6-dichloro-4-nitrophenol by *Bacillus* sp. strain PA-2. In the control (d), there was no chemotaxis away from 4-chloro-2-nitrophenol by the heat-killed cells of strain PA-2.

other chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Bacterial Strain. The bacterial strain PA-2 used in this study was isolated from soil collected from Yeungnam University. This strain was identified based on the 16S rRNA gene sequence using universal primers as previously described [13, 14].

2.3. Growth Studies. Strain PA-2 was grown on minimal medium containing 0.5 mM 4C2NP or 0.5 mM 4NP or 0.5 mM DCNP as the sole source of the carbon and energy. Strain PA-2 was also grown in trypticase soy broth in the presence or absence of 100 mM 4C2NP or 100 mM 4NP or 100 mM DCNP. The samples were collected at regular intervals and growth was measured by spectrophotometer at 600 nm.

2.4. Chemotaxis Away from 4-Chloro-2-nitrophenol. The negative chemotactic response of strain PA-2 to 4-chloro-2-nitrophenol was investigated by the drop plate assay [7] and the chemical-in-plug method [9].

For the drop plate assay, cells of strain PA-2 were grown on 250 mL trypticase soy broth and harvested at mid-log phase by centrifugation at 8,000 rpm for 15 min. Harvested cells were then washed twice with phosphate buffered saline, after which the bacterial solution was resuspended in minimal medium containing 0.3% bacto agar and poured into petriplates. A crystal of 4-chloro-2-nitrophenol was placed in the center of the petriplate, and the samples were then incubated at 30°C. The chemotactic response was observed after 6 h of incubation. Negative chemotaxis was indicated by the formation of a clearing zone around the crystal due to movement of the cells away from the 4C2NP crystal. In control, the heat-killed cells of strain PA-2 were used.

In the chemical-in-plug method, bacterial solution was prepared as described for the drop plate assay. The bacterial

FIGURE 2: Chemical-in-plug method showing negative chemotaxis of (a) 4-chloro-2-nitrophenol, (b) 4-nitrophenol, and (c) 2,6-dichloro-4-nitrophenol by *Bacillus* sp. strain PA-2.

solution was poured around hard agar plugs composed of minimal media, 2% bacto agar, and 4C2NP (100 mM) or 4NP (100 mM) or DCNP (100 mM). After solidifying, the plates were incubated at 30°C for 6 h, at which time they were evaluated for chemotactic response.

3. Results and Discussion

Strain PA-2 was identified as *Bacillus subtilis* PA-2 on the basis of 16S rRNA gene sequencing.

Strain PA-2 showed the highest sequence similarity (99.80%) with *Bacillus subtilis* subsp. *subtilis* NCIB 3610^T. The nucleotide sequence of the 16S rRNA sequence of strain PA-2 (1475 nt.) was deposited to the Genbank under accession number KP408227.

Drop plate assay showed that the cells of strain PA-2 moved away from 4C2NP crystal, forming a clear zone around the crystal (Figure 1(a)). The clear zone was also observed in the case of 4NP and DCNP (Figures 1(b) and 1(c)). However, a control in which the heat-killed cells of strain PA-2 were used showed no clear zone (Figure 1(d)). This is the first report of negative chemotaxis to 4C2NP, 4NP, and DCNP.

The chemical-in-plug method was also used to demonstrate negative chemotactic response in strain PA-2 to 4C2NP. The cells of strain PA-2 formed a clear zone around hard agar plugs containing 4C2NP (Figure 2(a)). Strain PA-2 also showed negative chemotactic response toward 4NP and DCNP (Figures 2(b) and 2(c)).

This is the first report of negative chemotaxis to 4NP, 4C2NP, and DCNP by a bacterium. Several studies have shown chemotaxis away from various chemicals including hydrocarbon [8], heavy metals [8], indole [9], 3-methyl indole [9], indole-3-acetic acid [9], thiocyanic acid [10], isothiocyanic esters [10], hydrogen peroxide [11], hypochlorite [11], and N-chlorotaurine [11]. However, there have been no reports of negative chemotaxis to NPs and CNPs. A few bacteria have shown positive chemotaxis toward NPs and CNPs [5–7, 15]. *Burkholderia* sp. SJ98 exhibited positive chemotaxis toward various compounds including NPs and

FIGURE 3: Growth of *Bacillus subtilis* strain PA-2 in (a) tryptone soy broth and (b) tryptone soy broth containing 100 mM 4C2NP or 100 mM 4NP or 100 mM DCNP.

CNPs. Another bacterium, *Pseudomonas* sp. JHN, showed positive chemotaxis toward 4-chloro-4-nitrophenol [15].

Negative chemotaxis has been demonstrated in micro-organisms using various methods [8–12], including the chemical-in-plug method, chemical-in-pond method, chemical-in-plate method, test tube method, and high throughput microwell method. Among these, the chemical-in-plug method has become widely accepted for demonstration of negative chemotaxis [9, 10]. Therefore, in this study, we used the chemical-in-plug method to demonstrate negative chemotaxis in strain PA-2. However, Li et al. [16] concluded that the chemical-in-plug method alone is not sufficient to demonstrate negative chemotaxis in bacteria because this method may give the false positive results; accordingly, any results obtained by the chemical-in-plug method should be confirmed with another type of assay [16]. Therefore, in this study, we also used a drop plate assay to demonstrate the negative chemotaxis response of strain PA-2. The drop plate assay has previously been used to demonstrate positive chemotaxis [5–7], and the results of our study clearly showed that the drop plate method is also suitable for negative chemotaxis. Overall, the results of this study showed that the drop plate assay is very sensitive and accurate and easy to apply for identification of negative chemotaxis.

The growth of strain PA-2 was monitored in the broth culture in presence of 4C2NP, 4NP, or DCNP. Strain PA-2 was unable to grow in the minimal medium containing 0.5 mM 4C2NP or 4NP or DCNP as the sole source of the carbon and energy. These data suggest that strain PA-2 did not utilize these compounds as the sole source of carbon and energy. Strain PA-2 was grown very well in the tryptone soya broth in the absence of 4C2NP or 4NP or DCNP (Figure 3). However, in the presence of 4C2NP (100 mM) or 4NP (100 mM) or DCNP (100 mM), the cells of strain PA-2 were unable to grow in the tryptone soya broth. These data suggest that 4C2NP, 4NP, and DCNP are very toxic to the cells of strain PA-2;

therefore, the cells of strain PA-2 showed chemotaxis away from these compounds.

Conflict of Interests

The authors declare that they have no conflict of interests.

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