HIGH-THROUGHPUT CHEMOTAXIS ASSAY OF PLANT-PARASITIC NEMATODE TOWARD GREEN AGRICULTURE
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ABSTRACT
This paper presents a new microsystem for simple and high-throughput chemotaxis assay of plant-parasitic nematodes Meloidogyne incognita. To improve an efficiency of chemotaxis assay, we developed a PDMS (Polydimethylsiloxane) microsystem, which can generate various chemical conditions in multiple microchannels at once. By using this system, we successfully identified a lower limitation of the concentration gradient of KNO₃ solution, which can act as a repellent for the nematode M. incognita.

KEYWORDS: Plant-parasitic Nematode, Chemotaxis, High-throughput Assay, Polydimethylsiloxane

INTRODUCTION
The plant-parasitic nematodes cause serious damage to agricultural products in the world [1]. Nevertheless, using environmentally harmful pesticides is only way to exterminate the nematodes so far. To realize an environmentally friendly extermination method, a behavior control of the nematodes with using harmless chemicals will be one of the efficient ways. However, in the conventional chemotaxis assays on agar plate [2], there are several technical issues such as low-efficiency of assay, difficulty to keep and define chemical conditions with high spatial resolution on agar-plate. To overcome these technical issues, we previously developed a method for on-chip chemotaxis assay of the nematodes and identified KNO₃ solutions as effective chemicals to Meloidogyne incognita, one of the notorious plant-parasitic nematodes [3]. This time, to improve efficiency and quantitative accuracy of the chemotaxis assay, we newly developed a PDMS microsystem, which can generate various chemical conditions at once.

EXPERIMENTAL
Newly developed PDMS microsystem for high-throughput chemotaxis assay consists of a main diffusion channel, four branched micro-slits connected with narrow microchannel-arrays, and nematode- and chemical-inlet (figure 1(a)). The narrow microchannel arrays can act not only to confine the nematodes within the micro-slits and but also to create concentration gradients by diffusion without an external pow-

Figure 1: PDMS microsystem for high-throughput chemotaxis assay of plant-parasitic nematode. (a) Microscopic view of the developed microsystem. Microchannel height: 30 µm, Micro-slit: 500 µm in width and 3000 µm in length. (b) Illustration of chemotaxis assay on PDMS microsystem.
er source. On this microsystem, four different chemical conditions can be generated at once by injecting the chemical solution, therefore we can efficiently and quantitatively analyze chemotaxis of the nematodes by observing behavior of the nematodes in each micro-slits as shown in figure 1(b).

To evaluate chemical concentration-distribution on the microsystem, we used a fluorescent substance, fluorescein (Wako Pure Chemical Industries, Ltd., Japan). Firstly, the microchannels were filled with 1 wt% ultra low-melting agarose gel (A2576 Agarose Type IX-A, SIGMA-ALDRICH) by power-free pumping method [4] and 15 μl of 1.5 mM fluorescein solution was injected into the chemical inlet. Then, we measured the time-dependent fluorescent intensity in each micro-slit.

Experimental protocols for chemotaxis assay on this microsystem were as below. First, the agarose gel containing the nematodes were loaded to the microsystem by using a protocol for allowing the nematodes to swim in microchannel [3]. After one hour, a candidate-chemical solution were applied by replacing agarose gel in one of the chemical inlets. Finally, the attractant/repellent behaviors were defined by counting time-dependent population of the nematodes in each micro-slits at room temperature. This time, for demonstrating high-throughput chemotaxis assay, we applied 15 μl of KNO₃ solutions with various concentrations to the chemical inlet.

RESULTS AND DISCUSSION

Figure 2 shows the time-dependent concentration distribution of fluorescein in micro-slits. We observed that the concentration gradients in micro-slit were sequentially formed from upstream side (slit #1) to downstream side (slit #4), and reached steady-state condition in every micro-slit within five hours (figure 2(a) and (b)). In addition, the ratio of concentration gradients among the micro-slits was kept under the steady-state condition as show in table 1. From these results, we confirmed that this method can be applied to high-throughput chemotaxis assay of the nematodes with forming various concentration-gradient at once.

In chemotaxis assay on this microsystem with applying 189 mM KNO₃ solution, we observed that the nematode *M. incognita* exhibited the repellent behavior in all the micro-slits from upstream side to downstream side serially, within 30 minutes (figure 3(a)). Compared with fluorescein (332.31 g mol⁻¹),
potassium (39.9 g mol$^{-1}$) and nitrate ion (62 g mol$^{-1}$) can diffuse more rapidly because the diffusion coefficient increases by decreasing molecular mass, thus the concentration gradient in every micro-slit may be created at least within one hour. In contrast, when applying lower concentration KNO$_3$ solution (48 and 95 mM), the repellent behavior of nematodes was not observed in downstream micro-slit #3 and/or 4 even after one hour (figure 3 (b)). These results are discussed as below. The value of concentration distribution in microchannel is proportional to the initial concentration as shown in figure 4. Therefore, when applying lower initial concentration of KNO$_3$ solutions, concentration gradients in downstream micro-slits might be less than an effective value which can act as repellent to the nematode *M. incognita*. These results suggest that we might efficiently estimate a quantitative value of the concentration gradient which can act as attractant/repellent to the nematodes with other candidate chemicals by using this analysis method.

**CONCLUSION**

This paper reported a high-throughput chemotaxis of the plant-parasitic nematode *M. incognita* on developed microsystem. We experimentally identified the lower limitation of the concentration gradient of KNO$_3$ solution, which can act as a repellent for the nematode *M. incognita*. As a result, we confirmed that the chemotaxis properties of the nematodes can be quantitatively and efficiently identified by using our developed microsystem and this system might be able to contribute to improve the global agriculture in the future.

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**REFERENCES**


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278