# Space Partitioning and Maze Solving by Bacteria

Ayyappasamy Sudalaiyadum Perumal[1], Monalisha Nayak[1], Viola Tokárová[1,2], Ondřej Kašpar[1,2], and Dan V. Nicolau[1(✉)]

[1] Department of Bioengineering, Faculty of Engineering, McGill University, Montreal, QC H3A 0C3, Canada
dan.nicolau@mcgill.ca
[2] University of Chemistry and Technology, Prague, Prague 166 28, Czech Republic

**Abstract.** Many bacteria dwell in micro-habitats, e.g., animal or plant tissues, waste matter, and soil. Consequently, bacterial space searching and partitioning is critical to their survival. However, the vast majority of studies regarding the motility of bacteria have been performed in open environments. To fill this gap in knowledge, we studied the behaviour of *E. coli K12-wt* in microfluidic channels with sub-10 μm dimensions, which present two types of geometries, namely a diamond-like network and a maze. The velocity, average time spent, and distance required to exit the networks, have been calculated to assess the intelligent-like behaviour of bacteria.

**Keywords:** Bacterial motility · Microfluidics · Maze

## 1 Introduction

Solving mazes is a nontrivial exercise that has been used for testing the intelligent-like behaviour of many organisms, e.g., ants [1], bees [2], rats [3], octopi [4, 5] and humans [6], as well as robots [7, 8] and rat-cyborgs [7]. Interestingly, even simple organisms, such as slime mold and fungi [9–11] possess complex and efficient biological algorithms employed in space searching and partitioning. However, despite their ubiquity, and despite studies regarding the motility characteristics of *E. coli* and its variants in straight microfluidics channels and directional preferences [12], or in simple geometries [13, 14], bacteria have not been the subject of an assessment of their intelligent-like behaviour via the classical exploration of mazes. Furthermore, earlier studies use chemotactic/attractant-based solution seeking studies in a maze [8–10], but chemotaxis is inferring with bacterial innate space searching capabilities, which are essential in nutrient poor environments. To fill this gap in knowledge, we studied the maze solving abilities of a common lab host, *Escherichia. coli K12*-wt, using simple microfluidics networks depleted of chemotactic clues.


A. Compagnoni et al. (Eds.): BICT 2019, LNICST 289, pp. 175–180, 2019.
https://doi.org/10.1007/978-3-030-24202-2_13

## 2 Materials and Methods

Poly di(methyl) siloxane, PDMS, was used to fabricate the confining network, via soft lithography replication using a silicon master, in turn fabricated using optical lithography. The designs of the microfluidics networks comprise a simple, uniform diamond-shaped maze, and a more complex, non-uniform maze. The height of the channels is set up to 6 μm and the widths are 3 μm. The protocol for the fabrication of microfluidics networks has been described earlier [12].

The PDMS microfluidics networks were plasma-treated, to render them hydrophilic, then sealed on to the coverslips, pre-wetted in a Petri dish with LB medium, and stored at low temperature before use for experiments. For the bacterial motility experiments, the PDMS structures were explored by a log phase culture of fluorescently labelled *E. coli K12* (Plasmid, pmf-440mcherry, add gene plasmid #62550 received a gift from Prof. Michael Franklin's lab). The fluid environment is a nutrient-rich medium, i.e., Luria-Bertani (LB) broth. Furthermore, the observation times were short enough to precluded and discernable consumption of nutrients resulting in concentration gradients. This experimental methodology would provide answers not only to explore the shortest paths but also various paths that may contribute to efficient searching of the space in a geometrically complex microenvironment.

The movement of bacteria in microfluidics networks was studied using a fluorescence microscope (Olympus IX83) with a 40X objective. The frames were analysed by ImageJ open software with plug-ins, e.g., track mate and MtrackJ [15]. We used density maps generated from background subtracted image stacks. The motility was recorded as tracks of x-y coordinates, which were used for the calculation of several other physical parameters.

## 3 Results and Discussion

### 3.1 Diamond Networks

The diamond structures present to bacteria two types of routes of the shortest path from entry to exit, i.e., the outer boundary routes (Fig. 1A(ii)), of and the zig-zagged path (Fig. 1A(iii)). Because of its symmetrical nature, in the uniform maze (diamond structure), the shortest path is equivalent irrespective of the route that the bacteria take (provided the bacterial agents do not revisit any path). The heat patterns of the trajectories in Fig. 2A suggest that *E. coli* prefers the outer straight-line paths (as shown in Fig. 1A(ii)). Although the zigzagged inner paths were also explored, these instances were rare and they happened when the outer boundary channels were crowded.

### 3.2 Maze Structures

Similarly, to the study of the bacterial motility in the diamond networks, we calculated the performance parameters for non-uniform mazes. Contrary to the results in diamond structures, the inner paths were well explored, and the heat-map reached saturation. It can be observed (table (Fig. 2C)) that *E. coli* spent more time in the non-uniform maze

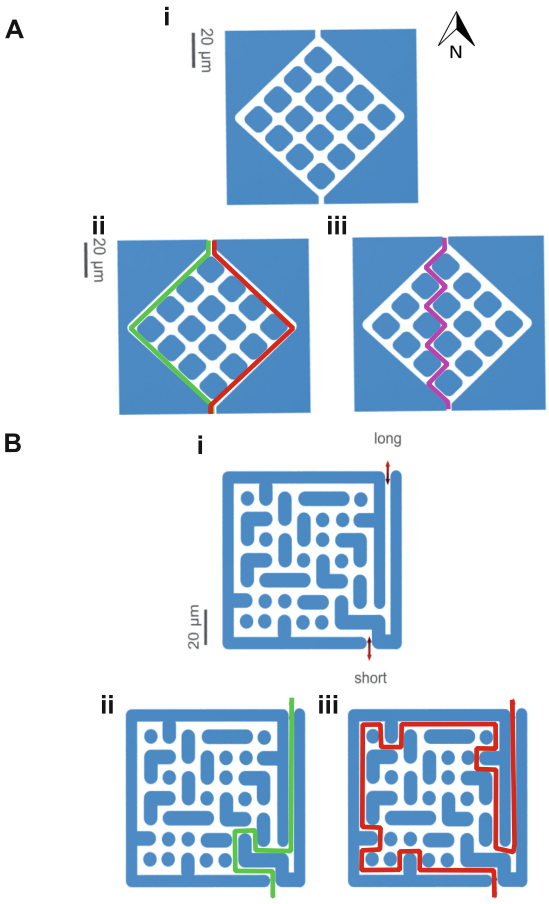
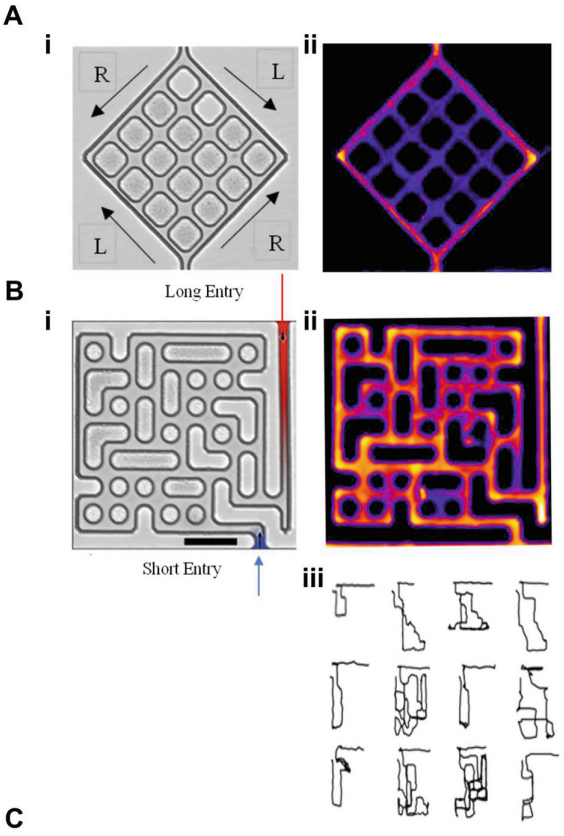



**Fig. 1.** A. Uniform maze (diamond structures): (i) layout of the diamond maze, (ii) and (iii) few different possible routes in the structure with the simplest (ii – green and red) and a zig-zag path (iii - pink). B. Non-uniform maze: (i) layout of the maze with two types of entry, short and long, (ii) and (iii) examples the shortest path possible (ii-green); and the longest path (iii – red). In both mazes, a bacterium could spend any range of time between seconds to hours, as the mazes are allowed for multiple solutions. Therefore, the paths presented here are only a few from possible paths. (Color figure online)

compared to the diamond structures, possibly due to the geometries like circular pillars, corners and barriers that deflected and diverted the bacteria visiting already explored paths.

Apart from the above-mentioned qualitative and empirical observations, we studied other motility characteristics, i.e., the preferences for long, and short points of the maze, and directional preferences. Interestingly, *E. coli K12* appears to use a 'wall-follower algorithm' which helps to solve the maze faster that would be otherwise achieved by a random exploration. This natural algorithm could be the result of the propensity of *E. coli* to swim closer to a surface ("wall accumulator" behaviour). This hypothesis




| Structure/parameter | Diamond | Maze |
|---|---|---|
| Average time (s) | 15.6±1.5 | 96±77 |
| Velocity (µm/s) | 9±0.5 | 8±3 |
| Distance (µm) | 127±12 | 651±472 |

**Fig. 2.** A Motility of *E. coli K12*-wt in diamond structures - (i) Optical image of the structure under a microscope. (ii) Heat-maps/density map of bacterial trajectories in the diamond structure B. Motility of *E. coli K12*-wt in maze structure. (i) Optical image of the microfluidic device. (ii) Heat-maps/density maps of the bacterial trajectories in a non-uniform maze, (iii) few actual representative trajectories of the *E. coli K12* in the maze. C. Tabular column comparing three major parameters calculated from the motility of bacteria in the network.

needs to be verified by further experiments seeking to compare this natural algorithm for bacteria having "wall escaper" behaviour, e.g., *Magnetococcus marinus MC-1*.

Another layer of complexity is the emerging behaviour of space search algorithms resulting from extremely varied architectures of the flagellar system. Finally, in many cases bacteria vary the ratio and frequency of the run & tumble machinery in response to the environment parameters, which in turn will impact on the natural space search and partitioning natural algorithms. Future more comprehensive experiments involving

various species of bacteria, each presenting different architectures, will advance in the understanding of the relationship between bacterial structure and resulting space search and partitioning algorithms.

## 4 Conclusion

In opposition to other studies, which studied bacterial motility in non-confining environments, the present work focused on the movement of bacteria in confining microfluidics structures, which could be conceived as a model of the microenvironments that they colonise. Furthermore, the present study operated in fluid conditions that suppress chemotaxis, which is one of the main driving forces of the directionality of bacterial movement. Consequently, the bacterial capacity to explore simple networks and more complex mazes, could be explored free of chemotaxis inference. This preliminary study suggests the further exploration of the motility patterns of other bacteria with different flagellar arrangements, and number, and exhibiting wall-escaping as well as wall accumulator character. Finally, this study suggests that bacteria can act as independent 'computational' agents solving mazes by employing space searching and partitioning natural algorithms.

**Acknowledgements.** The work presented here was financially supported by the Defence Advanced Research Projects Agency (DARPA) under Grant Agreement No. HR0011-16-2-0028.

## References

1. Kohler, M., Wehner, R.: Idiosyncratic route-based memories in desert ants, Melophorus bagoti: how do they interact with path-integration vectors? Neurobiol. Learn. Mem. **83**(1), 1–12 (2005)
2. Jin, N., Landgraf, T., Klein, S., Menzel, R.: Walking bumblebees memorize panorama and local cues in a laboratory test of navigation. Anim. Behav. **97**, 13–23 (2014)
3. Cohen, J.S., Burkhart, P., Jones, N., Innis, N.K.: The effects of an intramaze cue search rule on rat's spatial working memory. Behav. Processes **22**(1–2), 73–88 (1990)
4. Lee, P.G.: Chemotaxis by Octopus maya Voss et Solis in a Y-maze. J. Exp. Mar. Biol. Ecol. **156**(1), 53–67 (1992)
5. Schoenfeld, R., Moenich, N., Mueller, F.J., Lehmann, W., Leplow, B.: Search strategies in a human water maze analogue analyzed with automatic classification methods. Behav. Brain Res. **208**(1), 169–177 (2010)
6. Slusny, S., Neruda, R., Vidnerova, P.: Comparison of behavior-based and planning techniques on the small robot maze exploration problem. Neural Netw. **23**(4), 560–567 (2010)
7. Yu, Y., et al.: Intelligence-augmented rat cyborgs in maze solving. PLoS ONE **11**(2), e0147754 (2016)
8. Asenova, E., Lin, H.Y., Fu, E., Nicolau, D.V., Nicolau, D.V.: Optimal fungal space searching algorithms. IEEE Trans. Nanobiosci. **15**, 613–618 (2016)
9. Nakagaki, T.: Smart behavior of true slime mold in a labyrinth. Res. Microbiol. **152**(9), 767–770 (2001)

10. Hanson, K.L., Nicolau, D.V., Filipponi, L., Wang, L., Lee, A.P., Nicolau, D.V.: Fungi use efficient algorithms for the exploration of microfluidic networks. Small **2**(10), 1212–1220 (2006)
11. Held, M., Lee, A.P., Edwards, C., Nicolau, D.V.: Microfluidics structures for probing the dynamic behaviour of filamentous fungi. Microelectron. Eng. **87**(5–8), 786–789 (2010)
12. Nayak, M., Perumal, A.S., Nicolau, D.V., van Delft, F.C.: Bacterial motility behaviour in sub-ten micron wide geometries. In: 2018 16th IEEE International New Circuits and Systems Conference (NEWCAS), pp. 382–384. IEEE (2018)
13. Männik, J., Driessen, R., Galajda, P., Keymer, J.E., Dekker, C.: Bacterial growth and motility in sub-micron constrictions. Proc. Natl. Acad. Sci. U. S. A. **106**(35), 14861–14866 (2009)
14. Libberton, B., Binz, M., Van Zalinge, H., Nicolau, D.V.: Efficiency of the flagellar propulsion of Escherichia coli in confined microfluidic geometries. Phys. Rev. E **99**, 012408 (2019)
15. Schneider, C.A., Rasband, W.S., Eliceiri, K.W.: NIH Image to ImageJ: 25 years of image analysis. Nat. Methods **9**(7), 671–675 (2012)