ABSTRACT

E. coli is naturally capable of sensing substances in its environment and consequently moves directionally towards these, a phenomenon known as chemotaxis. Here, we apply directed evolution to chemoreceptors by targeting five amino acids residing in the ligand binding site to enable E. coli to perceive novel substances. In order to investigate mobility and directed movement towards a substance, an effective mobility selection method using special “swimming plates” is designed. Additionally, we attempt to improve E. coli’s swimming velocity by creating new parts derived from its own motility apparatus. Based on our selection system, we identify variants of chemoreceptors with new binding specificities in the mutant library. Once having established E. coli as our “tracking dogs”, the possible applications in medicine but also to environmental issues are virtually countless. As our planning moved on, we soon created three different focus groups which should work in parallel on the biggest and most crucial components of our project.

Strain and agar selection

Figure 1: We found the optimal swimming agar composition by testing various recipes.

Best taxis behavior: M9 swimming agar (defined minimal medium, 0.3% agar) Best swimming behavior: Tryptone swimming agar (0.3% agar, 1% tryptone, 0.5% NaCl) Fastest strain: MG1655 Utilized: BL21 (better genetic characterization)

The strains DH4 and XLI blue did not show any swimming at all.

Swimming improvement

Figure 2: The goal was to enhance the motility of E. coli in order to allow them to reach their targets more efficiently in later applications. We selected five different genes which should flagellum efficiency or number, if overexpressed. They were cloned into the vectors pCB18, pSS1C3 and pET100.

CONCLUSION

✓ Development of a swimming agar for...
✓ Establishment of a completely new selection assay – “Mutagenesis library selection system”
✓ Improvement of E. coli’s motility by overexpression of flic, yhfH and motB
✓ Generation of a mutant library with a final diversity of 2x10^9 clones
✓ Identification of two novel Tar receptors that are each associated with two and three novel chemoattractants, respectively
✓ Further characterization of several promoters from the Anderson promoter family of the parts registry

References: