



Definition of the quaternary amines specific sub-family of dCache sensor domains using bioinformatics and wet lab experimentation

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The signals recognized by the large majority of signal transduction receptors is unknown, and their identification currently represents a major research need in microbiology. Sensor domains show a significant degree of sequence divergence, as a consequence of which their ligand specificity is rarely predicted accurately based solely on overall sequence similarities.

We have reported the bioinformatics-based identification of a subfamily of dCache sensor domains that are specific for amino acids (Gumerov et al., 2022). Ligand recognition in this sub-family is primarily mediated by the establishment of hydrogen bonds. The aim of the present study is to determine whether a similar approach permits that identification of sensor domains that recognize hydrophobic signals and to that end we have chosen the binding of quaternary amines (QAs).

Based on three available structures of dCache sensor domains with bound QAs, the conserved residues of the ligand binding pocket were identified. Based on this information, bioinformatic approaches have resulted in the prediction of 8000 QA-responsive dCache domain sequences. We have overexpressed in *E. coli* the individual sensor domain of the QA-responsive chemoreceptor PctD from *Pseudomonas aeruginosa* (Matilla et al., 2022) along with 6 alanine-substitution mutants of amino acids in the binding pocket. Most of these side chains were of hydrophobic nature, confirming the hydrophobic nature protein-ligand interactions. ITC studies showed that these mutants bound choline with a 50- to 350-fold reduced affinity as compared to the native domain. These results assess the contribution of each residue in QA recognition. Using the same approach, we are currently studying QA recognition by ten of the predicted sequences. The identification of QA-responsive targets will allow us to further optimize the bioinformatic prediction algorithm.

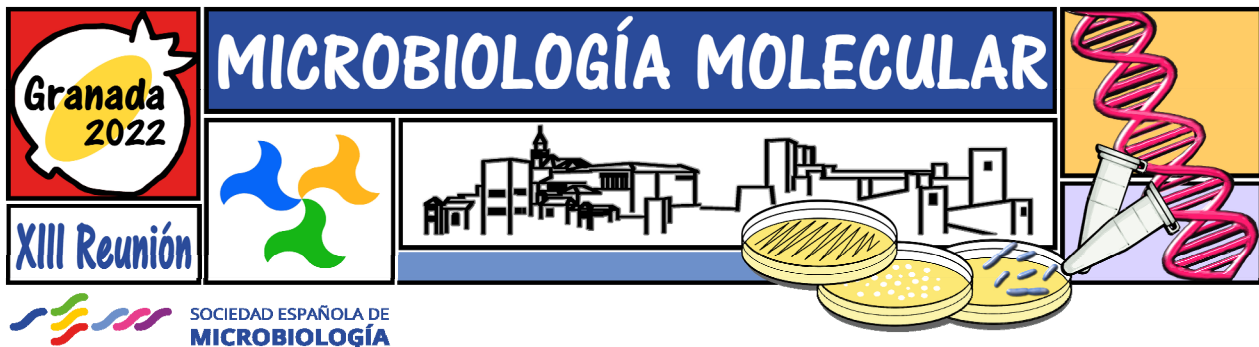
An analysis of the isolation source of the organisms that present the predicted QA-binding sensor domains show an overrepresentation of plant and soil-related isolation sources, which is in accordance with the fact that quaternary amines are abundantly present in plant root and seed exudates.

In conclusion, the combined bioinformatics-wet lab approach permits the sequence-based identification of sensor domains that recognize hydrophobic ligands.

References

Gumerov *et al.* (2022). Proc Natl Acad Sci U S A. DOI: 10.1073/pnas.2110415119

Matilla *et al.* (2022). mBio. DOI: 0.1128/mbio.03458-21



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