

# A novel concept combining experimental and mathematical analysis for the identification of unknown interspecies effects in a mixed culture.

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## Abstract

Bacteria in natural habitats only occur in consortia together with various other species. Characterization of bacterial species, however, is normally done by laboratory testing of pure isolates. Any interactions that might appear during growth in mixed-culture are obviously missed by this approach. Existing experimental studies mainly focus on two-species mixed cultures with species specifically chosen for their known growth characteristics, and their anticipated interactions. Various theoretical mathematical studies dealing with mixed cultures and possible interspecies effects exist, but often models cannot be validated due to a lack of experimental data. Here, we present a concept for the identification of interspecies effects in mixed cultures with arbitrary and unknown single-species properties. Model structure and parameters were inferred from single-species experiments for the reproduction of mixed-culture experiments by simulation. A mixed culture consisting of the three-species *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Staphylococcus aureus* served as a model system. For species-specific enumeration a

quantitative terminal restriction length polymorphism (qT-RFLP) assay was used. Based on models fitted to single-species cultivations, the outcome of mixed-culture experiments was predicted. Deviations of simulation results and experimental findings were then used to design additional single-cell experiments, to modify the corresponding growth kinetics, and to update model parameters. Eventually, the resulting mixed-culture dynamics was predicted and compared again to experimental results. During this iterative cycle, it became evident that the observed coexistence of *P. aeruginosa* and *B. cepacia* in mixed-culture chemostat experiments cannot be explained on the basis of glucose as the only substrate. After extension of growth kinetics, that is, for use of amino acids as secondary substrates, mixed-culture simulations represented the experimental findings very well. According to the model structure, as motivated by single-species experiments, the growth of *P. aeruginosa* and *B. cepacia* on glucose and amino acids could be assumed to be independent of each other. In contrast, both substrates are taken up simultaneously by *S. aureus*.

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