

Cooperation between food-associated microorganisms

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Introduction

Foodstuffs contain complex microbial consortia interacting with each other in a competitive or cooperative manner. Cooperative interactions of pathogens and/or spoilage microorganisms with commensal bacteria can threaten consumer health or reduce shelf life due to accelerated deterioration.

Signal molecules called autoinducers can be secreted to coordinate community-wide functions, e.g. bioluminescence, virulence, or biofilm formation. Autoinducer-2 (AI-2) molecules are produced by both Gram-positive and Gram-negative bacteria, and are thus assumed interspecies quorum sensing (QS) signals.

Conclusion

- Depending on the foodstuff of origin 40-50 % of isolates produced AI-2. The genus *Pseudomonas* was unable to produce AI-2, while lactic acid bacteria, *Enterobacteriaceae*, and different species of the genus *Bacillus*, as well as various strains of the pathogen *Listeria monocytogenes* were frequently tested positive for AI-2 production.
- Two-strain-combinations isolated from the same foodstuff frequently displayed enhanced growth and/or biofilm formation, stressing the importance of cooperation in food-associated habitats
- In most cases, the presence of both cells was pivotal for the observed behavior

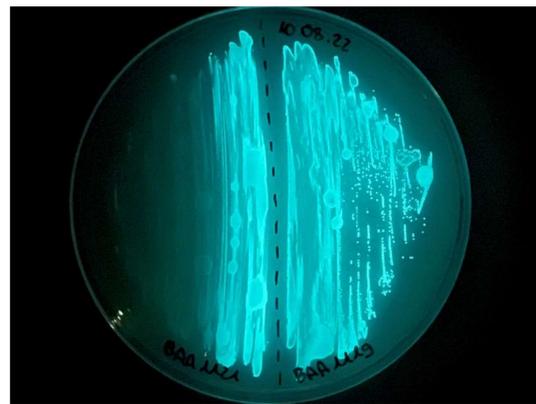
Methods

We assessed interspecies cooperation of strains isolated from a variety of foodstuffs, including meat, fish, salad, and ready-to-eat food.

Representative isolates were identified via 16S rRNA gene sequencing.



Isolates were tested for AI-2 production using the bioluminescent *Vibrio campbellii* reporter strain ATCC BAA-1121.



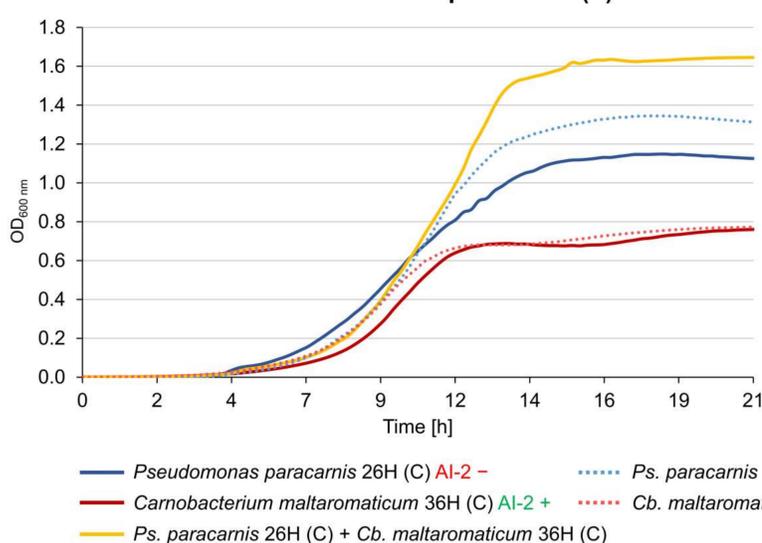
Pure and mixed cultures containing two strains originating from the same foodstuff were analysed for cooperative behavior:

Growth curves were generated in microplates by optical density measurement. Subsequently, biofilm formation was assessed by crystal violet staining.

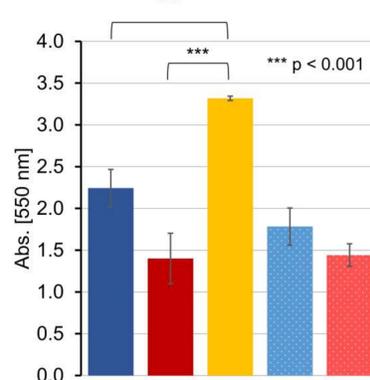
To determine the necessity of direct cell contact for the observed effects, pure cultures were additionally grown in a mixture of fresh medium and cell-free spent supernatant of their potential partner. Fluorescence microscopy was used to assess spatial proximity of partners in biofilms formed on stainless steel surfaces.

Results

Growth of pure and mixed cultures (C)
Effect of culture supernatants (S)



Biofilm formation

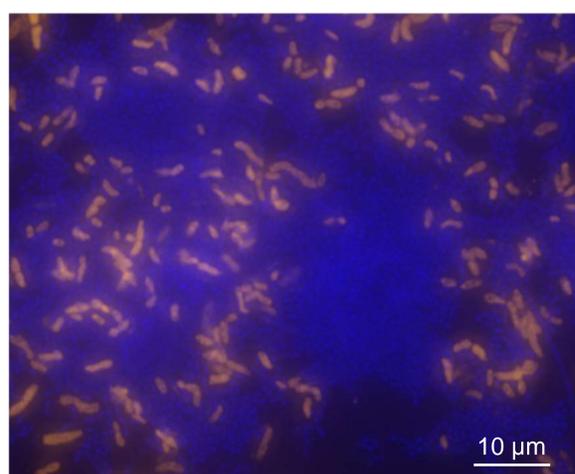


Many combinations of two isolates displayed enhanced growth and/or biofilm formation when incubated together compared to the pure cultures.

The presence of both partners was pivotal for the enhanced effect, since incubation of pure cultures with a mixture of fresh medium and the spent supernatant of the cooperation partner was insufficient for producing the same effect.

In many cases, cooperating combinations were composed of one AI-2 producer and one non-producer.

Fig. 1: Growth (OD_{600 nm}) and biofilm formation of pure and mixed cultures (C) containing both strains, isolated from minced meat, and effect of cell-free supernatants (S) on growth and biofilm formation of pure cultures of the respective cooperating partner



Fluorescence microscopy proved spatial proximity of the cooperating species formed on stainless steel surfaces, indicating beneficial effects of small distances between potentially cooperating partners.

Fig. 2: Fluorescence-*in-situ*-hybridization (FISH) of a co-culture of the isolates *Pseudomonas paracarnis* 26H, stained orange with probe PSE-1284-Cy3, specific for *Pseudomonas* sp., and *Carnobacterium maltaromaticum* 36H, counter-stained blue with DAPI.

Outlook

We aim to quantify AI-2 production by GC/MS and assess the effect of different environmental conditions relevant to the food industry on the amount of AI-2 production.

