LABORATORY FOR HEALTH, OMICS AND PATHWAY EXPLORATION

LAB Autoaggregation and Coaggregation Assays

A method reference for probiotic aggregation phenotypes, optical-density calculations, controls and interpretation.

DOCUMENT	VERSION	ISSUED
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Safety and scope boundary: This is a non-operational assay reference. It does not provide live-cell preparation, incubation or pathogen co-culture instructions. Obtain those details only from the supervisor-approved SOP for approved organisms.

Purpose and scope

Autoaggregation and coaggregation assays are used in probiotic research to examine cell-surface-associated aggregation behaviour. Autoaggregation describes the association of cells from the same strain; coaggregation describes the association between two different microbial partners. These are in-vitro phenotypic indicators and do not, by themselves, demonstrate intestinal adhesion, colonization or clinical benefit.

Scientific principle

Optical-density-based approaches compare the initial suspension signal with the signal remaining in a defined sampling region after a prespecified observation interval. A reduction in upper-phase turbidity can be consistent with sedimentation or aggregation, so mixing history, settling behaviour and sampling position must be standardized and reported.

Common calculations

Autoaggregation

Autoaggregation (%) = $[1 - (A_t / A_0)] \times 100$

A_0 is the initial optical density and A_t is the optical density at the selected observation time. Use the same measurement wavelength, sampling convention and time definitions across compared samples.

Coaggregation

Coaggregation (%) = $\{[(A_x + A_y) / 2 - A_{x+y}] / [(A_x + A_y) / 2]\} \times 100$

A_x and A_y are the optical-density values of the individual partner suspensions under the defined comparison condition; A_{x+y} is the value for the combined partners. State exactly how each value was obtained.

Materials and controls to plan

Item / control	Purpose
Approved, identified test strain	Provides traceability and defines the biological material under study.
Compatible buffer or matrix blank	Documents the background signal of the suspension medium.
Each partner assessed separately	Provides the single-partner reference needed for coaggregation calculation and reveals self-settling.
Non-aggregating or characterized reference, where available	Provides context for assay performance; its suitability must be established locally.
Independent biological replicates	Distinguish biological variability from repeated readings of the same preparation.

Workflow overview (non-operational)

A supervised method typically defines the organism identities and partner pairings, documents the standardized suspension and optical-density basis, records single-partner behaviour, evaluates the paired condition using the same measurement framework, calculates the selected percentage, and reports independent replicates. Exact suspension preparation, timing and sample handling belong in the approved local SOP.

Interpretation cautions

- Autoaggregation can be affected by sedimentation, cell morphology, surface properties and handling; it is not a direct adhesion assay.
- Coaggregation depends on the specific strain pair and cannot be generalized to all organisms or host settings.
- Pathogenic partner organisms require separate risk assessment and authorized containment. Do not adapt a probiotic screening method for pathogen handling without institutional approval.
- Report raw absorbance values, time points, wavelength, partner identity, replicate design, calculation and limitations.

References and further reading

1. Kos B, Susković J, Vuković S, Šimpraga M, Frece J, Matošić S. Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92. Journal of Applied Microbiology. 2003;94(6):981-987. doi:10.1046/j.1365-2672.2003.01915.x

2. Karbowiak M, Gałek M, Szydłowska A, Zielińska D. The Influence of the Degree of Thermal Inactivation of Probiotic Lactic Acid Bacteria and Their Postbiotics on Aggregation and Adhesion Inhibition of Selected Pathogens. Pathogens. 2022;11(11):1260. doi:10.3390/pathogens11111260

3. American Society for Microbiology. ASM Guidelines for Biosafety in Teaching Laboratories (2020). https://asm.org/guideline/asm-guidelines-for-biosafety-in-teaching-laboratories

Document status: Version 1.0. This reference should be checked against current literature, institutional requirements and the approved local method before use.