RESHAPE

APPLICATION NOTE

Microbial Antagonism

Investigating competition dynamics between microbes on solid media

APPLICATION

Automated time-resolved analysis of bacterial–fungal competition on solid media

Why is it a critical need?

Bacterial–fungal antagonism assays on solid media are widely used to evaluate inhibitory interactions relevant to food stability, biocontrol screening, strain engineering, and microbial discovery. However, these assays are traditionally assessed through manual inspection or endpoint measurements of inhibition zones, providing limited reproducibility and little insight into the temporal dynamics of antagonistic interactions. Fungal growth is highly dynamic and spatially complex, and inhibitory effects may emerge gradually, asymmetrically, or only after prolonged incubation, i.e. patterns that are difficult to capture reliably with manual workflows. As screening demands increase, there is a critical need for automated, time-resolved analysis that enables objective quantification of both colony expansion and growth inhibition.

How does Reshape help?

The Reshape Smart Incubator meets this need by integrating controlled incubation with high-resolution imaging and AI-powered colony detection and measurements. This enables continuous monitoring of bacterial and fungal growth, automated quantification of colony area and inhibition dynamics, and reproducible comparison across conditions — transforming a qualitative antagonism assay into a scalable, data-driven workflow.

Introduction

Traditional dual-culture assays rely on manual, endpoint measurements of inhibition zones or colony diameters. These approaches lack temporal resolution, introduce human-to-human variability and can miss critical interaction dynamics (e.g., onset of inhibition). In this study, we evaluated the interaction between *Bacillus subtilis* and four independent fungal isolates using the Reshape Smart Incubator. Continuous incubation and automated imaging enabled AI-driven colony detection and kinetic growth analysis over a 12-day period. This workflow transforms qualitative subjective assays into reproducible, time-resolved datasets suitable for comparative screening.

Microbial competition assays are central to screen strains for:

- Agricultural biocontrol
- Food preservation and spoilage prevention
- Strain engineering and microbial discovery

Image to the left highlights Reshape's AI analysis model which can detect colonies, measure area, and give time of first appearance. The user can view the raw image (above) or interact with the image to view instant insights (below). The data can also be downloaded as a CSV / Excel file for further post-processing if needed.

SHOWCASE STUDY

The objective of the study was to establish a quantitative, automated imaging workflow for time-resolved analysis of competition, or antagonistic interactions, between various fungal isolates and *Bacillus subtilis* on solid media.

Materials & Methods

Co-Cultivation Assay
Bacillus subtilis and four unknown fungal isolates derived from Danish soils were inoculated at a defined distance on potato dextrose agar (PDA). Plates were incubated at 30°C while imaging was performed automatically at fixed intervals (every 30 minutes) throughout the experiment (~350 hours total runtime).

Automated Image Analysis
AI models identified bacterial and fungal colonies separately, defined colony boundaries over time, quantified colony area (mm^2) at each time-point and generated normalized growth curves for comparative analysis.

All measurements were performed automatically without manual tracing or endpoint bias.

Results & Discussion

The automation and AI model workflow reliably distinguished between bacterial and fungal colonies throughout the full experimental duration, including after physical interaction zones developed (**Fig. 1A**). Automated boundary detection and colony area quantification were maintained consistently across all plates and isolates, eliminating manual tracing and reducing human bias. This ensured that data collection was standardized across experimental conditions, enabling direct, objective comparison between isolates.

Rather than focusing solely on endpoint measurements, the workflow generated structured, time-resolved growth curves for each bacterial–fungal pair. These growth profiles allowed straightforward comparison of competitive performance across four independent fungal isolates tested against *B. subtilis.*

While the degree of antagonism varied between isolates, the key outcome was the ability to rank isolates based on quantifiable growth suppression metrics using the same automated analytical pipeline. Because measurements were collected continuously over the full incubation period, divergence in growth trajectories could be detected objectively and reproducibly.

Continuous monitoring further enabled identification of key quantitative parameters such as time to growth deceleration, plateau onset, relative expansion rates, and final colony area differences. The shaded late-stage regions (**Fig. 1B**) illustrate how plateau behavior differed between isolates, providing a consistent framework for comparing inhibition strength without relying on subjective visual assessment. Importantly, the timing of interaction effects varied between isolates, demonstrating the added value of kinetic monitoring over traditional endpoint-only assays.

Overall, the automated workflow transforms classical confrontation assays into scalable screening experiments. It provides reproducible, structured datasets that support cross-isolate ranking, objective inhibition quantification, and standardized comparison across experimental runs. This makes the system particularly suited for screening strain libraries in biocontrol, food protection, and microbial discovery programs, where robustness, comparability, and throughput are more critical than detailed mechanistic characterization of individual interactions.

Results continued...

B. subtilis exhibited antagonistic activity against two of the four fungal isolates (isolates 2 and 3), indicating the production of inhibitory compounds effective against these soil-derived fungi. In contrast, isolate 4 showed no measurable inhibition, while isolate 1 displayed only minor, transient growth restriction that did not significantly affect overall colony expansion.

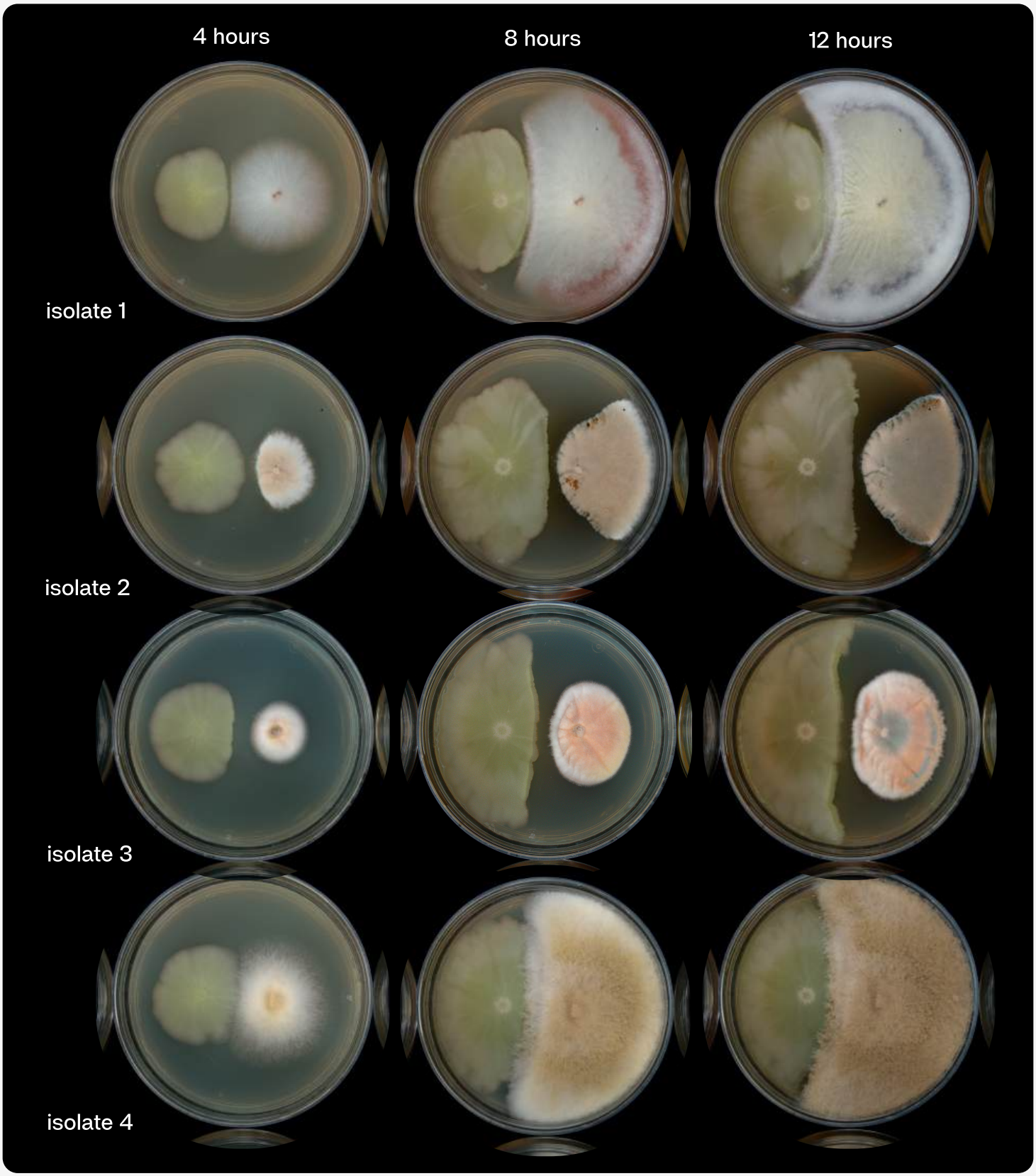

Figure 1A. Automated and consistent high-res images taken by the Reshape Smart Incubator, highlighting the antagonism behavior (or not) at 4, 8 and 12 hours of growth.

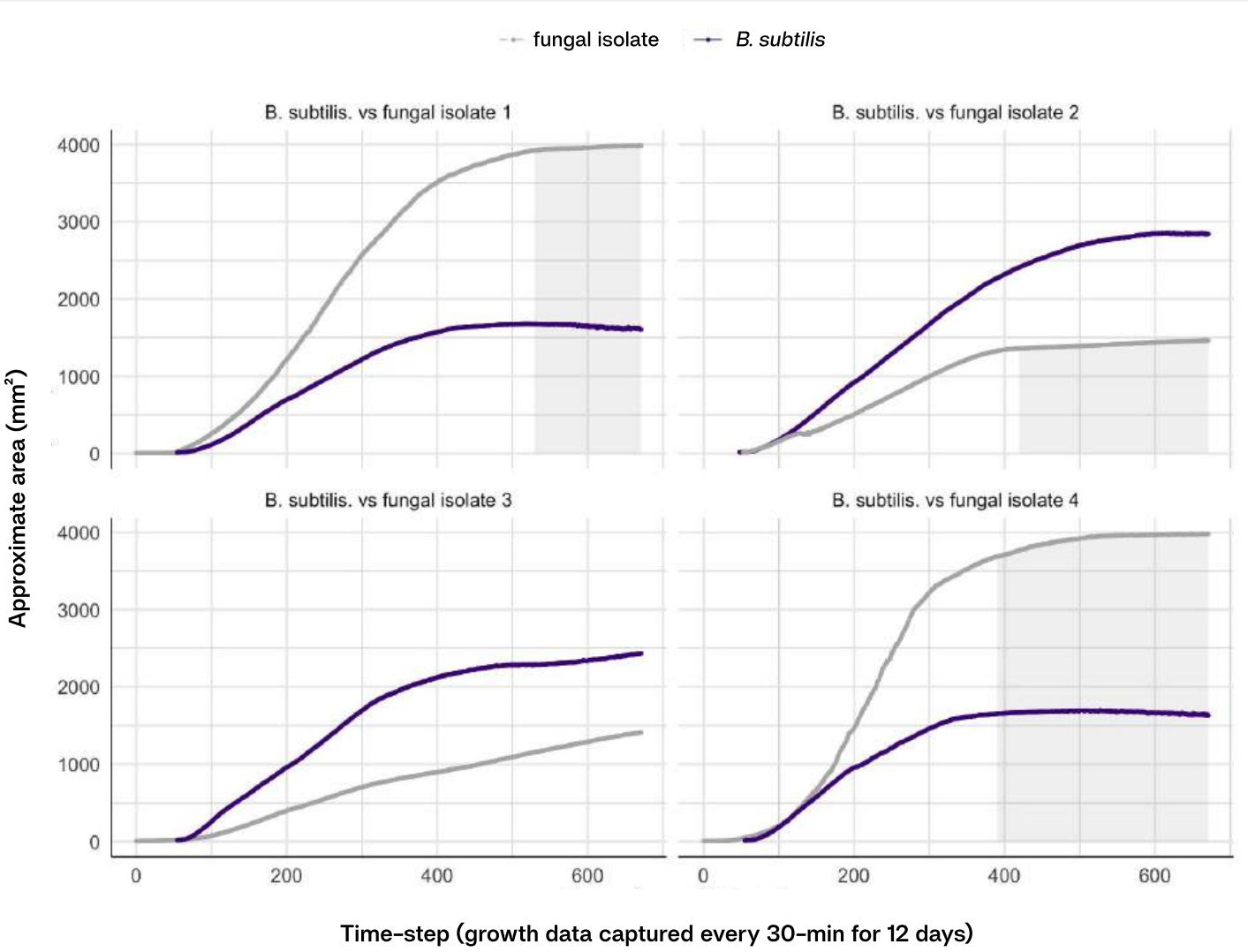

Figure 1B: Growth dynamics between *B. subtilis* and four fungal isolates showing the ability of *B. subtilis* to suppress the growth of two unknown fungal isolates with little to no effect on the other two.

Conclusion

This study demonstrates how automated incubation combined with AI-driven image analysis enables high-resolution, time-resolved quantification of bacterial–fungal antagonism. By capturing when and how growth divergence occurs, the platform provides deeper insight into microbial interaction dynamics, supporting biocontrol development, food protection validation, and strain engineering programs.

The combination of controlled incubation, time-resolved imaging, and automated analysis makes this approach particularly well-suited for modern R&D laboratories seeking higher data quality with reduced hands-on time.