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aNP-TRAP: a conceptual platform for *in situ* microbial cultivation and functional detection of antimicrobial activity

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Microbial natural products are central to drug discovery, yet many biosynthetic gene clusters remain transcriptionally silent under standard laboratory conditions. Conventional screening workflows—based on *ex situ* cultivation and metabolite extraction—can be labor-intensive and often fail to capture ecologically relevant microbial interactions. To address these limitations, we propose the aNP-TRAP (Activity-guided Natural Product Triaging and Recognition Assay Platform), a conceptual, field-deployable device designed to integrate *in situ* microbial cultivation with functional detection of bioactivity. The system consists of a honeycomb array of cultivation wells, semipermeable and gradient membranes to permit directional metabolite diffusion, and a detection layer containing biosensors responsive to antibacterial, antifungal, or quorum-sensing–inhibitory compounds. Three detection strategies are envisioned: *Escherichia coli* JW5503-1 with resazurin for antibacterial activity, *Candida albicans* for antifungal screening, and *Chromobacterium violaceum* CV026 for quorum-sensing inhibition. Microbial metabolites diffusing through the membranes interact with the biosensor matrices, potentially generating colorimetric or pigment-based signals. This platform is conceptual and currently lacks empirical validation; all performance expectations derive from simulation-based reasoning. In brief, simulations suggested a 0.2 μm membrane equilibrates nutrients within ~2–6 h, directional metabolite flux achieves >95% reflux suppression within ~6–10 h, and biosensor responses become detectable within ~4–10 h at representative inhibitory ranges. Although unvalidated, this integrated configuration may support early-stage triaging of microbial isolates and help guide the discovery of bioactive compounds from under-explored microbial communities. The platform should be viewed as a hypothesis-generating concept rather than a validated tool.

KEYWORDS

***in situ* cultivation, functional biosensing, natural product discovery, modular microenvironment, quorum sensing inhibition, field-deployable screening**

1 Introduction

Environmental microorganisms are an abundant and underexplored source of chemically diverse natural products that have led to life-saving therapeutics (Berdy, 2012; Newman and Cragg, 2020). Yet a substantial fraction of biosynthetic gene clusters (BGCs) remains silent under conventional cultivation owing to the absence of native cues

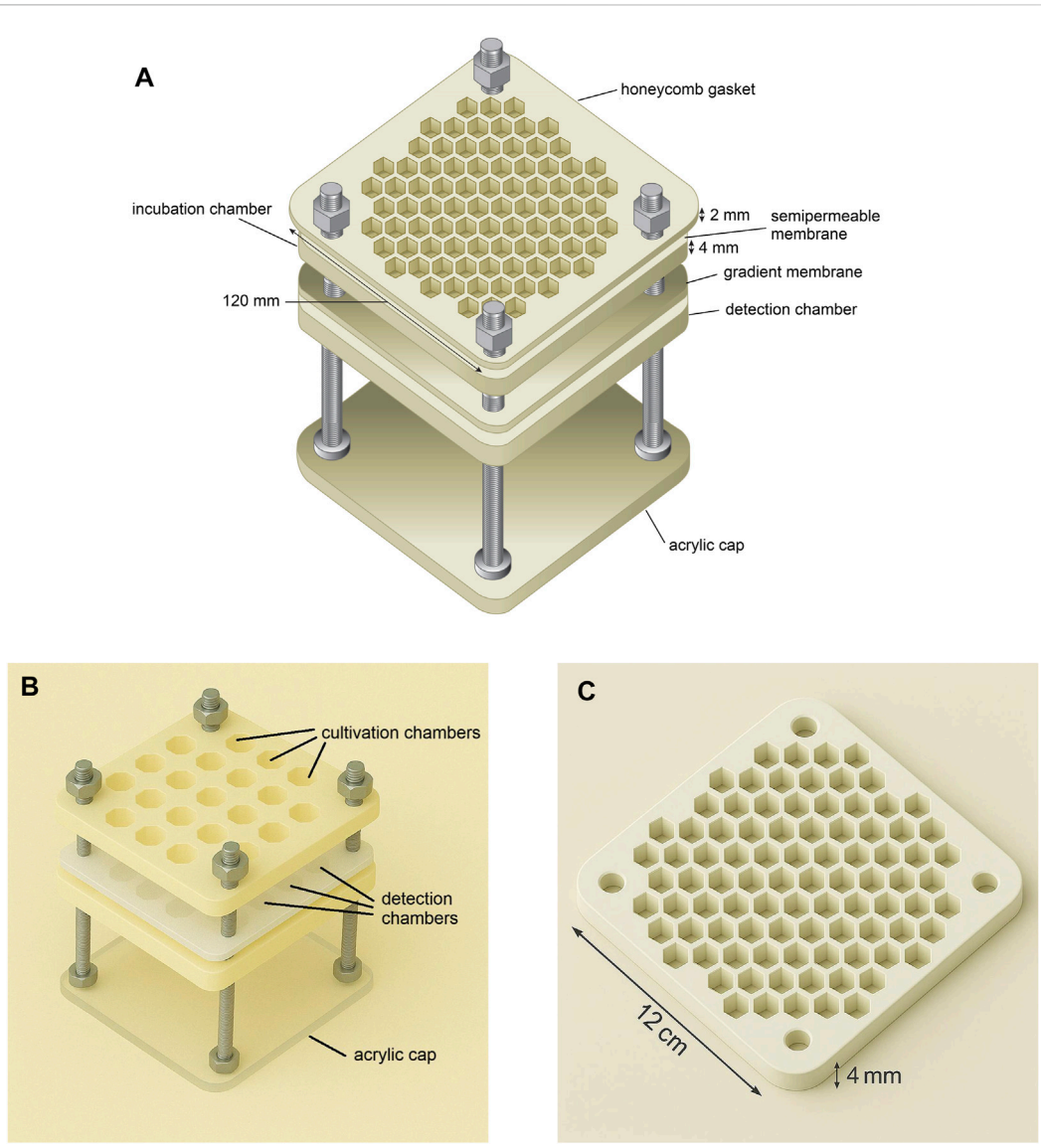

FIGURE 1
Exploded views of the aNP-TRAP device illustrating its modular configuration and functional components. **(A)** Conceptual exploded diagram showing the main structural layers of the device, including membranes, cultivation and detection modules, and acrylic base for visual readout. **(B)** Realistic exploded rendering illustrating the hexagonal cultivation wells, hexagonal biosensor detection layer, and the acrylic base. **(C)** Detailed view of a single honeycomb layer, highlighting hexagonal chambers that can serve for either microbial incubation or functional detection. Caption placement: per Reviewer #2, the full caption will appear immediately below the figure in the final layout.

and interactions (Rutledge and Challis, 2015; Ling et al., 2015; Ziemert et al., 2016; Bauman et al., 2021; Alain and Querellou, 2009; Kaeberlein et al., 2002). Traditional *ex situ* workflows—isolating organisms, cultivating them under artificial conditions, extracting metabolites, and screening—struggle to access this hidden potential and often rediscover known compounds (Berdy, 2012; Nichols et al., 2010; Epstein, 2013).

Recent methodological reviews reinforce the need for approaches that deliberately couple ecological context to functional detection and to distinguish field-deployable concepts from lab-only biosensor formats (e.g., Hossain, 2024). Here we introduce aNP-TRAP as a conceptual ("simulations only") *in situ* device that couples cultivation to embedded functional detection, explicitly distinguishing itself from lab-only biosensor and microfluidic formats by prioritizing field-deployable, low-infrastructure operation. Throughout, we emphasize that the present work is hypothetical pending experimental validation.

2 Device concept, workflow, and simulation-based feasibility

2.1 Device concept

The aNP-TRAP platform is conceived as a modular, field-deployable system enabling *in situ* microbial cultivation with simultaneous functional screening of diffusing metabolites. The device comprises three stacked layers and an acrylic base for readout:

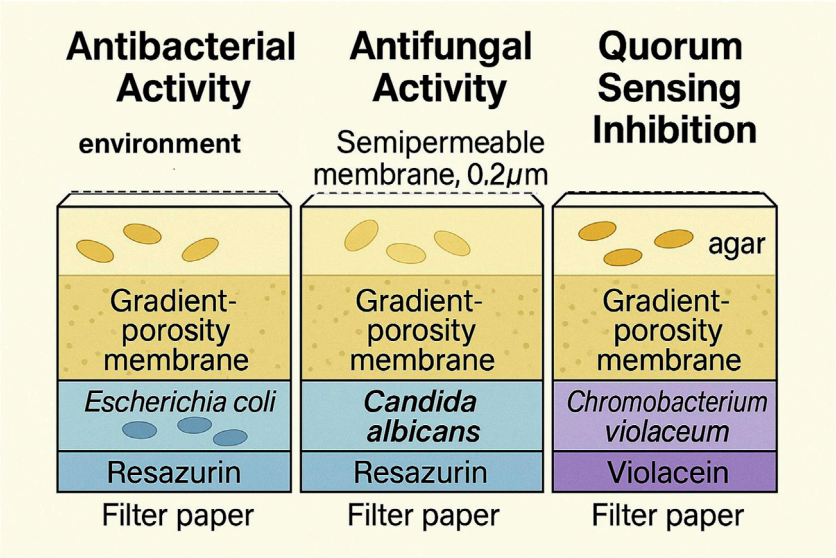

FIGURE 2
Lateral schematic view of the aNP-TRAP device showing the layered configuration and expected metabolite diffusion starting from the semipermeable membrane, followed by the cultivation chamber, gradient-porosity membrane, and reaching the hexagonal biosensor detection layer. The figure also illustrates the three initially proposed sensing systems: antibacterial (*Escherichia coli* JW5503-1 with resazurin), antifungal (*Candida albicans* with resazurin), and quorum-sensing inhibition (*Chromobacterium violaceum* CV026). Caption placement: per Reviewer #2, the full caption will appear immediately below the figure in the final layout.

i. Cultivation layer: 56 hexagonal wells (17 mm edge-to-edge, 4 mm depth) containing ~0.75 mL semisolid medium; the top is sealed with a 0.2 μm semipermeable membrane to allow nutrient influx while preventing microbial escape.
ii. Intermediate layer: a gradient-porosity membrane that favors downward metabolite diffusion and resists upward reflux to promote directional mass transfer.
iii. Detection layer: 56 biosensor matrices responsive to antibacterial, antifungal, or quorum-sensing (QS) inhibitory signals, aligned with the cultivation wells.
iv. Acrylic base: provides visual readout of colorimetric/pigment changes.

Components are held together with lateral bolts and gaskets to ensure structural integrity and sterility; the architecture accommodates sensor materials without compressive stress. While the standard top membrane is 0.2 μm, an optional anisotropic upper film can further restrict outward metabolite escape to enhance signal accumulation. The conceptual architecture and functional layers are illustrated in Figures 1A–C.

2.2 Sample preparation and chamber inoculation

Device assembly is performed under sterile conditions, drawing on precedents such as the iChip and diffusion chambers (Nichols et al., 2010; Berdy et al., 2017; Jung et al., 2020). Environmental samples (e.g., soil, rhizosphere, sediments) are suspended in native moisture or sterile buffer (PBS/Ringer). Preprocessing with mild bead agitation and mesh filtration reduces debris while preserving viability. Wells receive low-nutrient semisolid medium (1.5%–2% agar/gellan, cooled to 40°C–45 °C). Inoculation can target limiting dilutions for clonal isolation or be performed in bulk for consortia studies.

2.3 Device assembly and environmental deployment

After inoculation, the bottom of the cultivation layer is sealed with the gradient-porosity membrane and the top with a 0.2 μm membrane. Compression sealing with gaskets and fasteners yields a robust, portable unit. The device can be embedded 5–10 cm in the target matrix or suspended in water using tethers, enabling passive nutrient/signal exchange under near-native conditions. Alternative pore sizes can accommodate fungi/yeasts (Berdy et al., 2017). A lateral schematic of mass transfer and sensing modalities is provided in Figure 2.

2.4 Functional detection modules

The detection layer contains biosensors immobilized in paper/hydrogel/agarose matrices aligned to the wells. Three modalities are envisioned:

Antibacterial: *Escherichia coli* JW5503-1 + resazurin in hydrogel (PVA or low-melting agarose). Inhibitory activity suppresses metabolic reduction (blue → pink), generating a retained-blue signal (Allen et al., 2022; Sarker et al., 2007).

Antifungal: *Candida albicans* embedded in redox-sensitive hydrogel with resazurin. Depending on screening goals, alternative fungal sensors (e.g., *Saccharomyces cerevisiae*, *Aspergillus nidulans*) may be used.

TABLE 1 Proposed early benchmarking steps and minimal success criteria (mock-up integrity and sterile hold; sensor dose-response/LOD; ≥90–95% reflux suppression for tracers; ≤20% signal loss over 24–72 h; ≤10–15% unintended cross-responses; reproducible field signals vs. controls with traceable culture recovery). Includes imaging/quantification thresholds and use of antimicrobial/AHL standards.

Benchmarking step	Metric/readout	Minimal success criteria	Notes/tools
Benchtop mock-up and sterile hold	Mechanical integrity, leak tests, contamination check	No leaks; sterile hold ≥72 h	Gaskets/bolts; agar plates and sterility swabs
Mass-transfer directionality	Tracer flux ratio (downward vs. upward)	≥90–95% reflux suppression	Use dye or fluorescent tracer; quantify intensity ratio
Sensor dose–response	Signal vs. concentration curve	Monotonic response; LOD within intended range	Resazurin or violacein readouts; plate standards
Signal stability	Dye/AHL stability over time	≤20% signal loss over 24–72 h	Vary pH, temperature, and light exposure
Cross-sensor specificity	Off-target responses	≤10–15% unintended cross-response	Compare antibacterial/antifungal/QS assays
Pilot environmental deployment	Field signal vs. controls; culture recovery	Reproducible positives with traceable recovery	Barcode/grid map; LC–MS/MS dereplication
Imaging and quantification	RGB/hue extraction; thresholding pipeline	Consistent segmentation; SNR >3	Portable imaging; ImageJ or equivalent

Quorum-sensing inhibition: *Chromobacterium violaceum* CV026 in hydrogel with 10–20 μM C6-HSL as inducer; inhibitors suppress violacein pigmentation (McClean et al., 1997; Duddy and Bassler, 2021; Miller and Bassler, 2001). If CV026 stability is suboptimal, wild-type *C. violaceum* is a viable alternative. The layered configuration and mass flow are depicted in Figure 2.

Viability/specificity in mixed settings: No experiments were performed; feasibility and specificity remain hypothetical and are extrapolated from prior biosensor uses under controlled conditions.

2.5 Incubation and signal monitoring

In situ incubation is expected for 3–10 days under ambient conditions (shorter windows help dye/signal stability (Demir et al., 2024)). Monitoring can be visual or via portable imaging; simple image analysis (e.g., ImageJ) can extract RGB/hue measures. Internal controls include uninoculated wells and calibration wells with known compound concentrations. If weak signals are observed, devices may be redeployed to allow signal maturation and microbial proliferation. Thresholds and quantification steps follow standard colorimetric workflows and are specified in Table 1 (Imaging and quantification).

2.6 Recovery and characterization of positive cultures

Wells exhibiting reproducible biosensor responses (e.g., retained blue, pigment loss) are prioritized for isolation and scale-up. Downstream characterization includes MALDI-TOF MS, LC-MS/MS, and 16S/ITS sequencing for dereplication and producer prioritization (Ling et al., 2015). Each well's position is traceable via barcoding or a grid map, and active fractions are dereplicated to prioritize novel candidates. For early validation, we recommend using antimicrobial standards (e.g., ampicillin, nystatin) and AHL analogs to calibrate sensor response functions.

2.7 *In silico* modeling of diffusion and biosensor response

Clarification on "simulations": In this manuscript, "simulations" refers to conceptual, order-of-magnitude estimates and internal consistency checks (e.g., dimensional analysis, spreadsheet-level parametric sweeps) derived from literature parameters—not to the execution of numerical solvers (no FEM/CFD, COMSOL®, or MATLAB® runs were performed). Reported values are illustrative and for design guidance only.

To evaluate theoretical feasibility, estimates were structured following the logic typical of COMSOL®/MATLAB® model setups (again, not executed):

Nutrient diffusion: A 0.2 μm polyethersulfone (PES) membrane (~100 μm thick) with $D \approx 5–7 \times 10^{-6}$ cm^2 s^{-1} supports equilibration within ~2–6 h for common nutrients (Stewart, 2003).

Directional transfer: Anisotropic transport (forward $D = 5 \times 10^{-7}$; reverse $D = 5 \times 10^{-9}$ cm^2 s^{-1}) accumulates metabolites (e.g., violacein, rifamycin) in the detection zone within ~6–10 h, with >95% reflux suppression (Zhao et al., 2022; Hou et al., 2019).

Containment: Hydrophobic antibiotics (logP >1; MW > 400 Da) show >98% retention at 24 h; smaller/hydrophilic molecules exhibit polarity/size-dependent back-diffusion.

Sensor kinetics (illustrative): antibacterial—>50% viability-proxy drop in 4–6 h at ≥10 μg mL^{-1}; antifungal—>80% metabolic signal decline within ~8 h at ~25 μg mL^{-1}; QS inhibition—~70% violacein repression within ~10 h for $IC_{50} \approx 5–20$ μg mL^{-1}.

Table 2 Key modeling assumptions and parameter ranges (membrane pore size/thickness; diffusion coefficients; anisotropy targets; retention criteria by logP/MW; inducer dosing for CV026; time-to-signal windows; field incubation ranges), compiled for transparency and to guide early prototyping.

3 Discussion

aNP-TRAP is advanced here strictly as a conceptual innovation: a single, modular device that merges *in situ* cultivation with embedded functional detection, aiming to

TABLE 2 Key modeling assumptions and parameter ranges (membrane pore size/thickness; diffusion coefficients; anisotropy targets; retention criteria by logP/MW; inducer dosing for CV026; time-to-signal windows; field incubation ranges).

Parameter	References value(s)/ range	Notes/assumptions	Representative sources
Top semipermeable membrane pore size	0.2 μm	Prevents cell egress; permits ingress of nutrients and small metabolites	Nichols et al., 2010; Berdy et al., 2017
Membrane thickness	~100 μm (PES)	Used to estimate characteristic diffusion times	Stewart (2003)
Diffusion coefficient (small nutrients in membrane)	$D \approx 5–7 \times 10^{-6}$ $cm^2 \cdot s^{-1}$	Basis for equilibration-time estimates across the top membrane	Stewart (2003)
Nutrient equilibration time	~2–6 h	Order-of-magnitude estimate for small molecules	Derived from parameters above
Intermediate anisotropic (gradient-porosity) layer	Forward $D \approx 5 \times 10^{-7}$; reverse $D \approx 5 \times 10^{-9}$ $cm^2 \cdot s^{-1}$	Target >95% reflux suppression within ~6–10 h	Hou et al., 2019; Zhao et al., 2022
Downward metabolite accumulation window	~6–10 h	Representative for typical antimicrobial/metabolite sizes	Conceptual estimate
Containment of hydrophobic antibiotics	>98% retention at 24 h for logP >1; MW > 400 Da	Smaller/hydrophilic molecules may back-diffuse depending on polarity and size	Conceptual estimate
Antibacterial sensor configuration	*E. coli* JW5503-1 + resazurin	>50% viability-proxy drop in 4–6 h at ≥10 $\mu g \cdot mL^{-1}$	Allen et al., 2022; Sarker et al., 2007
Antifungal sensor configuration	*Candida albicans* + resazurin	>80% metabolic signal decline ~8 h at ~25 $\mu g \cdot mL^{-1}$	Conceptual estimate; Demir et al., 2024
QS-inhibition sensor configuration	*Chromobacterium violaceum* CV026	10–20 μM C6-HSL inducer; ~70% violacein repression ~10 h ($IC_{50} \approx$ 5–20 $\mu g \cdot mL^{-1}$)	McClean et al., 1997; Duddy and Bassler, 2021
Field incubation window	3–10 days	Balances growth/signal development with dye/AHL stability constraints	Nichols et al., 2010; Berdy et al., 2017

overcome limitations of *ex situ* workflows (Nichols et al., 2010; Berdy, 2012). Unlike the iChip—which excels at environmental cultivation without built-in detection (Nichols et al., 2010; Ling et al., 2015)—aNP-TRAP integrates a biosensor layer designed to report locally diffusing small molecules (<~1,000 Da) while restricting microbial translocation (Billings et al., 2015). In contrast to lab-only microfluidic/droplet systems (Aoi et al., 2009; Burmeister and Grünberger, 2020; Barakat et al., 2025), the focus here is field-deployability with minimal infrastructure.

To avoid any overstatement, we reiterate that aNP-TRAP is untested and hypothetical; empirical prototyping and validation are required before performance claims can be made. Potential confounders (e.g., environmental pigments/phenolics, AHL hydrolysis, dye photoreduction) warrant rigorous controls and cross-sensor comparison. An initial benchmarking plan is summarized in Table 1, including standards-based calibration (ampicillin, nystatin, C6-HSL) to quantify dose-response, LOD, and specificity. As an alternative readout, GFP-based reporters may provide fluorescence-based viability signals (Chalfie et al., 1994; Andersen et al., 1998). Preserving ecological signals may prime otherwise silent pathways during *in situ* interactions (Traxler et al., 2013).

4 Conclusion

aNP-TRAP is presented as a hypothesis-generating, conceptual, untested device that integrates *in situ* cultivation with functional detection to help triage microbial producers under ecologically relevant conditions. Conceptual "simulations" outline plausible timescales for nutrient equilibration, downward metabolite flux, and biosensor response, but these remain illustrative. The system should be viewed as unvalidated and requiring experimental prototyping and benchmarking as a next step.

Author's note

This manuscript is conceptual and unvalidated experimentally. It was developed with language/figure assistance by AI tools under the author's direction. All scientific ideas, device logic, and design choices are the author's responsibility.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MA: Methodology, Investigation, Conceptualization, Writing – review and editing, Writing – original draft, Formal Analysis.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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