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Whole-Cell SELEX Isolates Species-Specific DNA Aptamers Targeting Aspergillus niger Conidia to Overcome Fungal Cross-Reactivity

Genomics * Biotech & Cellular Systems * Microbiology

The filamentous fungus Aspergillus niger occupies a paradoxical position in modern biotechnology and environmental medicine [1, 2] . Industrially, it is an indispensable workhorse organism utilized worldwide for the submerged fermentation of citric acid, gluconic acid, and recombinant industrial enzymes such as glucoamylase and pectinase [1] . In clinical, food safety, and agricultural settings, however, airborne A. niger spores (conidia) pose severe risks: they cause food spoilage through mycotoxin contamination (including ochratoxin A and fumonisins), provoke severe allergic bronchopulmonary reactions, and trigger life-threatening invasive aspergillosis in severely immunocompromised patients [1, 5] .

Rapid, reliable detection of A. niger has historically been hindered by the extreme morphological and biochemical similarity among members of Aspergillus section Nigri (the black aspergilli), where conventional monoclonal antibodies cross-react with related species such as Aspergillus tubingensis [1, 5] . In a study published in Fungal Biology and Biotechnology , researchers at TU Wien (Vienna University of Technology) led by Valeria Ellena, Andreas Farnleiter, and Matthias Steiger developed an optimized whole-cell SELEX (Systematic Evolution of Ligands by EXponential enrichment) pipeline [1] . By screening randomized single-stranded DNA (ssDNA) libraries against intact conidia, the team successfully isolated high-affinity DNA aptamers that bind specifically to A. niger while completely discriminating against closely related black and green aspergilli [1, 3] .

The Molecular Challenge of the Fungal Spore Surface

Traditional diagnostic assays for fungal pathogens rely on either time-consuming microbiological cultures (which require 3 to 7 days for sporulation), polymerase chain reaction (PCR) amplification, or antibody-based immunoassays such as enzyme-linked immunosorbent assays (ELISA) [1, 5] . While PCR provides genetic specificity, it requires cell lysis, DNA extraction, and thermal cycling instrumentation, making rapid on-site environmental monitoring difficult [1] . Conversely, antibody-based detection targets surface-exposed epitopes directly on intact spores but frequently suffers from high batch-to-batch variation, high production costs in animal hosts, and severe cross-reactivity [1, 3] .

The fungal spore wall is a dense, multilayered composite of beta-1,3-glucan, beta-1,6-glucan, chitin , and galactomannan, topped by an outer hydrophobic sheath termed the rodlet layer [1, 5] . In Aspergillus species, this outer protective coat is assembled from small, cysteine -rich proteins known as hydrophobins (such as RodA), cross-linked by melanin pigments [1, 5] . Because hydrophobin architectures are conserved across Aspergillus section Nigri , antibodies generated against

isolated recombinant proteins frequently fail to distinguish between different black aspergilli in their native, intact conidial state [1, 5] .

Whole-Cell SELEX: Evolving Oligonucleotides Against Intact Cells

To overcome this barrier without requiring prior purification of fragile surface antigens, the TU Wien team implemented whole-cell SELEX (Cell-SELEX), a directed evolution technique pioneered by Tan and colleagues [1, 3] . The process operates on a combinatorial synthetic library containing approximately 10 14 unique 80-nucleotide single-stranded DNA molecules, each featuring a central randomized 40-nucleotide sequence flanked by constant primer-binding sites [1, 3] .

Rather than panning against immobilized recombinant proteins on microtiter plates, whole-cell SELEX exposes the oligonucleotide pool directly to viable, intact fungal conidia in liquid suspension [1, 3] :

* Positive Selection : The folded ssDNA library is incubated with intact A. niger conidia. Spore-binding oligonucleotides partition onto the cell surface via shape complementarity, electrostatic interactions, and hydrogen bonding [1, 3] .

* Centrifugal Partitioning & Thermal Elution: Unbound oligonucleotides are removed through repeated centrifugal washing. Conidia-bound sequences are subsequently released into solution by thermal denaturation at 95 deg C [1, 3] .

* Negative Counter-Selection: To eliminate cross-reactive sequences that bind conserved fungal polysaccharides or shared hydrophobin motifs, the enriched pool is incubated with counter-target species -- specifically Aspergillus tubingensis and the model green aspergillus Aspergillus nidulans [1, 3] . Only oligonucleotides remaining in the supernatant (unbound to non-target cells) are collected.

* Asymmetric PCR & Strand Separation: The counter-selected pool is PCR-amplified using biotinylated primers and converted back to pure single-stranded DNA using streptavidin magnetic beads, generating an enriched input library for the next selection cycle [1, 3] .

Biophysical Telemetry & Binding Kinetics of Selected Aptamers

By coupling nine iterative rounds of selection with high-throughput next-generation sequencing (NGS), the researchers monitored sequence convergence and identified dominant candidate clusters, led by aptamers AN01-R9-006 and AN02-R9-185 [1] .

| Aptamer / Metric | | AN01-R9-006 | | AN02-R9-185 | | Diagnostic & Mechanistic Significance |

| Oligonucleotide Class | | Single-stranded DNA (80 nt) | | Single-stranded DNA (80 nt) | | Chemically stable, synthetic recognition probe [1, 3] . |

| Target Cell Stage | | Intact A. niger conidia | | Intact A. niger conidia | | Binds native surface epitopes without spore lysis [1] . |

| Apparent Binding Affinity (K d) | | Nanomolar range (high affinity) | | Nanomolar range (high affinity) | | Matches or exceeds typical monoclonal antibody affinities [1, 3] . |

| Cross-Reactivity vs. A. tubingensis | | None detected (<2% background) | | None

detected (<3% background) | | Successfully discriminates cryptic sibling species within section Nigri [1] . |

| Cross-Reactivity vs. A. nidulans | | Negligible | | Negligible | | Confirms complete selectivity against divergent aspergilli [1] . |

| Structural Motif | | Stem-loop hairpin structure | | G-quadruplex / bulge domain | | Forms rigid tertiary folds required for target binding [1, 4] . |

Structural Mechanism of Target Discrimination

Flow cytometry and confocal fluorescence microscopy confirmed that fluorophore-labeled AN01-R9-006 and AN02-R9-185 decorate the outer surface of A. niger conidia with dense, uniform ring-like patterns [1] . In contrast, incubation with A. tubingensis -- a sibling species that differs from A. niger by subtle variations in cell-wall mannoprotein glycosylation and secondary metabolite profiles -- yielded no significant fluorescence signal above baseline controls [1] .

Secondary structure modeling indicates that the randomized 40-nucleotide cores fold into stable stem-loop hairpins and guanine-rich tertiary pockets stabilized by magnesium cations (Mg^{2+}) [1, 4] . These folded DNA pockets fit precisely into unique spatial arrangements of surface glycoproteins on the A. niger rodlet layer, providing exquisite target discrimination that conventional antibodies raised against denatured antigens routinely fail to achieve [1, 3, 5] .

Applications Across Industrial Fermentation and Clinical Mycology

The successful isolation of species-specific conidial aptamers provides a versatile platform for real-time fungal surveillance across multiple biotechnology sectors [1, 2] :

* In-Line Bioprocess Quality Control: In large-scale industrial fermentation plants producing citric acid and technical enzymes, early contamination by wild-type or foreign fungal strains can disrupt production runs [1] . DNA aptamers can be integrated into microfluidic biosensors to monitor seed cultures and verify strain purity in real time [1, 3] .

* Rapid Food and Feed Safety Testing: Black aspergilli are notorious post-harvest contaminants of grapes, coffee, maize, and tree nuts, where they produce hazardous mycotoxins [1] . Aptamer-functionalized lateral flow strips (aptamer dipsticks) allow field inspectors to screen agricultural commodities in minutes without laboratory culturing [1, 2] .

* Environmental and Point-of-Care Diagnostics: In clinical settings and hospital heating, ventilation, and air conditioning (HVAC) systems, rapid detection of airborne mold conidia is vital for protecting neutropenic and organ-transplant patients [1, 5] . Aptamers offer superior thermal stability, lower synthesis costs, and simple fluorescent or electrochemical conjugation compared to animal-derived antibodies, providing a scalable foundation for next-generation biosensors [1, 3, 4] .

References & Primary Sources

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