Biosecurity Threats about the Combination of Aerobiology, Morphology and Genetic Manipulation of Fungal Spores

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Abstract: The passive flight of large numbers of mitospores ensures propagation and survival of many fungal species. Moreover, many fungi have a surplus DNA context which allows for easier manipulation and tampering, especially when inserting eukaryotic genes and translation systems. These facts taken together suggest a high prominence of the sporogonic airborne fungi in biosecurity/biodefence priority lists, but due to the low virulence and mortality of the fungal pathogens, they do not figure high, or, sometimes, not at all. The disruptive effect of biosecurity fungal threats is important per se and genetic manipulations are to further it. Novel, long range and high-persistence sampling methods, using robotics and unmanned systems already fielded in other applications may extend surveillance and responsive sampling. Effects-based, proteomic approaches may provide a solution for deliberate incidents, while polyphasic taxonomy may resolve spontaneous incidents and assist medical diagnosis, but in the biosecurity context, where mass collection and processing of samples and speed in producing robust results are vital, complex approaches might be proved more of a hindrance than an asset.

Key words: Aerobiology, biosecurity, spore aerodynamics, genetic manipulation.

1. The Benign Present: Aerobiology, Morphology and Dispersion

Insightful identification of emerging threats, not restricted to the near-term, is of paramount importance for focusing vigilance and developing countermeasures. In the case of biosecurity, attention is currently paid almost exclusively to highly virulent agents considered as immediate threats. Thus, a gap in projected biosecurity and biodefence capability will occur in the near future stemming from microorganisms of lesser immediate weaponization potential but possessing considerable “growth margin”, defined as an existing set of qualities favoring focused manipulation for enhancement of

desired attributes. Fungi comprise the most flyable microorganisms. Fungal spores are produced with flight provisions and complex flight initiation mechanisms (best example of the latter being the ballistospores). No other microorganism shows such provisions: bacterial spores more often than not are simply hardened hypobiotic forms, which might or might not disperse by passive flight [1]. Viruses, on the other hand, in some cases are meant for airborne dispersion, thus causing cough or sneezing, but their size and limited genetic space deny evolutionary application of any custom aerodynamics, which, in any case, might well not function at such extremely small sizes. The viral airborne dispersion is accomplished rather by droplet formation than by any other passive flight mechanism [2].

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The passive flight of large numbers of mitospores ensures propagation and survival of the fungal species. Spores have been found near the polar cycle and typing patterns suggest very distant origin [3]. By such a mechanism, diverse habitats can be reached and colonized by large numbers of spores, thus ensuring propagation of the species when conditions are favourable, and increasing probability of survival of the species whenever conditions turn disfavourable. The larger the flight distance and the number of spores flying, the higher the probability for a favourable habitat to be reached and colonized somewhere.

Such dispersion and survivability provisions are not necessarily made with human virulence as their epicenter. Usable habitats usually comprise of crops and rotting/dead matter; mobility and transportability are of prime importance in such cases, and a hydrophobic layer coating the spore allows decent buoyancy even in high humidity. Perhaps the best flyer is *Aspergillus fumigatus* mitospore [4]. It is relatively shallow, long strakes actually form multiple wings [5]. As these entail low aspect ratio (span square by wing surface or wingspan by wingcord), they produce ample lift surface individually (compared with spiked forms, as with *Aspergillus niger*), whereas being small of span (2-3 μm) [6], they allow better evasion through non-solid surfaced barriers, and most importantly, they remain under the 5- μm threshold of the upper respiratory tract epithelial cilia [7]. As a whole, the strakes produce a triplane (or, rather, multiplane) effect generating ample lift. Taking into consideration that the spore flight is unpowered and, most importantly, unsteered and largely probabilistic, the lack of axial form, which would improve aerodynamic qualities, is well compensated by the increased probability inherent in point-symmetry shapes to take advantage of any microflow and microcurrent to move to some/any direction without the confines of destination that is, to disperse.

2. Evolution and Development of the Threat: Virulence, Applicability and Weaponization

Pathogenicity and virulence factors of *A. fumigatus* are moderately evolved and dedicated: they range from attachment mechanics (spores' strakes shape) and adhesion methods (by biochemical interactions, i.e., by adhesins [7]) to immune system deactivation and distraction/evasion strategies [8]. But the net result is the overall low pathogenicity and virulence of the species and fungal species in general (and perhaps of the kingdom as a whole [9]), to immunocompetent, air- or ingestion-infected populations. This fact clearly negates any serious bioterrorism/biowarfare applications, with the possible exception of food degradation and agroterrorism. Fungi, though, have a most interesting feature: relatively huge quantities of "spare" DNA [10-12], especially compared to the more compact viral genomes [13], a fact of unknown firm aetiology. It possibly implies poor genome control mechanisms, but it still allows for genetic manipulation experiments far more freely than in stricter and tighter genomes. New insertion techniques, coupled with the latest, high-throughput molecular methods and almost-instant extracting and amplification protocols allow short confirmation of the manipulation results and fast operation (trial-and-error) cycles. Extremely lethal eukaryotic toxins of plant or animal origin, possibly accompanied by their whole regulatory transcription system can be inserted in the "empty" DNA space without much interaction problems, since the molecular mechanisms are eukaryotic in both receptor and donor cells; targeted insertion, once the full receptor genome is known, facilitates the issue. Thus, tampered fungal strains with *A. fumigatus* volatility and dispersion and cobra's venom lethality could be created and dispersed readily and perhaps stealthily, causing unexplained massive syndromes. Dynamic transcriptome studies, especially of the biofilm vegetative phase [6], provide a map and a manual for inserting and regulating such allogeneous gene products. Moreover, surface toxins on the conidial

phase, as is the case with *Stachybotrys chartarum* trichothecenes [14], could be inserted, thus disposing of any need of regulation through and after germination, and making inhaled conidia spontaneously toxic or hypotoxic.

3. Responding to the Threat: Vigilance and Surveillance

Compared to *Bacillus anthracis*, the prominent bioweapon [1], *Aspergillus fumigatus* spores travel much further without costly fabrication/manipulation techniques to improve flight characteristics of its conidia. Due to the low virulence and mortality of the fungal pathogens, they are not included in such lists but sporadically. This is so, mainly because they are compiled by medical-oriented and trained personnel [15]. Though, the disruptive effect of fungal-based biosecurity threats is important and genetic manipulations are to further destructive potential as well to unheard-of levels.

These facts taken together suggest a high prominence of the sporogonic airborne fungi in biosecurity/biodefence priority lists [9]. As aerial delivery is by far more cost-effective and massive than any liquid or solid-body contamination, it is obviously the epicenter of our vigilance or it should be. Years of biotechnological research and development can be used to insert exogenous genes and regulatory mechanisms, creating hybrid bioweapons which must be detected using binary algorithms, one for the vector cell and one for the effector molecule, organelle or mechanism. This binary detection and classification leads by definition to a merge of very different techniques, since some techniques are suitable for detecting and classifying cells, but very different ones must be used for effector molecules.

In such cases, even the most modern monitoring approaches, based on database compilation and imaging procedure of collected data may prove inadequate. Public health-minded procedures are ineffective against well-prepared and informed

perpetrators. Much more costly, but hugely more responsive and adaptable monitoring and sampling practices should be investigated [16], making full use of unmanned vehicles' technology, as proposed by Boeing with the Scan Eagle UAV in 2007 [17]. Effects-based, proteomics or metabolomics approaches may well be a possible solution for perpetrator-induced incidents in the mid-term, whereas polyphasic taxonomy [18] may revolutionize the resolution of spontaneously originated incidents and medical diagnosis and taxonomy, allowing a better, multi-aspect characterization of isolates, thus circumventing any problems posed to enlisted, inadequately specialized personnel by atypical or cryptic factors. Robust identification and taxonomic tools are needed for developing a correct fungal and microbial airflora picture. This picture will be the background against which samples will be compared by decision algorithms to establish perpetrated incidents and/or initiate staged alerts.

Nonetheless, in actual biosecurity procedures, such complex approach—polyphasic taxonomy might be proved more of a hindrance than an asset and in any case require considerable development time, and once fielded, it might still require multiple preparations and sensitive and expensive instrumentation to run and other high fidelity practices, which consist of efficiency in quick procession of large numbers of samples. It is also most probable that the perpetrator shall have taken every practical precaution to hinder detection, classification and tracking of the agent used. Some of the polyphasic taxonomy's discrete tools, though, such as secondary metabolite characterization and molecular taxonomy could be of interest, as their individual use may provide classification of the vector (the latter) and detection of the deleterious agent (the former) in a rather tight lab context, or, at the near future, in a combined and integrated sensor package.

Near-term surveillance and detection methods, fusing suitable equipment and protocols from both the security/military and the medical communities, might

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prove eventually handy, with minimal adaptation. Anti-contamination clothing, such as silver containing fabric (Pandis et al., 2010, unpublished result), could be issued to enlarged high-risk groups as standard. Sampling, (collection and handling of samples) can be improved in remote sites as an addition to automated surveillance equipment. Such improvements refer to protocols, materials, transportation, delegation of processing steps and networking for distribution of results and informed, prompt decision-making. Beyond such steps, only a concerted, long-term developmental effort with clear objectives and contingency priority roadmaps to adapt to recurring data on perpetrators' progress could eventually shut the Pandora's box opened by diffusion of potent genetic modification technologies.

References

- [1] T.V. Inglesby, T. O'Toole, D.A. Henderson, J.G. Bartlett, M.S. Ascher, E. Eitzen, et al., Anthrax as a biological weapon, *Journal of American Medical Association* 287 (17) (2002) 2236-2251.
- [2] A.I. Donaldson, S. Alexandersen, Predicting the spread of foot and mouth disease by airborne virus, *Scientific and Technical Review International Office of Epizootics* 21 (3) (2002) 569-575.
- [3] D.W. Denning, K.V. Clemons, L.H. Hanson, D.A. Stevens, Restriction endonuclease analysis of total cellular DNA of *Aspergillus fumigatus* isolates of geographically and epidemiologically diverse origin, *Journal of Infectious Diseases* 162 (1990) 1151-1158.
- [4] A.R. Asif, M. Oellerich, V.W. Armstrong, B. Riemenschneider, M. Monod, U. Reichard, Proteome of conidial surface associated proteins of *Aspergillus fumigatus* reflecting potential vaccine candidates and allergens, *Journal of Proteome Research* 5 (2006) 954-962.
- [5] B. Jahn, A. Koch, A. Schmidt, G. Wanner, H. Gehringer, S. Bhakdi, et al., Isolation and characterization of a pigmentless-conidium mutant of *Aspergillus fumigatus* with altered conidial surface and reduced virulence, *Infection and Immunity* 65 (12) (1997) 5110-5117.
- [6] J.G. Gibbons, A. Beauvais, R. Beau, K.L. McGary, J.P. Latgé, A. Rokas, Global transcriptome changes underlying colony growth in the opportunistic human pathogen *Aspergillus fumigatus*, *Eukaryotic Cell* 11 (1) (2012) 68-78.
- [7] J.P. Latge, *Aspergillus fumigatus* and aspergillosis, *Clinical Microbiology Reviews* 12 (1999) 310-350.
- [8] M.E. Kambouris, A. Velegraki, *Aspergillus fumigatus*, *A. flavus* and *A. niger*: Aerodynamic, immunological and metabolic virulence determinants, *Archives of Hellenic Medicine* 18 (1) (2001) 20-34. (in Greek with English abstract)
- [9] A. Casadevall, L. Pirofski, The weapon potential of human pathogenic fungi, *Medical Mycology* 44 (2006) 689-696.
- [10] W.C. Nierman, A. Pain, M.J. Anderson, J.R. Wortman, H.S. Kim, J. Arroyo, et al., Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*, *Nature* 438 (2005) 1151-1156.
- [11] D.W. Denning, M.J. Anderson, G. Turner, J.P. Latgé, J.W. Bennett, Sequencing the *Aspergillus fumigatus* genome, *The Lancet Infectious Diseases* 2 (4) (2002) 251-253.
- [12] S.E. Ahnerta, T.M.A. Finkb, A. Zinovyev, How much non-coding DNA do eukaryotes require, *Journal of Theoretical Biology* 252 (2008) 587-592.
- [13] A.E. Firth, C.M. Brown, Detecting overlapping coding sequences in virus genomes, *BMC Bioinformatics* 7 (2006) 75-81.
- [14] D.G. Panaccione, C.M. Coyle, Abundant respirable ergot alkaloids from the common airborne fungus *Aspergillus fumigatus*, *Applied and Environmental Microbiology* 71 (6) (2005) 3106-3111.
- [15] A. Casadevall, L. Pirofski, The weapon potential of a microbe, *Trends in Microbiology* 12 (2004) 259-263.
- [16] K. Grivas, A. Velegraki, M.E. Kambouris, Mid-term deployability and geointegration concerns in biodefense sampling and detection hardware design and procedures, *Defensor Pacis* 22 (2008) 111-116.
- [17] B. Alkire, J.G. Kallimani, P.A. Wilson, L.R. Moore, Applications for Navy Unmanned Aircraft Systems [online], RAND Corporation, Santa Monica, CA, USA, 2010, pp 20-21, http://www.rand.org/content/dam/rand/pubs/monographs/2010/RAND_MG957.pdf
- [18] R.A. Samson, S. Hong, S.W. Peterson, J.C. Frisvad, J. Varga, Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*, *Studies in Mycology* 59 (2007) 147-203.