Indoor microbes and the future of indoor environmental assessment

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Disclosures

- Sporometrics Inc (sole owner)
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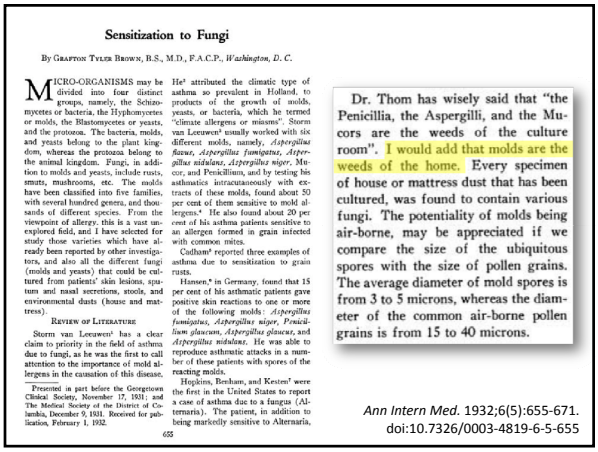


Sensitization to Fungi

By Grafton Tyler Brown, B.S., M.D., F.A.C.P., *Washington, D. C.*

MICRO-ORGANISMS may be divided into four distinct groups, namely, the Schizomycetes or bacteria, the Hyphomycetes or molds, the Blastomycetes or yeasts, and the protozoa. The bacteria, molds, and yeasts belong to the plant kingdom, whereas the protozoa belong to the animal kingdom. Fungi, in addition to molds and yeasts, include rusts, smuts, mushrooms, etc. The molds have been classified into five families, with several hundred genera, and thousands of different species. From the viewpoint of allergy, this is a vast unexplored field, and I have selected for study those varieties which have already been reported by other investigators, and also all the different fungi (molds and yeasts) that could be cultured from patients' skin lesions, sputum and nasal secretions, stools, and environmental dusts (house and mattress).

Review of Literature

Storm van Leeuwen[1] has a clear claim to priority in the field of asthma due to fungi, as he was the first to call attention to the importance of mold allergens in the causation of this disease.

Presented in part before the Georgetown Clinical Society, November 17, 1931; and The Medical Society of the District of Columbia, December 9, 1931. Received for publication, February 1, 1932.

He[2] attributed the climatic type of asthma so prevalent in Holland, to products of the growth of molds, yeasts, or bacteria, which he termed "climate allergens or miasms". Storm van Leeuwen[3] usually worked with six different molds, namely, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, Mucor, and Penicillium, and by testing his asthmatics intracutaneously with extracts of these molds, found about 50 per cent of them sensitive to mold allergens.[4] He also found about 20 per cent of his asthma patients sensitive to an allergen formed in grain infected with common mites.

Cadham[5] reported three examples of asthma due to sensitization to grain rusts.

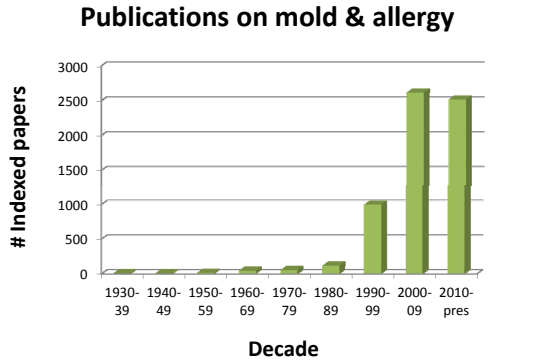
Hansen,[6] in Germany, found that 15 per cent of his asthmatic patients gave positive skin reactions to one or more of the following molds: *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium glaucum*, *Aspergillus glaucus*, and *Aspergillus nidulans*. He was able to reproduce asthmatic attacks in a number of these patients with spores of the reacting molds.

Hopkins, Benham, and Kesten[7] were the first in the United States to report a case of asthma due to a fungus (Alternaria). The patient, in addition to being markedly sensitive to Alternaria,

655

Dr. Thom has wisely said that "the Penicillia, the Aspergilli, and the Mucors are the weeds of the culture room". I would add that molds are the weeds of the home. Every specimen of house or mattress dust that has been cultured, was found to contain various fungi. The potentiality of molds being air-borne, may be appreciated if we compare the size of the ubiquitous spores with the size of pollen grains. The average diameter of mold spores is from 3 to 5 microns, whereas the diameter of the common air-borne pollen grains is from 15 to 40 microns.

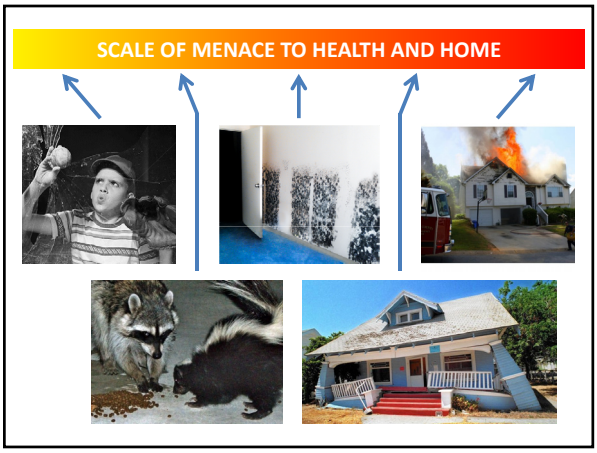
Ann Intern Med. 1932;6(5):655-671.
doi:10.7326/0003-4819-6-5-655



source: ISI Web of Science, accessed 2016-07-30
search term: (mold OR mould OR fung*) AND (allerg* OR asthma)

What have we learned about health?

- Allergy and asthma is epidemic in children in westernized countries
  - about half of the burden of disease appears to have an environmental basis
- Few large population health studies have included environmental measurement
  - from those and others, we know that:
    - children living in moldy houses are sicker
    - complex gene-environment interactions alter the trajectory of allergic/ asthmatic phenotype

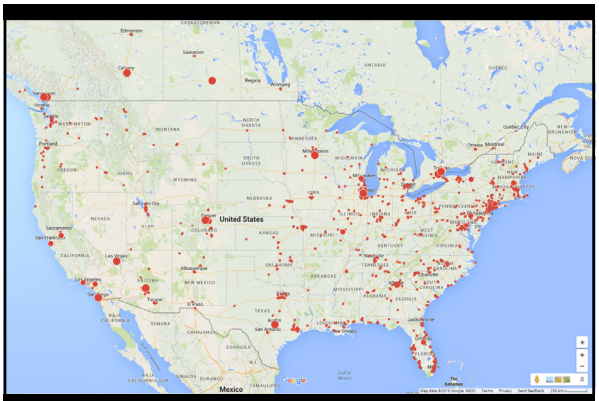


Why have we wanted to assess microbial populations?

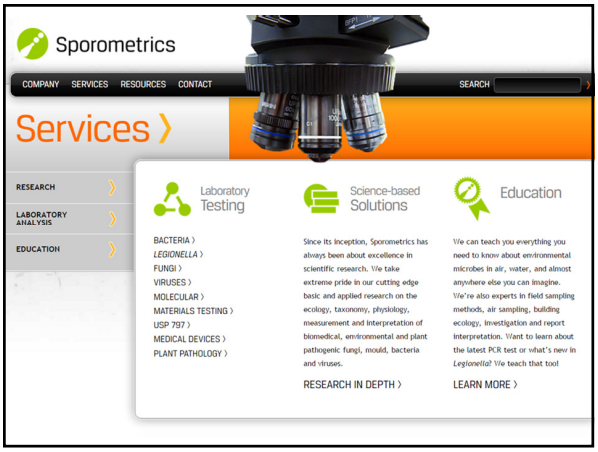
- Exposure assessment
  - evaluates exposure to predict health risk
  - problematic for reasons AIHA & ACGIH have given
    - airborne microbes are complex mixtures; measurement units are not dose-relevant; individual susceptibility, etc.
- Building health
  - queries the presence of indoor growth
  - informs actions to forestall biodeterioration
    - useful for building sustainability and health protection

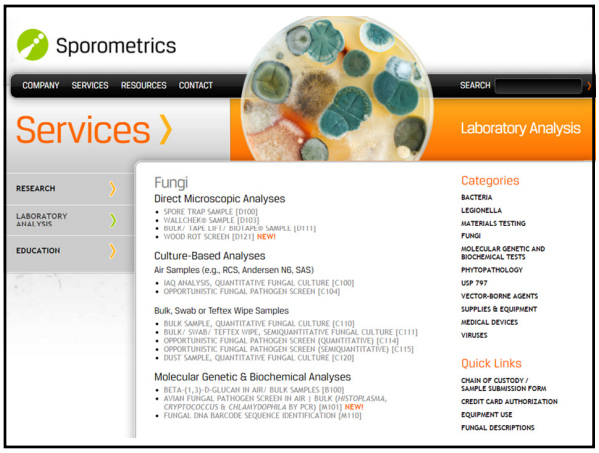
Indoor microbiological testing

- fundamental tenet
- common types of testing

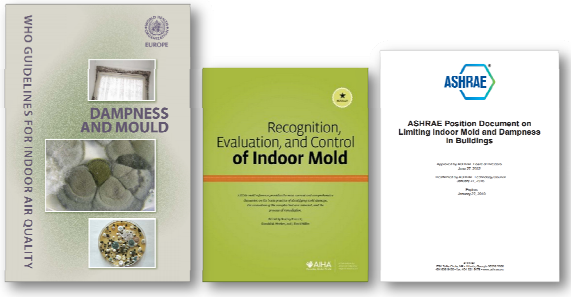


Mold consultants/ environmental testing laboratories in North America (●; larger = paid listing; data from Google Maps query on search term *mold+testing*; identities anonymized); 05/2016





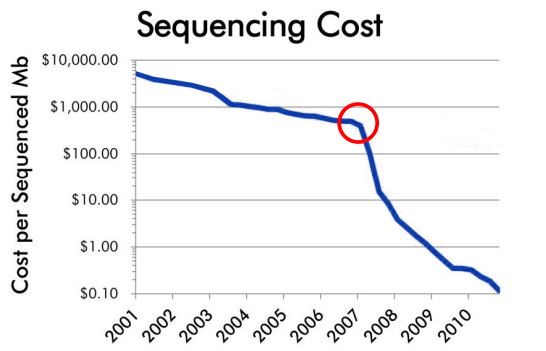
Merits & limitations of current approaches



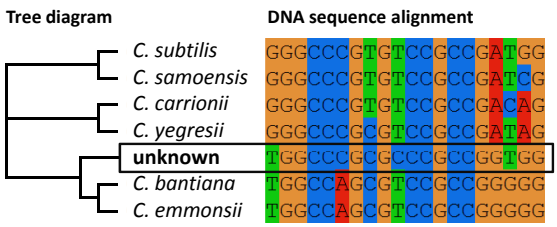
What have we learned about sampling?

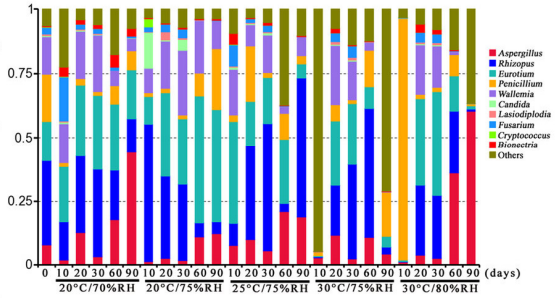
1. Routine air sampling is uselessness for assessing health-relevant microbial exposure
   - exceptions are in health care and manufacturing
2. Assessing indoor microbial growth is better accomplished by inspection than sampling
3. Current analytical approaches yield poor quantitive/ qualitative agreement and reproducibility between laboratories
   - culture, microscopy, qPCR, etc.

Microbiology of the Built Environment 2.0:
High Throughput Sequencing (HTS)

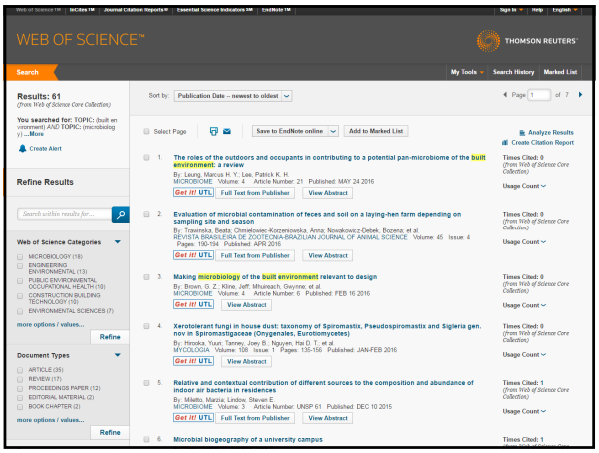


adapted from doi:10.3835/plantgenome2012.05.0005



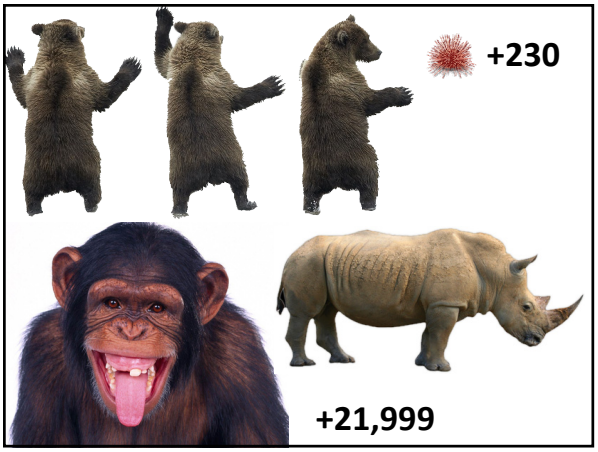


doi:10.1038/srep25930



High-throughput DNA sequencing

- Stuff we already knew or suspected
  - conditions of use/ outdoor climate/ biogeography
  - [– dampness]
- Some things we didn't know
  - many fungi that are "sterile" in culture are probably of outdoor origin (e.g., plant pathogens)
  - "indoor mould" is an unexpectedly subtle perturbant
  - human-associated yeasts are extremely common
  - membership in decay assemblages on building materials is poorly understood from standard methods (esp. basids)
  - but some caution is needed...



Research applications of HTS methods

BUILDINGS

- Microscopy/ culture are cheap and easy but alone give poor resolution of decay communities
  - HTS → better characterization of decay agents facilitating the understanding of materials failures

HEALTH

- qPCR methods have shown some promise in predicting health outcomes (with limitations)
  - HTS → vastly better information at less cost

Practical uses of HTS methods

(assuming we can figure out what it all means)

- evaluation of stringent environments
  - health care (nosocomial agents); manufacturing; etc.
  - (may need a robust way to "filter" for living/ non-living)
- "next-level" investigation for hidden mould
  - the limitation of HTS to report moisture damage can probably be over by better ecological signal processing
  - better detection of structure-relevant agents, e.g., dry rot
- rapid, sensitive testing for other organisms
  - mites, insects, bedbugs, roaches, etc.

What is neccessary before practitioners adopt HTS methods for building assessments?

(in other words, what answers do I need before I can sell HTS as a test?)

Consultants will ask:

- How much does it cost?
  - I think we're almost there
- What do the results mean?
  - helpful if there is a geospatial database of "normal"
- Do my clients need it?
  - mostly the answer for any type of testing is "probably not"
- How long does it take to get the results?
  - nobody will buy the test if it takes longer than 48 hr

Acknowledgements

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

AllerGen Allergy, Genes and Environment Network / Le réseau des allergies, des gènes et de l'environnement

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