RESEARCH ARTICLE

# REVISED Phenotype profiling of white-nose syndrome pathogen *Pseudogymnoascus destructans* and closely-related *Pseudogymnoascus pannorum* reveals metabolic differences underlying fungal lifestyles [version 2; referees: 3 approved]

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**v2** **First published:** 25 May 2018, **7**:665 (doi: 10.12688/f1000research.15067.1)
**Latest published:** 17 Jul 2018, **7**:665 (doi: 10.12688/f1000research.15067.2)

## Abstract

**Background:** *Pseudogymnoascus destructans*, a psychrophile, causes bat white-nose syndrome (WNS). *Pseudogymnoascus pannorum*, a closely related fungus, causes human and canine diseases rarely. Both pathogens were reported from the same mines and caves in the United States, but only *P. destructans* caused WNS. Earlier genome comparisons revealed that *P. pannorum* contained more deduced proteins with ascribed enzymatic functions than *P. destructans*.
**Methods:** We performed metabolic profiling with Biolog PM microarray plates to confirm *in silico* gene predictions.
**Results:** *P. pannorum* utilized 78 of 190 carbon sources (41%), and 41 of 91 nitrogen compounds (43%) tested. *P. destructans* used 23 carbon compounds (12%) and 23 nitrogen compounds (24%). *P. destructans* exhibited more robust growth on the phosphorous compounds and nutrient supplements (83% and 15%, respectively) compared to *P. pannorum* (27% and 1%, respectively.). *P. pannorum* exhibited higher tolerance to osmolytes, pH extremes, and a variety of chemical compounds than *P. destructans*.
**Conclusions:** An abundance of carbohydrate degradation pathways combined with robust stress tolerance provided clues for the soil distribution of *P. pannorum*. The limited metabolic profile of *P. destructans* was compatible with *in silico* predictions of far fewer proteins and enzymes. *P. destructans* ability to catabolize diverse phosphorous and nutrient supplements might be critical in the colonization and invasion of bat tissues. The present study of 1,047 different metabolic activities provides a framework for future gene-function investigations of the unique biology of the psychrophilic fungi.

## Keywords

Psychrophilic fungi, phenotype microarray, metabolism, catabolism, gene function

**Open Peer Review**

**Referee Status:** ✓ ✓ ✓

| | Invited Referees 1 | 2 | 3 |
|---|---|---|---|
| REVISED **version 2** published 17 Jul 2018 | ✓ report | | ✓ report |
| **version 1** published 25 May 2018 | ? report | ✓ report | ? report |

1 **Christine Salomon**, University of Minnesota, USA

2 **Christopher T Cornelison**, Kennesaw State University, USA

3 **Flavia Pinzari**, Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Italy

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**Corresponding author:** Vishnu Chaturvedi (Vishnu.Chaturvedi@health.ny.gov)

**Author roles: Chaturvedi V**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **DeFiglio H**: Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – Review & Editing; **Chaturvedi S**: Conceptualization, Funding Acquisition, Investigation, Project Administration, Resources, Supervision, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** This study was supported by the National Science Foundation (Award Number 1203528).
*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*



**How to cite this article:** Chaturvedi V, DeFiglio H and Chaturvedi S. **Phenotype profiling of white-nose syndrome pathogen *Pseudogymnoascus destructans* and closely-related *Pseudogymnoascus pannorum* reveals metabolic differences underlying fungal lifestyles [version 2; referees: 3 approved]** *F1000Research* 2018, **7**:665 (doi: 10.12688/f1000research.15067.2)

**First published:** 25 May 2018, **7**:665 (doi: 10.12688/f1000research.15067.1)

**REVISED** **Amendments from Version 1**

We modified/added a number of items in response to the reviewers' comments. The main changes are:

1. Growth comparison and heat map scale details were added
2. The original Figure 1 was deleted on the advice of Reviewer 3. The results for the entire study are available as Dataset 1–Dataset 4
3. Figure legends were improved
4. Two additional references were added to provide a wider context for the phenotype microarray applications and statistical tools for the data analysis
5. The preliminary nature of the findings and study limitations are explicit
6. We have added Biolog maps of PM 1-10, and PM 21, 23-25 plates as Supplementary File 1 to allow for easy access to list of test compounds tested
7. We have also posted detailed response to each reviewer

**See referee reports**

## Introduction

*Pseudogymnoascus destructans* causes white-nose syndrome (WNS), a disseminated disease afflicting hibernating bats in North America since 2006[1–3]. WNS is linked to mass mortality and now afflicts bats over large geographic areas in the United States and Canada. *P. destructans*' pathogenic mechanisms remain mysterious especially as no other human or animal fungal pathogen expresses virulence attributes at such low temperatures. *Pseudogymnoascus pannorum*, a closely related fungus, is widely distributed in the soil and substrates of caves and mines in North America[3]. *P. pannorum* grows both at psychrophilic and mesophilic temperature ranges and causes human and canine diseases rarely[4]. However, *P. pannorum* does not cause any disease in hibernating bats. These facts raise the exciting possibilities that *P. destructans* is more specialized for the pathogenic lifestyle on bats while *P. pannorum* successfully colonizes a broader range of substrates in nature.

Environmental studies on the psychrophilic and psychrotolerant fungi documented the versatility of *Pseudogymnoascus* (*Geomyces*) *pannorum* for the utilization of complex carbohydrates and keratin-enriched substrates, and tolerance to high salt[5–7]. Additional laboratory studies demonstrated extensive saprotrophic enzymatic activities that would allow resource capture by the non-pathogenic *Pseudogymnoascus* species vis-a-vis *P. destructans*[8,9]. *P. destructans* is known to secrete proteolytic, lipolytic, and keratinolytic exoenzymes, and possesses specialized catabolic activities that contribute to its growth and survival in the nutrient-poor caves and mines[2,10].

Although their draft genomes are similar in size (~30 Mb), there are numerous repeats and far fewer proteins and enzymes in *P. destructans* (2,052 proteins) than in *P. pannorum* (2,734 proteins)[11]. In the present study, we report the results of extensive Biolog Phenotype Microarray metabolic profiling to confirm *in silico* gene predictions, and find clues for the different lifestyles of these psychrophilic fungi.

## Methods

The metabolic analysis was conducted using *P. destructans* (M1379) and *P. pannorum* (M1372)[11]. The PM1-10 and PM21, 23–25 phenotype microarray plates were procured from Biolog, Hayward, CA. The fungal spores were harvested in sterile water from 3 - 5-week-old, heavily sporulating culture on potato dextrose agar (PDA) flasks at 15°C. In preliminary experiments, spore counts and viability were determined on agar plates using a hemocytometer and colony forming units (CFU). For the final tests, the spores were harvested, washed once in sterile water by centrifugation, and the suspension adjusted to an $OD_{600}$ = 0.2 (transmittance = 62%). This suspension equated to between 550 and 950 spores per well via hemocytometer count, and 250–500 spores per well by CFU. In preliminary experiments, the two fungi grew at different growth rates and comparable growth was observed after day 7 for *P. pannorum* and day 10 for *P. destructans* (details not shown). Further incubation of the plates beyond the observation period did not change the observed growth pattern.

The PM plates were inoculated per Biolog protocol and incubated at 15°C[12,13]. The presence or absence of growth was measured by $OD_{600}$ on day 10 for *P. destructans*, and day 7 for *P. pannorum*. Negative control wells were weakly growth positive for both *P. destructans* and *P. pannorum*. This observation was also reported for Biolog PM plates in another study[13] Therefore, the corresponding negative control well reading from each experiment were averaged together and used to normalize the OD values averages for each test compound. For the heat map visualization, the negative control reading was assigned a score of 0.0 and the positive growth scored on a 0.0 – 1.0 scale. The phenotypic assay was repeated once. The limited dataset precluded any quantitative statistical analysis.

## Results

Nearly 1,047 different metabolic activities were analyzed for each test fungus (Datasets 1–4[14]). *P. pannorum* metabolized far more carbon and nitrogen compounds; *P. destructans* exhibited prominent activity on phosphorous compounds and nutrient supplements (Figure 1). *P. pannorum* utilized 78 of 190 carbon compounds (41%), and 41 of 91 nitrogen compounds (43%) tested. *P. destructans* used 23 carbon compounds (12%) and 23 nitrogen compounds (24%). *P. destructans* exhibited more robust growth on the phosphorous sources and nutrient supplements (83% and 15%, respectively) compared to *P. pannorum* (27% and 1%, respectively.). *P. pannorum* metabolized nearly all carbon intermediates in the major fungal metabolic cycles[13] (Figure 2). *P. destructans* utilized only a few simple sugars in glycolysis with no activity on a range of carbon intermediates. *P. pannorum* used a wider variety of nitrogen sources including amino acids, amino bases, and alkanes while *P. destructans* had a preference for the simple N sources and dipeptides[13] (Figure 3). Most phosphorous sources tested supported the growth of *P. destructans* while *P. pannorum* only grew on few phosphosugars and phosphorylated nucleosides (Figure 4). Both fungi did not utilize sulfur intermediates (Datasets 1–4[14]). Fifteen of ninety-five nutrient supplements supported good growth of *P. destructans* while *P. pannorum* grew only on D-Pantothenic acid (Supplementary files). *P. pannorum* grew at very high salt concentrations and extreme acidic and basic pH ranges while *P. destructans* was sensitive to high salt (diminished growth ≥ 1% NaCl) and basic pH (diminished growth > pH 8.5) (Figure 5). *P. pannorum* showed extreme tolerance to 96 xenobiotics in PM21, PM23 - PM25 plates in contrast to severe sensitivity observed in *P. destructans* (details not shown).

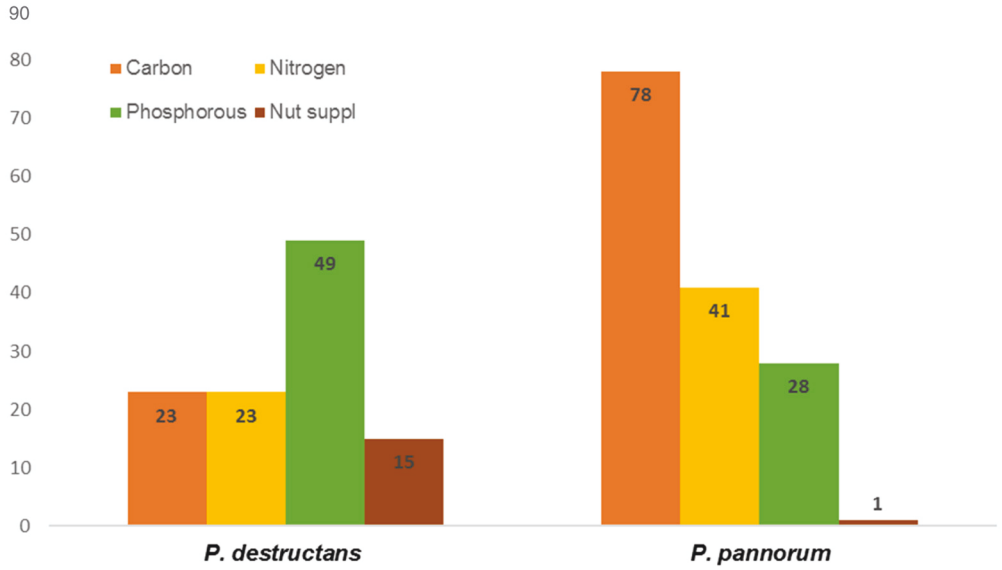


**Figure 1. A comparison of carbon, nitrogen, phosphorous and nutrient supplements utilized by *Pseudogymnoascus destructans* and *Pseudogymnoascus pannorum*.**

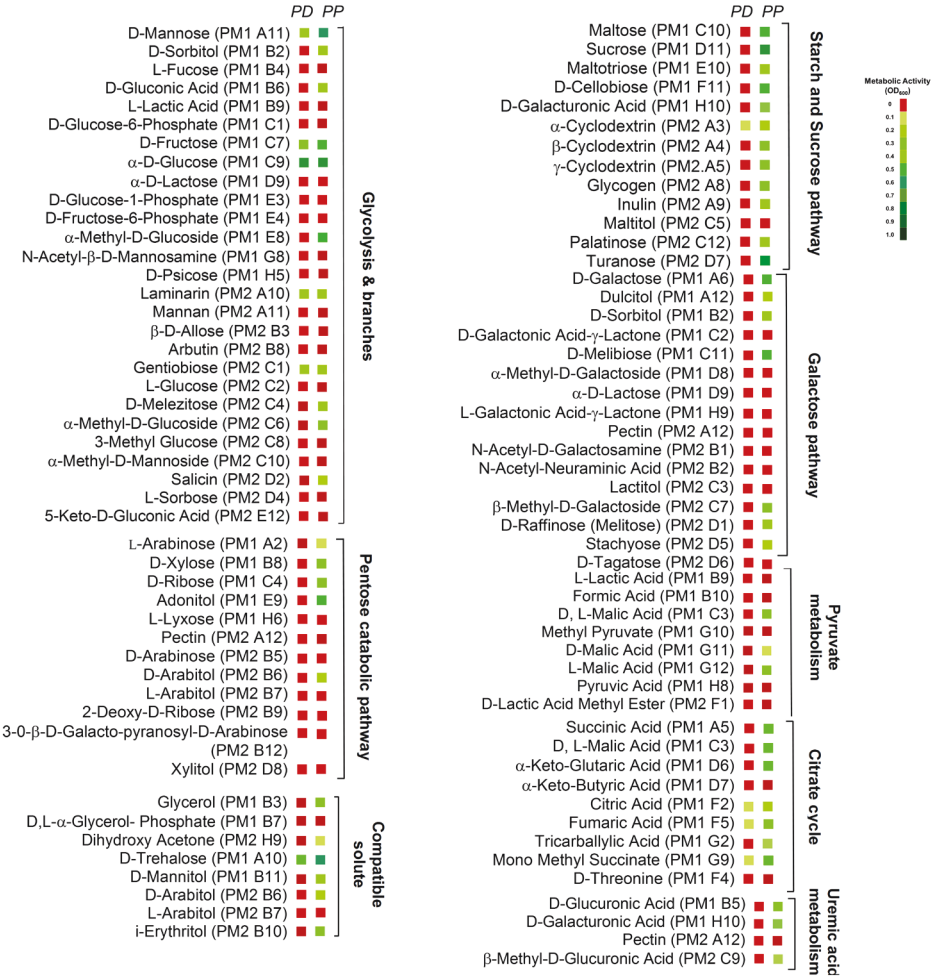


**Figure 2. Catabolism of Carbon compounds by *Pseudogymnoascus destructans* (PD) and *Pseudogymnoascus pannorum* (PP).** The details of test set-up and end point reading are described in the methods. For the heat map visualization, the negative control reading was assigned a score of 0.0 and positive growth scored on a 0.0 – 1.0 scale.

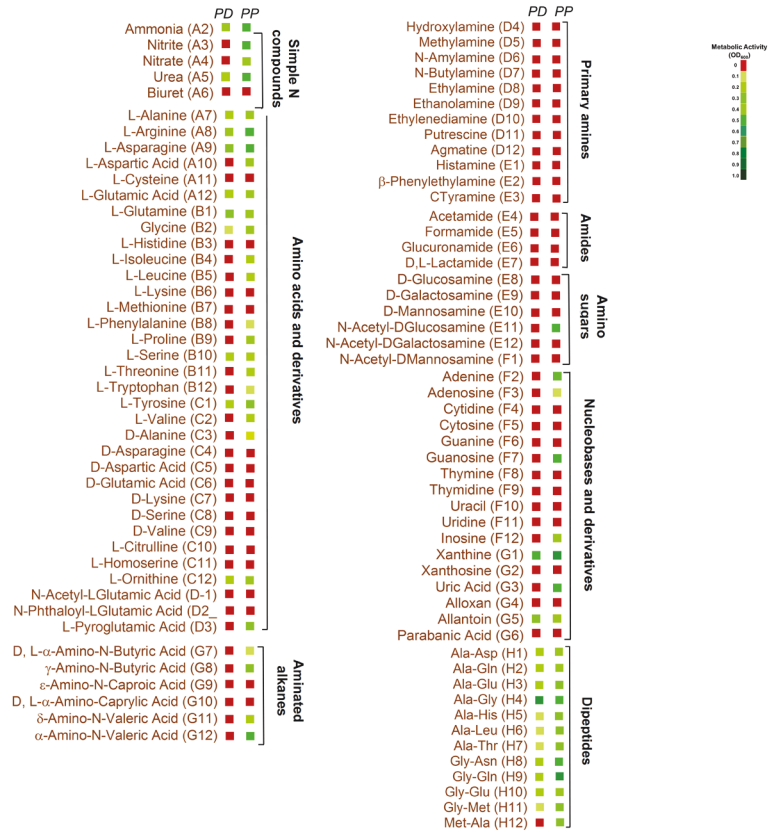


**Figure 3. Use of nitrogen compounds by *Pseudogymnoascus destructans* (PD) and *Pseudogymnoascus pannorum* (PP).** The details of test set-up and heat map are similar to Figure 2.

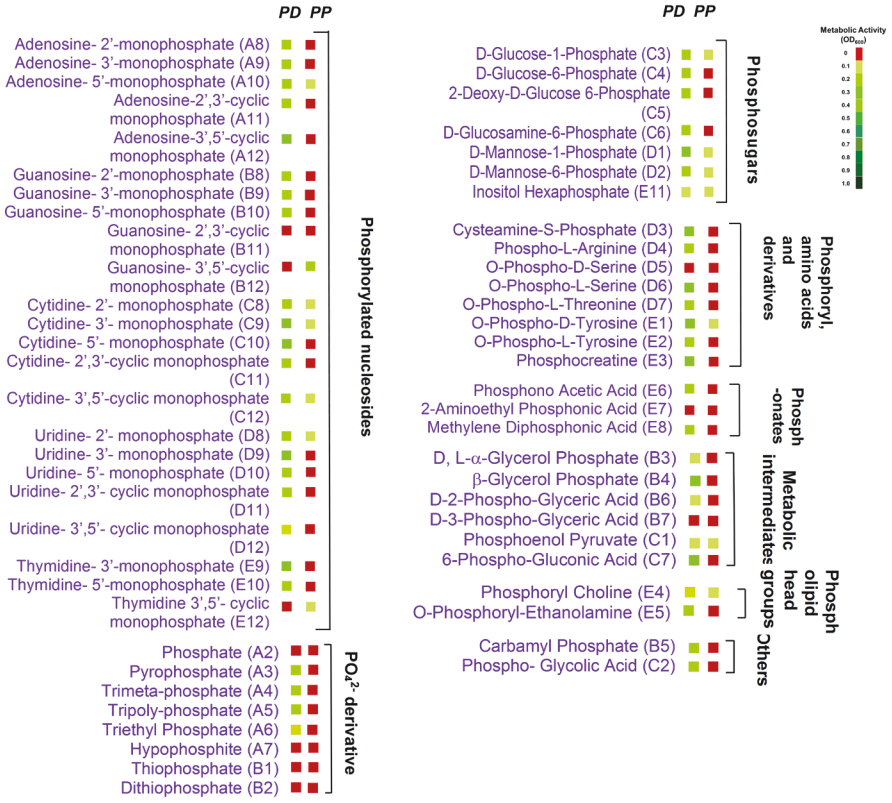


**Figure 4. Use of phosphorous compounds by *Pseudogymnoascus destructans* (PD) and *Pseudogymnoascus pannorum* (PP).** The details of test set-up and heat map are similar to Figure 2.

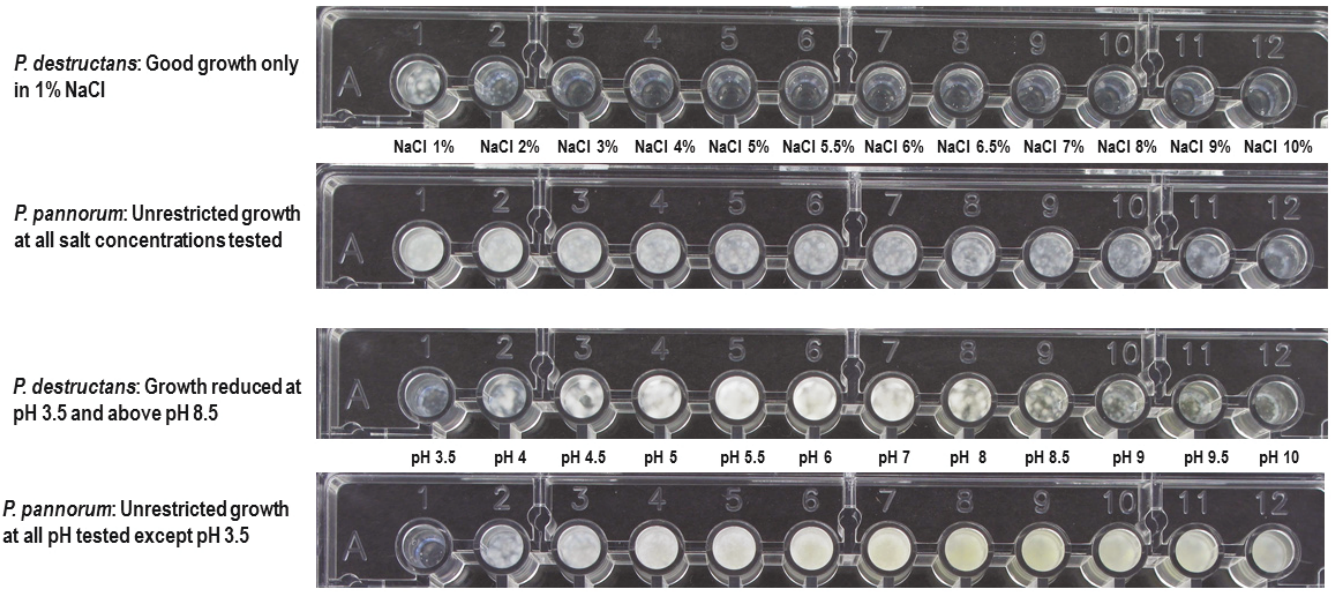


**Figure 5. Growth of *Pseudogymnoascus destructans* and *Pseudogymnoascus pannorum* under high salt and pH extremes.**

**Dataset 1. Excel sheets with $OD_{600}$ values for all Biolog plates tested in this study**

http://dx.doi.org/10.5256/f1000research.15067.d204679

## Discussion

Metabolic profiles of *P. destructans* and *P. pannorum* validated *in silico* predictions about the notable differences in the number of protein-encoding genes in their genomes[11]. *P. destructans* contained enzymes and catabolic pathways that support fungal growth on a limited range of substrates of non-plant origin and showed high sensitivity to stress. *P. pannorum* was remarkably adapted for the nutrient poor environments of the caves and mines ('extremophile') with oligotrophic metabolism, osmotolerance, xerotolerance, and xenobiotic tolerance.

The findings in the present study confirm and expand on results from other reports on *P. destructans*' adaptation and persistence in the North American caves and mines in the face of possible competitive interactions with the native fungal species[8–10]. Both Raudabaugh and Miller (2013) and Reynolds and Barton (2014) used a variety of biochemical tests to probe the metabolic activities in a collection of *Pseudogymnoascus* species isolates[9,10]. The authors of the former study surmised the suitability of *P. destructans* as a saprobe in the affected caves and mines in limited biotic competition ('resource island')[10]. Reynolds and Barton (2014) found a reduced saprotrophic ability in *P. destructans* isolates vis-à-vis *P. pannorum* and other *Pseduogymnoascus* species, which suggested 'co-evolution with the host'[9]. Wilson *et al.* (2017) performed a variety of tests including Biolog FF Microplate with 95 different substrates, and found limited saprotrophic ability in *P. destructans* in comparison to other *Pseudogymnoascus* species[8].

Further Phenotype Microarray profiling of *P. destructans* and *P. pannorum* would be crucial to fill-in current gaps in their genome sequences, define gene functions, and elucidate pathophysiological attributes[11,15,16].

The limitations of the current study include the use of single strains of two fungal species, and single end points instead of growth curves, which allow curve analysis for more accurate data interpretation as highlighted by other investigators.

We and others hope to accomplish these milestones with the recent availability of a high-quality *P. destructans* genome and data pipelines to automate Biolog analysis[15,17–20].

## Data availability

Datasets 1–4: Excel sheets with $OD_{600}$ values for all Biolog plates tested in this study. DOI, 10.5256/f1000research.15067.d204679[14]


### Competing interests

No competing interests were disclosed.

### Grant information

This study was supported by the National Science Foundation (Award Number 1203528).

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

## Supplementary material

**Supplementary File 1.** Biolog maps of PM 1 – 10, and PM 21, 23 – 25 plates

Click here to access the data.

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PubMed Abstract | Publisher Full Text | Free Full Text

# Open Peer Review

## Current Referee Status: ✔ ✔ ✔

**Version 2**

Referee Report 02 August 2018

**doi:**10.5256/f1000research.17010.r36149

✔ **Christine Salomon**
Center for Drug Design, University of Minnesota, Minneapolis, MN, USA

The authors have addressed my concerns and questions in their revision.

***Competing Interests:*** No competing interests were disclosed.

***Referee Expertise:*** Microbial ecology, microbial natural products chemistry, fungal and bacterial infectious disease, biological control

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 01 August 2018

**doi:**10.5256/f1000research.17010.r36150

✔ **Flavia Pinzari**
Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Rome, Italy

I think the answers and related changes to the text clarified most of the points.

***Competing Interests:*** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Version 1**

Referee Report 29 June 2018

**doi:**10.5256/f1000research.16406.r35120

?

**Flavia Pinzari**
Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Rome, Italy

The manuscript is focussed on a comparison between two *Pseudogymnoascus* fungal species, which belong to different species but have a partly overlapped ecological niche, based on the use of metabolic profiling with Biolog Phenotype Microarray commercial multiwell plates.
These plates are used with redox dyes to evaluate substrate use. With fungi this colorimetric approach is very complicated, and as the authors actually did, the fungal growth in the wells is usually measured as a change in optical density.

The interesting aspects and merits of this work are the following:

- This is a brilliant use of this technique, since the comparison between a pathogenic/parasitic species and a mainly saprophytic one can really highlight important clues on the nutritional requirements for the pathogenic organism to spread and develop.
- The two compared species *P. destructans* and the closely related species *P.pannorum are* truly interesting from different points of view. They live in caves, they live at low temperatures, they are very close but behave differently, they attack mammals, etc.

The main criticisms regard:

- The description of the methods (very poor: it is difficult to understand the procedure followed both in data production and analysis), and the way data are presented.
- Figure 1 is really useless. Figure 3 is informative, but little or nothing is reported on data analysis in the methodological section.
- Apparently, the authors harvested the two fungi for inoculum preparation in two different moments (different sporification time: 3 to 5 weeks, it can be very different). They also chose two different incubation times for comparing the catabolism of the two fungi. These choices should be discussed and justified. The two fungi have different development times. This can be the main reason for the differences observed. A better description and motivation of the chosen approach would make the work stronger and clearer.
- Instead of single time-point comparison the authors could have used empirical models and regression splines that allow extrapolation of curve parameters of biological interest, namely, lag time, maximum rate of increase and maximum absorbance. Curve integration and the resulting area under the curve condenses these three parameters into a single estimate that can also be used to compare kinetics across substrates and samples. Curve parameters offer the main advantage of being independent of incubation time, while also accounting for potential differential rates of colour development across substrates and plates. An example of this approach is given in the following work: Canfora et al (2017)[1], and theory is reported in the references therein.
- The authors tested only one strain for each species (I agree that PM plates are very expensive, but drawing a result using only one strains in the comparison is a limitation in case of intraspecific variability, even if they used two replicates)
- The lowest value on the heat map is an OD of “zero”. How was data scaling performed?
- What the red and green lines stand for in Figure 3? Legends are needed.
- References list is lacking of some important elements. One is the following: Atanasova L, Druzhinina IS[2].
- The authors reported that predicted enzymes are related to the number of carbon sources that can be utilized by the two fungal species however, a better definition of the kind of correlations observed between genotype and phenotype is needed to better understand this connection.

**References**
1. Canfora L, Abu-Samra N, Tartanus M, Łabanowska BH, Benedetti A, Pinzari F, Malusà E: Co-inoculum of Beauveria brongniartii and B. bassiana shows in vitro different metabolic behaviour in comparison to single inoculums.*Sci Rep*. 2017; **7** (1): 13102 PubMed Abstract | Publisher Full Text
2. Atanasova L, Druzhinina IS: Review: Global nutrient profiling by Phenotype MicroArrays: a tool complementing genomic and proteomic studies in conidial fungi.*J Zhejiang Univ Sci B*. 2010; **11** (3): 151-68 PubMed Abstract | Publisher Full Text

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
No

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Partly

***Competing Interests:*** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 11 Jul 2018

**Vishnu Chaturvedi**, New York State Department of Health, USA

We appreciate the thoughtful comments of the reviewer.

**The main criticisms regard:**
**The description of the methods (very poor: it is difficult to understand the procedure followed both in data production and analysis), and the way data are presented.**
We have added required details in the methods.

**Figure 1 is really useless. Figure 3 is informative, but little or nothing is reported on data analysis in the methodological section.**
Figure 1 provided a bird's-eye view of the scope of work and different trends noticed in this comparative study. We appreciate that someone knowledgeable about Biolog typing might find this information redundant and therefore, figure 1 was
removed from the revised version. We expanded the legend for figure 3 (now figure 2).

**Apparently, the authors harvested the two fungi for inoculum preparation in two different moments (different sporification time: 3 to 5 weeks, it can be very different). They also chose two different incubation times for comparing the catabolism of the two fungi. These choices should be discussed and justified. The two fungi have different development times. This can be the main reason for the differences observed. A better description and motivation of the chosen approach would make the work stronger and clearer.**
The reviewer's criticism is valid similar to the reviewer 1. We have added details in the revised version to ally concern that "differences observed were due to different development times".

**Instead of single time-point comparison the authors could have used empirical models and regression splines that allow extrapolation of curve parameters of biological interest, namely, lag time, maximum rate of increase and maximum absorbance. Curve integration and the resulting area under the curve condenses these three parameters into a single estimate that can also be used to compare kinetics across substrates and samples. Curve parameters offer the main advantage of being independent of incubation time, while also accounting for potential differential rates of colour development across substrates and plates. An example of this approach is given in the following work: Canfora et al (2017)[1], and theory is reported in the references therein.**
We have used the biolog phenotype plates as per the manufacturer's instructions. There are many publications on Biolog profiling that use single end-point reading for trend analysis. The reviewer refers to a more elaborate setup, and we agree that studies similar to Canofora et al. (2017) provide more accurate and dynamic data for building predictable models. This reference was included in the bibliography and a sentence added in the revised text about the limitation of this qualitative study, and the scope for more dynamic data generation. We shall pursue future opportunities to conduct such a study with the two fungi.

**The authors tested only one strain for each species (I agree that PM plates are very expensive, but drawing a result using only one strains in the comparison is a limitation in case of intraspecific variability, even if they used two replicates)**
We believe that a trend analysis is possible and meaningful with one well-defined strain representative from each species. This is also the basis for whole genome sequencing of most fungi.

**The lowest value on the heat map is an OD of "zero". How was data scaling performed?**
We have added more details to describe the heat map.

**What the red and green lines stand for in Figure 3? Legends are needed**
We have modified the legends for the figure 6 (now figure 5). The red and green lines were deleted.

**References list is lacking of some important elements. One is the following: Atanasova L,Druzhinina IS[2].**
We have included the article by Druzhinina et al.

**The authors reported that predicted enzymes are related to the number of carbon sources that can be utilized by the two fungal species however, a better definition of the kind of correlations observed between genotype and phenotype is needed to better understand this connection.**

We modified the text to suggest that the trend analysis of Biolog data indicated correlation with the *in silico* analysis of genome sequencing data.

***Competing Interests:*** No competing interests

Referee Report 15 June 2018

**doi:**10.5256/f1000research.16406.r34574

**Christopher T Cornelison**
Division of Research and Advanced Studies, Kennesaw State University, Kennesaw, GA, USA

The manuscript, "Phenotype profiling of white-nose syndrome pathogen *Pseudogymnoascus destructans* and closely-related *Pseudogymnoascus pannorum* reveals metabolic differences underlying fungal lifestyles", describes the comparative analysis of metabolic profiling of 2 closely related fungal pathogens with vastly different hosts and virulence. The manuscript utilizes the well known BioLog system to accomplish the comparison. In general the the study is well designed and executed and the manuscript well written. The conclusions are not surprising considering previous publications regarding the genomics of Pd and its loss of carbon utilization related gene content. Accordingly the impact of the findings on the field are modest and the sophistication of the analysis is simplistic. Regardless the manuscript does support previous findings and although the methods are limited in scope they are sound and well vetted. Accordingly it is my recommendation that the manuscript is acceptable as it is.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

***Competing Interests:*** No competing interests were disclosed.

***Referee Expertise:*** Emerging fungal pathogens, microbial control, applied microbiology.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 11 Jul 2018

**Vishnu Chaturvedi**, New York State Department of Health, USA

We appreciate very much the encouraging comments of the reviewer and the approval of the manuscript as it is.

***Competing Interests:*** No competing interest

Referee Report 07 June 2018

**doi:**10.5256/f1000research.16406.r34411

**Christine Salomon**
Center for Drug Design, University of Minnesota, Minneapolis, MN, USA

This paper describes a relatively straightforward study focused on comparing the nutrient utilization capacity of the fungal bat pathogen *Pseudogymnoascus destructans* versus the closely related species *P. pannorum* using the well established Biolog phenotype system. In general, the results suggest that *P. pannorum* can more readily utilize most carbon and nitrogen sources compared to *P. destructans* under the experimental conditions tested (15 deg C, and 7 and 10 days, respectively). The bat pathogen was also more sensitive to pH extremes and less tolerant to high salt. The authors conclude that these results validate their previous whole genome studies which compared the predicted protein numbers between these two species. In general, the results also support previous metabolic capacity studies of *P. destructans* and other non-pathogenic *Pseudogymnoascus* species.

There are some important questions that should be addressed, and additional details that would improve this manuscript:

- How was the incubation time of 10 and 7 days for each species determined? Presumably by comparison of equivalent growth in the control wells, but this detail should be provided. If this time is increased, does the utilization capacity of *P. destructans* eventually catch up?

- More details should be provided about the cut-off value determination for growth versus no growth. Also, there should be some analysis of the range of results, versus simply using the average of the two readings. For example, what was the standard deviation for replicates?

- It would be helpful to have a map of the nutrient sources, xenobiotics, etc. in the supplementary data to accompany the OD data (the numerical data alone is impossible to interpret without any other identifying information)--presumably Biolog provides this as a document.

- It's not clear how the heat map values were generated, if the starting spore inoculum had an OD of 0.2. (since the lowest value on the heat map is "0" OD). Presumably, if no growth occurred under a given condition, it would remain at the starting OD? Also, is 1.0 the highest OD obtained or was the data scaled to 0-1.0?

- The heat map figure for the “nutrient supplements” is missing (also, I’m not sure what compounds this category encompasses, so some information about this would also be helpful, perhaps even just referring to the plate map in the supplementary data if that is added).

- For Figure 6, the solid and dotted green and red lines seem to indicate relative growth, but the numerical cutoffs should be provided in the methods (or figure legend). For example, the growth in the well for 2% NaCl looks (labeled with a solid green line above) looks similar to the well for pH 9 with a dotted green line, but presumably are numerically different.

- Part of the justification for doing this work is stated as confirming the in silico gene predictions (from a previous publication by the authors). However, it’s not entirely clear that the just comparing the overall numbers of predicted proteins is actually correlated to the overall number of nutrient sources that can be utilized. This seems likely to be true, but the two studies don’t necessarily test/confirm this connection. The reference cited is a short report on the overall sequencing of the *P. destructans* and *P. pannorum* genomes, and prediction of encoded proteins, but no significant functional analysis. It might be more relevant to include references that include more functional data on metabolic and enzymatic capacities.

Overall, this work adds important information about the competitive ability and metabolic specificity of *P. destructans* and could provide additional insight into fungal life history strategies and potential ways to control or mitigate white nose syndrome in bats. Some additional details (highlighted above) would provide critical information that would allow others to replicate or expand on this work.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
No

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Partly

***Competing Interests:*** No competing interests were disclosed.

***Referee Expertise:*** Microbial ecology, microbial natural products chemistry, fungal and bacterial infectious disease, biological control

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 11 Jul 2018

**Vishnu Chaturvedi**, New York State Department of Health, USA

Thanks very much for your insightful comments on the manuscript. We have modified the manuscript given your suggestions.

**How was the incubation time of 10 and 7 days for each species determined? Presumably by comparison of equivalent growth in the control wells, but this detail should be provided. If this time is increased, does the utilization capacity of *P. destructans* eventually catch up?**
In preliminary experiments, both fungi grew at different growth rates and comparable growth was observed after day 7 for *P. pannorum* and day 10 for *P. destructans* (details not shown). Further incubation of the plates beyond the observation period did not change the observed growth pattern.

**More details should be provided about the cut-off value determination for growth versus no growth. Also, there should be some analysis of the range of results, versus simply using the average of the two readings. For example, what was the standard deviation for replicates?**
The PM plates were inoculated per Biolog protocol and incubated at 15° C. The presence or absence of growth was measured by $OD_{600}$ on day 7 for *P. pannorum* and day 10 for *P. destructans*. The negative control wells were weakly growth positive for both *P. destructans* and *P. pannorum*. The observation was also reported for the Biolog PM plates in another study (Nai C, et al. Fungal Genet Biol. 2013; 56:54-66). Therefore, the corresponding negative control well reading from each experiment were averaged together and used to normalize the OD values averages for each test compound. For the heat map visualization, the negative control reading was assigned a score 0.0 and the positive growth scored on a 0.0 -1.0 scale. The phenotypic assay was repeated once. The limited dataset precluded any quantitative statistical analyses.

**It would be helpful to have a map of the nutrient sources, xenobiotics, etc. in the supplementary data to accompany the OD data (the numerical data alone is impossible to interpret without any other identifying information)--presumably Biolog provides this as a document**.
The Biolog maps for all test compound are now included as a supplementary file.

**It's not clear how the heat map values were generated, if the starting spore inoculum had an OD of 0.2. (since the lowest value on the heat map is "0" OD). Presumably, if no growth occurred under a given condition, it would remain at the starting OD? Also, is 1.0 the highest OD obtained or was the data scaled to 0-1.0?**
Please see details provided earlier about the heat map visualization.

**The heat map figure for the "nutrient supplements" is missing (also, I'm not sure what compounds this category encompasses, so some information about this would also be helpful, perhaps even just referring to the plate map in the supplementary data if that is added).**
The nutrient map from Biolog was uploaded as a supplementary file.

**For Figure 6, the solid and dotted green and red lines seem to indicate relative growth, but**

**the numerical cutoffs should be provided in the methods (or figure legend). For example, the growth in the well for 2% NaCl looks (labeled with a solid green line above) looks similar to the well for pH 9 with a dotted green line, but presumably are numerically different.**
The legend for figure 6 (now figure 5) was modified for clarity; the summary numerical cutoff values were included in the revised text.

**Part of the justification for doing this work is stated as confirming the in silico gene predictions (from a previous publication by the authors). However, it's not entirely clear that the just comparing the overall numbers of predicted proteins is actually correlated to the overall number of nutrient sources that can be utilized. This seems likely to be true, but the two studies don't necessarily test/confirm this connection. The reference cited is a short report on the overall sequencing of the *P. destructans* and *P. pannorum* genomes, and prediction of encoded proteins, but no significant functional analysis. It might be more relevant to include references that include more functional data on metabolic and enzymatic capacities.**
Instead of 'confirm,' we switched to 'assess correlations with *in silico* gene predictions.' The two studies provide initial information about the genomes and metabolic pathways. The findings indicate a trend towards unique genomic and phenomic attributes in two psychrophilic fungi with different lifestyles. We agree with the reviewer that there is need to carry out more detailed functional analysis to identify unique genes, enzymes, and other proteins that differentiate these two psychrophiles. Such studies, necessitating considerable resources, are planned for the future.

***Competing Interests:*** No competing interests.