Review of: "Deciphering the potential niche of novel black yeast fungal isolates in a biological soil crust based on genomes, phenotyping, and melanin regulation"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Summary: In this paper, the authors describe the isolation and characterization of two novel black yeast fungal isolates: JF 03-3F “Goopy” Exophiala viscosium and JF 03-4F “Slimy” Exophiala limosus. These were isolated from biological soil crusts and underwent many analyses for this work. The genomes were constructed and annotated; effects of abiotic stressors including temperature, metals, and UV exposure were assessed; melanin production using tailored extraction procedures was analyzed; the type of melanin produced was assessed; and the melanin pathway was assessed using a mutant study. The major findings of this work include that both of these isolates were capable of producing melanin and withstanding high levels of abiotic stress; both had genes for potential production of all 3 types of melanin, however there was some uncertainty of which pathway was preferred, and both species could excrete melanin-a trait the authors propose is related to symbiotic relationships with other members of the soil crusts.

Summarized of Concerns: The major concerns with this paper are mostly related to a need for clarification and improvement of the communication of the science. Some sections are missing crucial details which are likely known to the authors and would help readers understand the work better. The tone of the article is at times informal and vague which makes both the rationale and conclusions less clear. For these reasons, we recommend acceptance of this work contingent upon major manuscript revisions.

Major Concerns:

1. Lines 288-302: In this section describing UV resistance testing, the rationale given to test this trait is that these species have “highly melanized cell walls and constant exposure to sunlight in their natural habitat.” However, in the testing, the comparison was between samples kept in the dark and samples exposed to only 120 seconds of UV light before also being placed in the dark. Would not a more meaningful test include longer exposure since that would more closely match the conditions the melanin would be naturally providing protection within?
2. Lines 420-421: This section needs clarification. Was the test to see if the extraction method was lysing cells or is it testing the hypothesis that the melanin present in the cultures is due to natural amounts of

cell death and then release following cell degradation? Currently the wording makes it sound like the latter, however the method you describe would only check for the former. You would still centrifuge out the dead cells that had already lysed and released their melanin. Could the melanin potentially be from cells releasing their melanin upon death within the culture?

3. Has a phylogenetic tree been constructed to place these species amongst known species? If so, please include.
4. It is not clear why these 2 particular isolates were chosen. Lines 748-754 should be expanded upon to explain better why only 2 of the 25 were chosen. As it stands it is not clear why these 2 particular isolates achieve your purpose and why none of the others were explored. For the purpose of this work- Were you wanting to find unique species? High melanin producers? Compare two isolates with different phenotypes? or simply add to the understanding of black fungi?

Minor Concerns:

1. Line 93: Daily and seasonally instead of daily and annually?
2. Lines 97-100: needs citation
3. Lines 118-120: Broken hyperlink. Perhaps these links would be better placed in the supplemental media?
4. Line 251: Could cite McFarland standard (McFarland J. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc 1907; 14:1176-8.)
5. Lines 260-261: Need to define the 1-5 scale and how it fits with the coding already described
6. Lines 295-296: This statement is confusing- were there three placement conditions tested? Or were the lids that were taken off placed inside the cross linker?
7. Line 316: “once the plates were observed to have grown enough” is very passive and not detailed.
8. Lines 547 & 549: Should video be labelled as video 1 instead?
9. Line 739: “REF” needs replaced with a citation
10. Could end with a concluding statement rather than on a list of questions.
11. Overall: Write out full term on first use of acronyms- (ex. WT, DF, ITS in lines 124, 130, 139 respectively). Same throughout rest of paper.

Grammatical issues:

1. Line 75: Organism should be organisms.
2. Line 78: End with a single period instead of two.
3. Lines 80-81: “These novel fungi are of the genus Exophiala, this genus has previously 81 been found in BSCs (Bates et al., 2006)” Comma should be a period.
4. Line 96: Need to start with “An”
5. Line 480: two spaces between “this” and “occurs”

6. Lines 492-493: “Figure 2 and Figure 4” should possibly be “Figure 2 & Figure 4” or “Figures 2 & 4”?
7. Paragraph Lines 516-528: Gene names should be italicized.
8. 867-869: awkward wording
9. Throughout: Several instances of conversational tone, ex. “did not necessarily observe” in line 795 and more throughout the discussion. Could be reworded to sound more objective.
10. Throughout: Temperature references need space adjustments, example: 36° C should be 36°C or 36 °C.