Genomic characterization of Laetiporus subspecies utilizing Nanopore MinION sequencing

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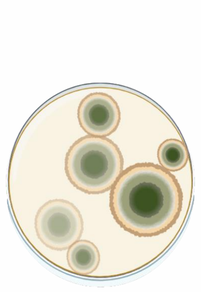
Date: 18 January 2024

Introduction

Laetiporus *sulphureus* and *cincinnatus* are species of fungi which grow on decomposing wood of oak and other deciduous trees, while degrading biomass they produce a bright orange pigment. This pigment, laetiporic acid is a non-fossil fuel based and potentially high value colorant. Therefore, we have tried to genomically characterize Laetiporus subspecies to secure that this pigment can be used as a non-food safe dye. During this study cultivation, DNA extraction, whole genome sequencing and genomic characterization was performed on Laetiporus subspecies.

Cultivation & DNA extraction



L. Cincinnatus & L. Sulphureus - cultivated on Czapek Dox (20g/l pepton) 2 weeks @ RT

1cm2 agar cubes added to 100ml Czapek dox liquid media (20g/L pepton). Incubated 3 days @ 23°C & 100 RPM in a rotary incubator.

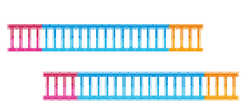
Vacuum filtration, separating biomass from media.

The mycelium is ground using liquid nitrogen & pestle and mortar.

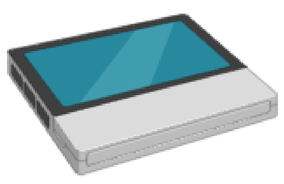
Pulverized cells suspended in DNA extraction buffer with RNase.

Phenol:chloroform:iso amyl alcohol protein & contaminant removal

NaAc:Iso-propanol DNA precipitation & DNA rehydration

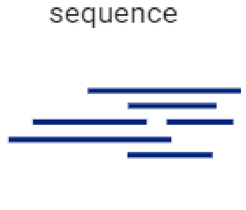


Nucleospin plant II wash protocol, DNA bead purification, barcode ligation & library preparation.



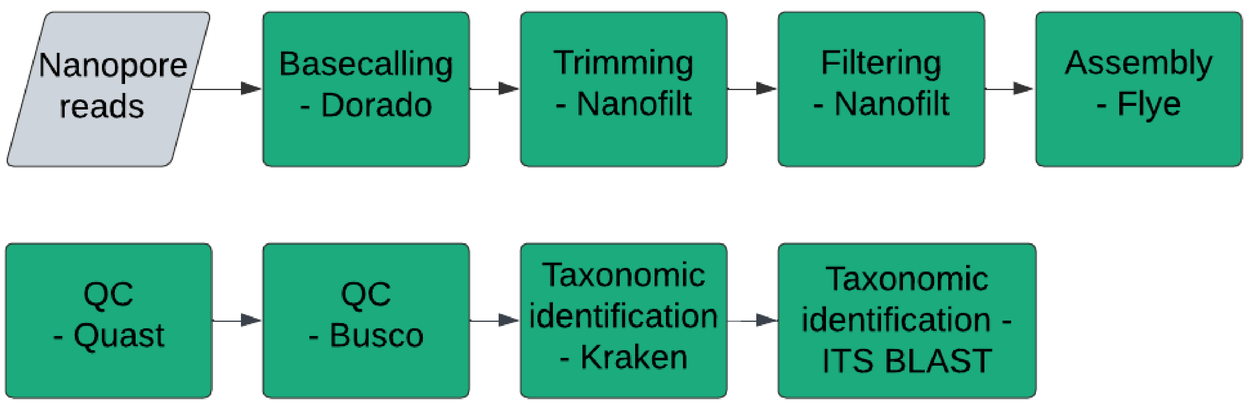
Libraries are loaded onto MinION Mk1C.

Nanopores read DNA in real-time, generating long-read sequences.



Sequencing reads are processed and analyzed to assess the taxonomy.

Bio-informatic pipeline



Results

Sample average	A260/280	A260/230	Concentration – ng/µl	DIN
L. *sulphureus*	2,13	2,02	56,2	8,0
L. *cincinnatus*	2,05	1,86	78,0	9,2

Schematic results of the 260/280, 260/230 Nanodrop absorption ratio's, the amount of ng/µL according to Qubit and the DIN values determined with Tapestation regarding isolates from L Sulphureus and L. Cincinnatus.

Laetiporus Sulphureus					
C: 96,9%	S: 96,2%	D: 0,7%	F: 0,2%	M: 2,4%	N: 4494
Laetiporus Cincinnatus					
C: 96,6%	S: 96,2%	D: 0,4%	F: 0,3%	M: 3,1%	N: 4494

BUSCO results of L. Sulphureus and L. Cincinnatus. C: complete BUSCO's, S: single copy orthologs, D: duplicated, F: fragmented, M: missing, N: number of BUSCO groups searched in the eukaryotic and Hypocreales database.

O Hypocreales
F Nectriaceae
G Fusarium
G1 **Fusarium solani species complex**
S **Fusarium keratoplasticum**
S Fusarium falciforme
G1 Fusarium sambucinum species complex
S Fusarium venenatum
S Fusarium pseudograminearum

O Hypocreales
F Cordycipitaceae
G Akanthomyces
S Akanthomyces muscarius
F Nectriaceae
G Fusarium
G1 Fusarium sambucinum species complex
S Fusarium pseudograminearum
S1 **Fusarium pseudograminearum CS3096**

Kraken2 output where the most abundant taxa was Fusarium keratoplasticum for L. *sulphureus(L)* & Fusarium pseudograminearum CS3096 species for L. *Cincinnatus(R)*.

Name	Query cover	Identity %	Acc. length
L. *sulphureus*			
Fusarium ambrosium	55%	97,31%	628
Fusarium solani	55%	96,54%	632
Fusarium falciforme	55%	96,68%	627
L. *cincinnatus*			
Lecanicillium coprophilum	43%	100%	573
Emericellopsis robusta	62%	88,52%	729
Gamszarea kalimantanensis	43%	95,28%	631

ITS sequence BLAST against the NCBI fungal ITS reference sequence database. L. sulphureus yielded a 97-99% identity score with a query coverage of 55% on the ITS region of Fusarium species complex, where L. cincinnatus showed a 100% match with Lecanicillium coprophilum with a query coverage of 56%.

Conclusion & discussion

Analysis of L. *Sulphureus* and L. *Cincinnatus* genomes showed unexpected placement in the Hypocreales order by BUSCO. Kraken identified Fusarium keratoplasticum in L. Sulphureus and Akanthomyces muscarius/Fusarium pseudograminearum CS3096 in L. Cincinnatus. BLAST revealed high identity with Fusarium subspecies complex for L. Sulphureus and Lecanicillium coprophilum for L. Cincinnatus. Indicating that the species which were used for this study were different strains than Laetiporus. However, multiple species were found during analysis suggesting that there might be a contamination. A higher sequencing depth needs to be achieved to get a better resolution of the genome and resolve any identification and contamination issues. Morphological identification, MALDI-TOF and direct ITS sequencing could prove useful for better identification.

References

1. Images created with: https://www.biorender.com/
2, Flowchart created with: https://www.lucidchart.com/