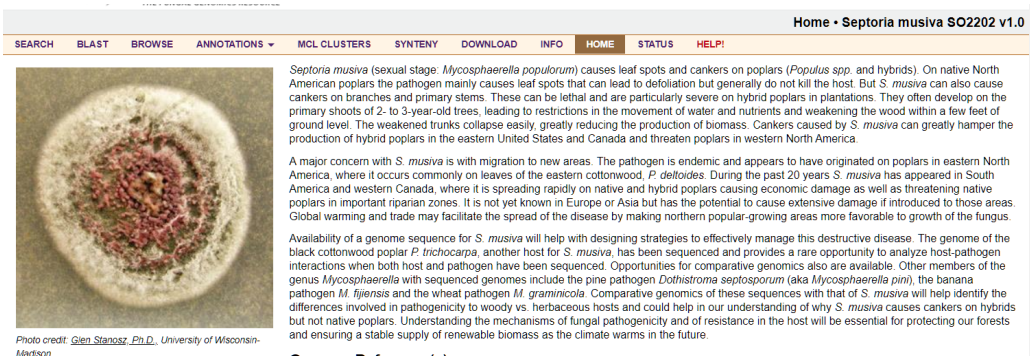
MycoCosm Secondary Metabolism Clusters Browser

In fungi, Secondary metabolite (SM) genes are often organized in chromosomal clusters dedicated to that metabolite's biosynthetic pathway. Each portal's SM Clusters Browser facilitates display and discovery of MycoCosm's SM-annotated genes.

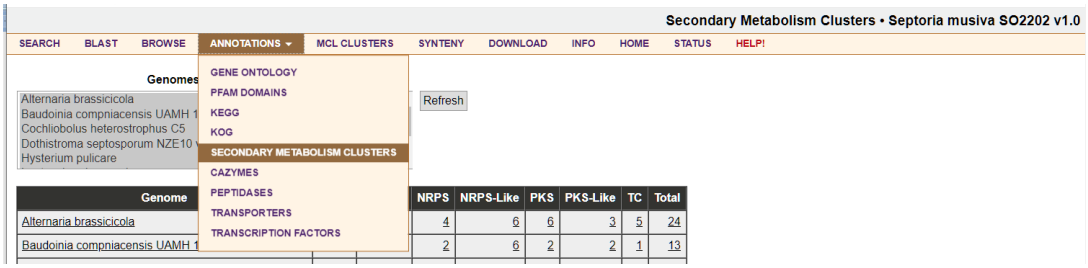
Scenario: You have identified a toxic SM produced by *Septoria musiva*, a pathogenic fungus that induces cankers in the poplar tree, but not produced by *Septoria populicola*, which infects a different species of poplar and does not induce cankers. The SM's structure suggests that its biosynthetic pathway may have as its core enzyme a hybrid PKS-NRPS (polyketide synthase-nonribosomal peptide synthetase). Use MycoCosm to find candidate gene clusters for this pathway.

1) Go to the MycoCosm Septoria PhyloGroup at genome.jgi.doe.gov/Septoria. Both species are represented in the group:

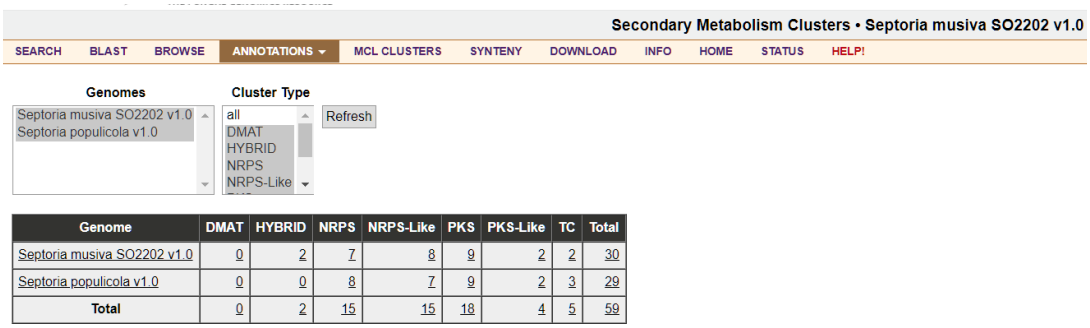
2) Click on '*Septoria musiva*' to go to its genome portal:



3) Click on '"ANNOTATIONS => SECONDARY METABOLISM CLUSTERS" to go to the portal's SM clusters browser:

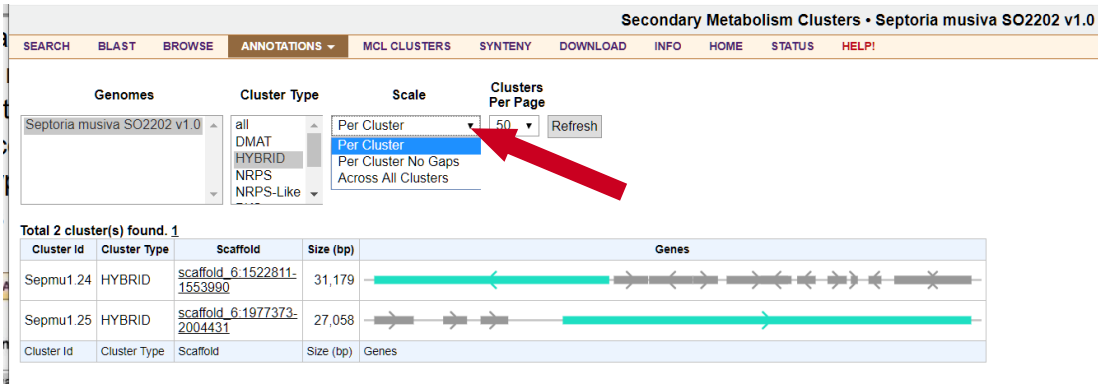


4) Scroll through the ‘Genomes’ list box and select both ‘*Septoria musiva*’ and ‘*Septoria populica*’, and only those 2 species. Click the ‘Refresh’ button. Only the SM cluster core gene counts of the 2 *Septoria* sp. are shown, and may be directly compared. *S. musiva* has 2 hybrid core genes (PKS-NRPS genes) while S. populicola has none:

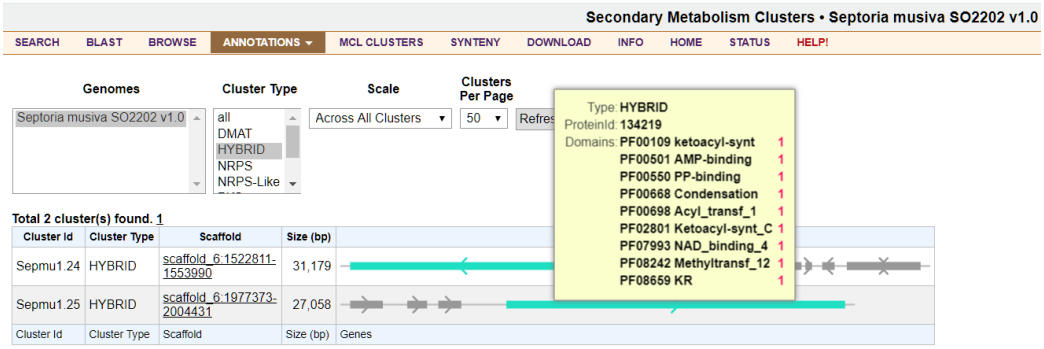


Genome	DMAT	HYBRID	NRPS	NRPS-Like	PKS	PKS-Like	TC	Total
Septoria musiva SO2202 v1.0	0	2	7	8	9	2	2	30
Septoria populicola v1.0	0	0	8	7	9	2	3	29
Total	0	2	15	15	18	4	5	59

5) There is a total of 2 genes in the Hybrid column. Click on the number to show a graphical representation of the 2 *S. musiva* gene clusters. The ‘Size’ column displays each cluster’s length, and the ‘Genes’ column displays each cluster’s core PKS-NRPS gene (in color) and its accessory, decorator, and other genes (in gray). A core hybrid gene is typically very large, but the total cluster size can be highly variable. To resize the 2 clusters to scale to each other, go to the ‘Scale’ pull-down menu, select ‘Across All Clusters’, and click on the ‘Refresh’ button:



6) Each gene in the clusters is represented by an arrow with a single pair of fletching that indicates the gene’s 5’ → 3’ direction. Mouse-over the top cluster’s core gene to get more information about the PKS-NRPS hybrid. The listed domains are typical of a hybridenzyme:



7) To get domain information about the other genes in the SM cluster, mouse-over them too. The next gene 3' to the core gene has a p450 domain:

8) To get more detailed information about a gene, click on it directly. Click on the gene with the p450 domain to see its 'protein page'. Examination of the protein page reveals that:
   a) The gene is expressed. The blue bars represent UTRs, which can be inferred only from transcriptomic data.
   b) The protein has p450 Pfam and other annotations indicative of a cytochrome p450 monooxygenase.
   c) The best Blast hit in nr is a cytochrome p450 monooxygenase from *Aspergillus nidulans*, which belongs to a different class of fungi (Eurotiomycetes) from *Septoria* (Dothideomycetes).

Description:

Best Hit: gi|67902848|ref|XP_681680.1| hypothetical protein AN8411.2 [Aspergillus nidulans FGSC A4] >gi|40747877|gb|EAA67033.1| hypothetical protein AN8411.2 [Aspergillus nidulans FGSC A4] >gi|259484346|tpe|CBF80485.1| TPA: Cytochrome P450 monooxygenase (Eurofung) [Aspergillus nidulans FGSC A4] (model%: 91, hit%: 90, score: 1905, %id: 71) [Aspergillus nidulans FGSC A4]

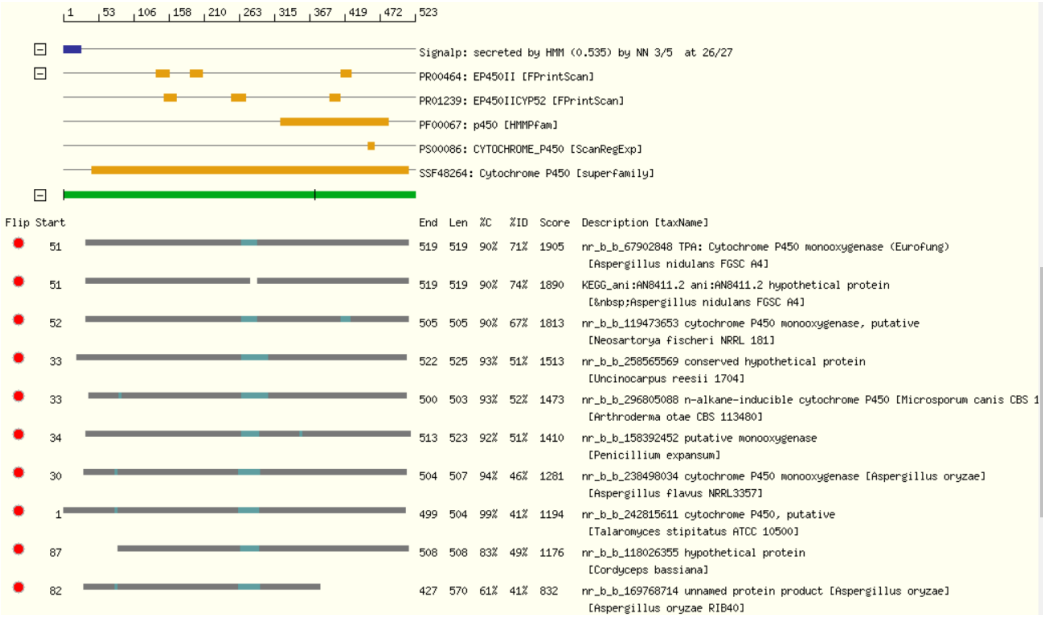
total hits(shown) 683 (10)

ASPECT	GO Id	GO Desc	Interpro Id	Interpro Desc
Molecular Function	0016712	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	IPR002974	Cytochrome P450, E-class, CYP52
	0004497	monooxygenase activity	IPR002402 IPR001128	Cytochrome P450, E-class, group II Cytochrome P450
	0020037	heme binding	IPR002402 IPR002974 IPR001128	Cytochrome P450, E-class, group II Cytochrome P450, E-class, CYP52 Cytochrome P450
	0005506	iron ion binding	IPR002402 IPR002974 IPR001128	Cytochrome P450, E-class, group II Cytochrome P450, E-class, CYP52 Cytochrome P450
Biological Process	0006118	electron transport	IPR002402 IPR002974 IPR001128	Cytochrome P450, E-class, group II Cytochrome P450, E-class, CYP52 Cytochrome P450

KOG GROUP	KOG Id	KOG Class	KOG Desc
Metabolism	KOG0158	Secondary metabolites biosynthesis, transport and catabolism	Cytochrome P450 CYP3/CYP5/CYP6/CYP9 subfamilies

View/modify manual annotation
View nucleotide and 3-frame translation To Genome Browser
NCBI blastp Predicted number of transmembrane domains: 1

9) So this really is a cytochrome p450 monooxygenase, a class of enzymes that often modify core structures of SM biosynthetic pathways. Similar perusal of the other genes of the cluster says that this cluster is an excellent candidate for synthesis of your SM.



10) One explanation for *S. musiva* having this cluster and the congeneric *S. populica* not is that the former acquired the cluster by horizontal gene transfer from a phylogenetically distant source. The 'best Blast hit' of the cytochrome p450 enzyme supports this hypothesis. To see if the core enzyme can shed some light, click the web browser back button to go back to the SM CLUSTERS graphic, and click on the same PKS-NRPS core gene we moused over earlier. The protein page is rich in details, including domains and the top 10 hits. All of the hits are high quality and are from Eurotiomycetes. This cluster is an excellent candidate for horizontal gene transfer from the Eurotiomycetes!

References

- Dhillon B, Feau N, Aerts AL, Beauseigle S, Bernier L, Copeland A, Foster A, Gill N, Henrissat B, Herath P, LaButti KM, Levasseur A, Lindquist EA, Majoor E, Ohm RA, Pangilinan JL, Pribowo A, Saddler JN, Sakalidis ML, de Vries RP, Grigoriev IV, Goodwin SB, Tanguay P, Hamelin RC. Horizontal gene transfer and gene dosage drives adaptation to wood colonization in a tree pathogen. Proc Natl Acad Sci U S A. 2015 Mar 17;112(11):3451-6. doi: 10.1073/pnas.1424293112. Epub 2015 Mar 2. PubMed PMID: 25733908
- Schümann J, Hertweck C. Molecular basis of cytochalasan biosynthesis in fungi: gene cluster analysis and evidence for the involvement of a PKS-NRPS hybrid synthase by RNA silencing. J Am Chem Soc. 2007 Aug 8;129(31):9564-5. Epub 2007 Jul 18. PubMed PMID: 17636916.