Exercise: Exploring host-pathogen interactions in Ensembl Fungi

Zymoseptoria tritici, also known as *Septoria tritici* and *Mycosphaerella graminicola*, is a fungal pathogen that causes septoria leaf blotch disease in wheat. This fungus is considered a major threat to wheat production world-wide, and its ability to rapidly adapt to fungicides and host plants makes it a significant challenge for disease management.

You can explore molecular interactions of genes in Ensembl Fungi, ranging from pathogen-host interactions to symbiotic relationships across microbes and other Ensembl species.

Step 1: Find all genes with molecular interactions for *Zymoseptoria tritici*. From the endpoint API doc page https://interactions.rest.ensembl.org, search for all zymoseptoria_tritici genes

https://interactions.rest.ensembl.org/ensembl_gene?scientific_name=zymoseptoria tritici

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You should get the following output:

zymoseptoria_tritici": ["Mycgr3G53658", "Mycgr3g88451",

"Mycgr3G85040", "Mycgr3G40048", "Mycgr3G111221", "Mycgr3G103264",

"Mycgr3G89160", "Mycgr3G80707", "Mycgr3G65552", "Mycgr3g105487",

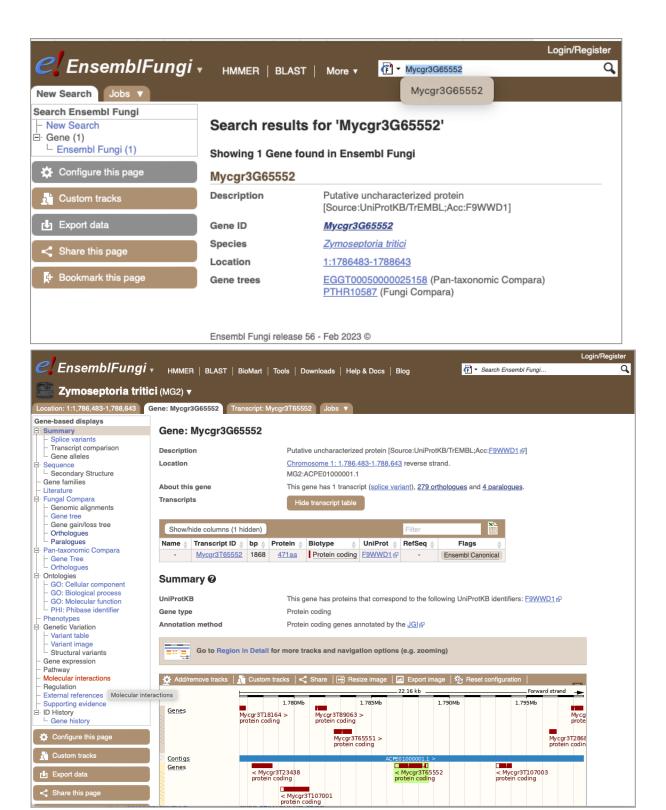
"Mycgr3G70181", "Mycgr3G46840", "Mycgr3G93828", "Mycgr3G31676",

"Mycgr3G51018", "Mycgr3G36951", "Mycgr3G77528", "Mycgr3G39611",

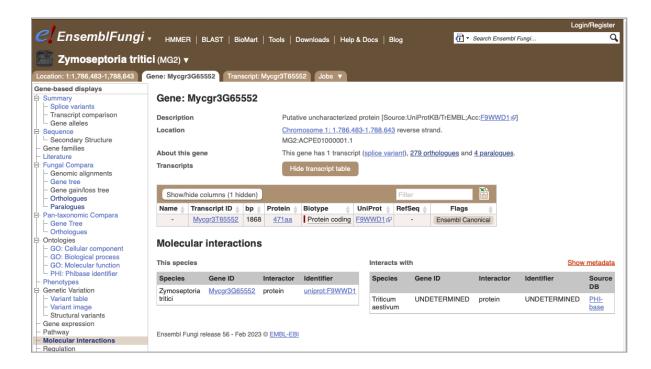
"Mycgr3G96592", "Mycgr3G86705", "Mycgr3G107320", "Mycgr3G74194",

"Mycgr3G87000", "Mycgr3G100355", "Mycgr3G92404", "Mycgr3G69942"]
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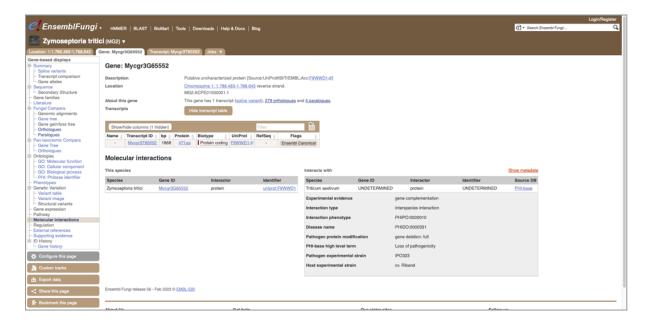
Step 2: Let's find out more about the gene Mycgr3G65552 in Ensembl Fungi. On the homepage, enter the gene ID Mycgr3G65552 in the top right-hand corner and hit Search. Click on the Gene ID Mycgr3G65552 to open the Gene tab.



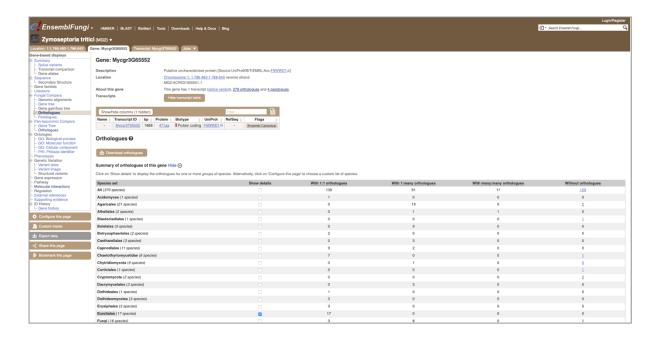
To find a list of species with which this particular *Z. tritici* gene has molecular interactions with, click on Molecular interactions in the left-hand panel. From this page, we can see that *Z. tritici* is known to interact with *Triticum aestivum* (wheat). Can you find the wheat Gene ID that Mycgr3G65552 interacts with? Look at the Interacts with table. The Gene ID is UNDETERMINED. This means a molecular interaction has been experimentally verified between Mycgr3G65552 and wheat, but the former gene hasn't been identified yet.



Can you find out what the phenotype for this interaction is? Click on Show metadata at the top right-hand corner of the Interacts with table. Based on PHI-base, the interaction is associated with Loss of pathogenicity.

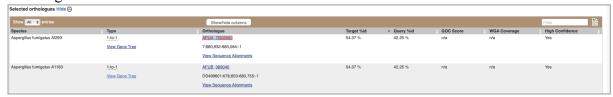


Step 3: Next, let's find all fungal orthologues. There are several ways of doing this. One way is to go to Fungal Compara: Orthologues in the left-hand panel.

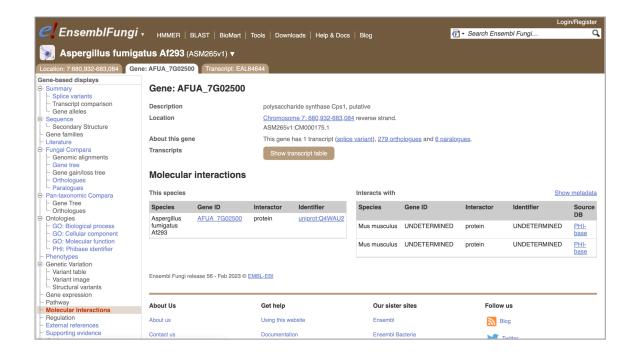


Can you find out if there are any orthologues in *Aspergilus fumigatus* with molecular interactions entries?

Step 4: You can hide the Summary of orthologues of this gene table by clicking the Hide button. Enter *Aspergilus fumigatus* in the filter box on the top right-hand corner of the Orthologues table.



There are two orthologues in *A. fumigatus*. Click each of the gene IDs to find out which one has an entry under the Molecular interactions Gene-based display. Molecular interactions are available for the second orthologue, AFUA_7G02500. What is the phenotype of the interaction for this orthologue with mice?



Additional host-pathogen exercise 1 – Exploring GO terms and phenotypes

Botrytis cinerea is a necrotrophic fungus that infects a wide range of crops and ornamental plants, causing significant economic losses in agriculture and horticulture industries. It is known to cause botrytis bunch rot in various species. Use Ensembl Fungi to find out more information about molecular interactions in the species and answer the following questions:

- (a) Using the Ensembl REST API, can you retrieve all genes with molecular interactions information for *B. cinerea*?
- (b) Open the Molecular interactions page for the Bcin07g00720 gene in *B. cinerea*. What plant species does the gene interact with?
- (c) Can you find the phenotype that is reported for each of the species the gene interacts with?
- (d) Find all fungal orthologues. Is there any orthologue in *Magnaporthe oryzae* for Bcin07g00720? For which orthologue is molecular interaction information available?
- (e) Which species does the *M. oryzae* orthologue interact with?

"Bcin01g11360", "Bcin15g00450",

"Bcin09g05050", "Bcin15g03580", "Bcin05g02590"]

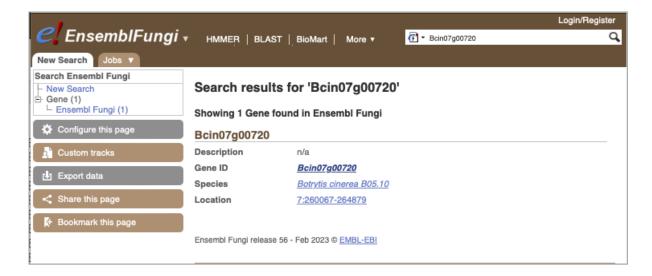
(f) Compare the Molecular interaction phenotypes between the *B. cinerea* and *M. oryzae* orthologues. Can you find any common molecular functions that may explain this phenotype?

Answers:

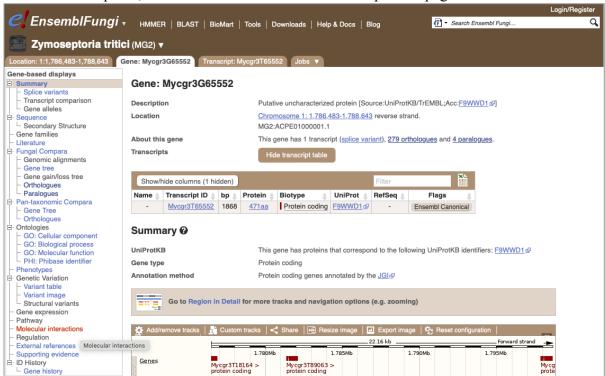
(a) From the endpoint https://interactions.rest.ensembl.org/interactions_by_prodname/. Search for botrytis_cynerea, which will give you the following output:
"botrytis_cinerea": ["Bcin07g00720", "Bcin02g02570", "Bcin12g04900",
"Bcin16g00630", "Bcin02g06770", "Bcin03g07190", "Bcin09g02390",
"Bcin09g01800", "Bcin07g03050", "Bcin08g05150", "Bcin10g01250",
"Bcin14g01870", "Bcin06g04870", "Bcin06g00240", "Bcin06g03440",
"Bcin03g07900", "Bcin03g06840", "Bcin10g02530", "Bcin08g02990",
"Bcin07g02610", "Bcin03g08710", "Bcin10g05590", "Bcin16g01820",
"Bcin03g01540", "Bcin14g00650", "Bcin09g05460", "Bcin10g02650",
"Bcin02g02780", "Bcin05g03080", "Bcin08g00160", "Bcin01g06010",
"Bcin01g11360", "Bcin01g06010", "Bcin01g06010",

(b) Go to the Ensembl Fungi homepage and search for Bcin07g00720. In the results, click on the Gene ID to open the Gene tab.

"Bcin03g04600", "Bcin09g01910",

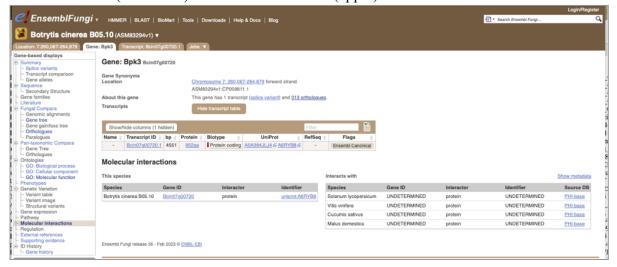


In the left-hand panel, click on Molecular interactions to open the page.

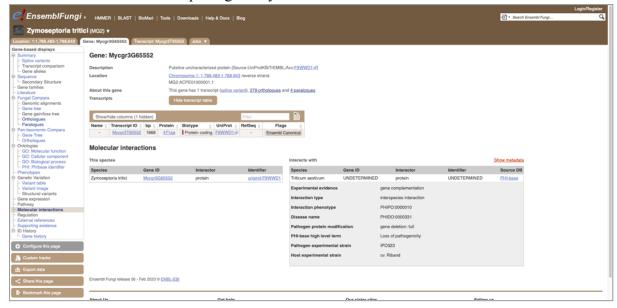


In the Molecular interactions page, you can find all species the gene interacts with in the right-hand table. These include *Solanum lycopersicum* (tomato), *Vitis vinifera* (grape),

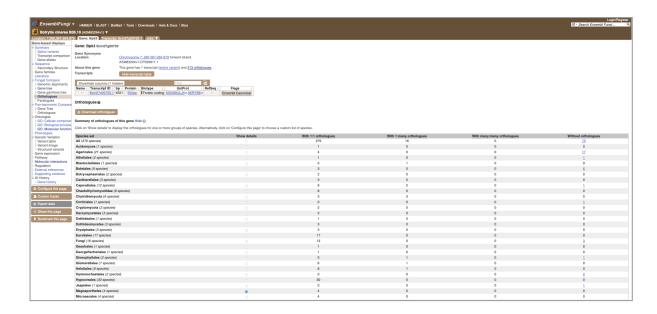
Cucumis sativus (cucumber) and Malus domestica (apple).



(c) Click on Show metadata in the right-hand corner of the Interacts with table. You can find associated phenotypes under PHI-base high level term. The gene is associated with "Reduced virulence" and / or "Loss of pathogenicity".



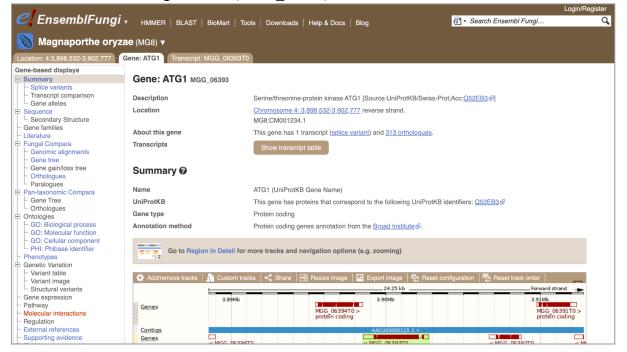
(d) To retrieve all fungal orthologues, go to Fungal Compara: Orthologues in the left-hand panel.



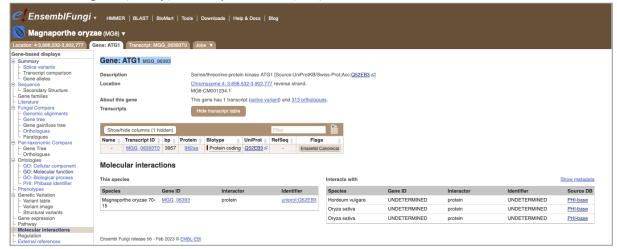
Scroll down to the Orthologues table and use the filter box in the top right-hand corner to search for *Magnaporthe oryzae*.



Click on each of the orthologue gene IDs to open their respective gene tab and find out if the Molecular interactions Gene-based display is available. Molecular interactions information is available for the orthologue ATG1 (MGG 06393).



(e) Click on Molecular interactions in the left-hand panel. The ATG1 protein interacts with *Hordeum vulgare* (barley) and *Oryza sativa* (rice).



- (f) Click on Show metadata to view the phenotypes associated with the molecular interactions. In *B. cinerea*, the phenotype is "Loss of pathogenicity" and in *M. oryzae* the phenotype is "Loss of pathogenicity" and "Reduced virulence".
- (g) Go to Ontologies: GO: Molecular function for both *B. cinerea* and *M. oryzae*. Comparing the GO terms for the two orthologues we can see that they have identical GO annotations: "nucleotide binding", "protein kinase activity", "protein serine/theonine kinase activity", "ATP binding", "kinase activity", "transferase activity" and "protein serine kinase activity".

