Genomic analysis of Cryptococcus neoformans var. grubii in Colombia

Objective: Recent genome-wide genotype-phenotype association studies (GWAS) of sets of clinical fungal isolates have reported problems resulting from strong phylogenetic clustering (apparent quasi-clonal population structures), difficulties in localizing association signals on chromosomes. We wished to directly investigate such difficulties, for whole-genome sequence data, and correspoding phenotype data we had generated.

Methods: Our recently released Illumina reads sequences (Project PRJNA660951) for 29 clinical Colombian isolate genomes of Cryptococcus neoformans var. grubii (28 VNI and 1 VNI as outgroup) were assembled and aligned to the annotated H99 VNI reference genome. Single nucleotide polymorphisms (SNPs) were called for the VNI alignment. MLST types, phenotypes, and source metadata of the same isolates were integrated with the genomic data. Analyses were interpreted in the light of published literature.

Results: The 28 VNI isolates were assigned an order considering MLST classification and whole-genome phylogenetic distances. Most SNPs were biallelic, allowing straightforward calling. Each of the 28 isolates was given a bar if it had the same allele. Barcode profiles (SNP-stem matrices) were found repeated numerous times within genes and across entire chromosomes, indicating that neither fine nor rough mapping of associations would succeed, except where flagged genes had already been characterised by relevant molecular biology experiments. The same repititions can allow amplification of individual MLST loci to tag and inform on variation present in other loci in the genome. The gene for the capsule-associated protein CAP59 and its promoter region illustrate such implications at a small (gene) scale.

Conclusions: Most associations of phenotypes with fungal genomes from modestly sampled clinical isolate populations cannot be localized to one or a few regions in a genome. This problem is less restrictive in human GWAS studies or (possibly) in studies of recombining fungi sampled from the environment. It limits the benefits for molecular etiologic understanding that one might expect from GWAS of isolates obtained in clinical contexts, where key fungal phenotypes can be under stronger selection pressure. Exploratory power may be low if one compares just MLST types or phylogenetic clusters, or examines only variation within individual MLST types. More informative results may be gained by restricting the genomic-watch space a priori to a subset of genes that are likely to be related to a phenotype, and then zooming in to individual SNPs that are known/suspected to affect protein structure/function or influence transcription/translation of the gene. Where the modes of...
Isolation of keratinophilic fungi of the genus Microsporum from the soils of Moscow, Russia

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It is reported about the discovery of asexophilic and phyllophilic dermatophytes in the soils of city parks and adjacent territories of Moscow, Russia. Microscopic fungi of the Microsporum genus were found in 2750 samples (54%). Microsporum gypseum (42%) and Microsporum canis (22%), their number was higher in the soils taken from the adjacent areas where domestic animals are walked. This increases the risk of infection of animals upon contact with soil.

Objectives: The research was conducted to determine the frequency of occurrence of potential pathogens of animal dermatophytosis in those areas of the soil cover where pets are walking. Soil samples were taken from several areas: adjacent territories along the streets of Vohinnovskiy and Kostyakov, designated as areas 1 and 2, the park ‘Dishki’, Lianozovskiy, Timirovskiy, designated as 3, 4, and 5, respectively.

Areas 1 and 2 are narrow patches of soil adjacent to residential buildings and sidewalks where pets are most often walked. Areas 3 and 4 are areas of soil adjacent to park paths, where animal walking is less common. Area 5 is a relatively little-trafficked place in the forest park, where animals are rarely walked.

Methods: Before sampling, vegetation cover and rubbishes were removed from the soil area, then ~ 100 g of soil was taken from the surface using aseptic, sterile plastic containers. A total of 10 soil samples were taken from each plot. The soil was dried at room temperature, sieved to remove rubbish, and placed in sterile Petri dishes.

Isolation of fungi was carried out by the ‘bait’ method: samples of sterile pet hair about 1 cm long were placed on the surface of the soil, the soil was moisturized with sterile distilled water with the addition of a selective additive for the isolation of dermatophytes (Dermaid, Ossel). Petri dishes were periodically inspected for fungal growth on the surface of the wool and monitored for 6 weeks.

When the growth of microscopic fungi was detected on the surface of the wool, they were sterilely transferred to Petri dishes with yeast extract medium (MYE medium) to obtain a pure culture and identification. Only microscopic fungi of the genus Microsporum were taken into account.

Results: As a result of the research, a total of 27 isolates of microscopic fungi, 21 of Microsporum gypseum and 6 of Microsporum canis were isolated from the soils of Moscow; 7 isolates at the area 1 (6 of M. gypseum, 1 of M. canis), 8 isolates at the area 2 (5 of M. gypseum, 3 of M. canis), 9 isolates at area 3, and 4 (4 of M. gypseum, 1 of M. canis); 2 isolates of M. gypseum at the area 5. The results are illustrated in Figure 1.

Conclusions: Soils located in adjacent areas are more polluted by the hair of pets that walk there. Therefore, these soils also contain keratinophilic microscopic fungi, which includes the pathogens of animal dermatophytosis. In such places, animals are susceptible to infection with fungal infections directly from the soil.