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Development of a bioinformatic tool for the treatment of WGS data for dermatophytes typing and characterization: Focus on squalene epoxidase mutations and terbinafine resistance.

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Objectives

The present work aims to use the Whole Genome Sequencing (WGS) as a tool to characterize dermatophytes strains. Data generated by WGS are analyzed by using a bioinformatic tool called “WGS typer” and several markers are highlighted, such as genes implicated in resistance to antifungals or genes linked with high virulence in dermatophytes. The tool will also permit to analyze dermatophytes following their genetic diversity and provide similarity dendrograms. The present work focus on squalene epoxidase (SQLE) gene characterization among *T. rubrum* and *T. indotineae* strains by the WGS typer. Indeed, SQLE mutations have been linked with a resistance to terbinafine (TERB) in dermatophytes.

Methods

-15 strains of *T. rubrum* (7 R to TERB and 8 S)
-19 strains of *T. indotineae* (8 R to TERB and 11 S) from a previous study

(Multicentre validation of a EUCAST method for the antifungal susceptibility testing of microconidia-forming dermatophytes, J Antimicrob Chemother, *Arendrup et al, 2020*)

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Illumina Whole Genome Sequencing (GIGA-genomics platform, ULG, Liège, Belgium)

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WGS data analysis with « WGS typer » from **Hedera22** (Liège, Belgium), search for SQLE mutations

Results

1) Presentation of the “WGS typer” tool

The WGS typer is a commercial bioinformatics tool developed by Hedera-22 (http://www.hedera22.com) and licensed to the Department of Clinical Microbiology of the University of Liège. This tool enables high-throughput typing of pathogen isolates based on raw sequencing data and a collection of relevant markers (single genes, gene variants, gene clusters, MLST). The analysis reports the presence/absence of targeted markers or genotypes from a sequence homology search against the assembled sequencing data according to a set of sequence identity/coverage thresholds.

2) SQLE analysis

We evaluated the ability of the tool to detect amino acid (AA) substitutions in the SQLE protein that are responsible for terbinafine resistance in dermatophytes. Seven *T. rubrum* showed a resistant profile to terbinafine (MIC values >0.25µg/µl) with the previously tested microdilution method . **Figure 1** shows the distribution of AA substitutions on SQLE among our tested strains. Among these, four shared the F397L substitution on SQLE, one was wearing L393F substitution while two other shared the L393S substitution. All these substitutions in AA were efficiently highlighted by the **WGS typer.** Among the eight strains presenting a MIC value under 0.25µg/µl, no substitution was found on SQLE. Regarding *T. indotineae*, 8 strains were previously characterized to be resistant to terbinafine with the previously tested microdilution method (MIC values >0,25µg/µl). Among them, the WGS typer detected seven strains with the AA substitution F397L and one strain with the substitution L393F on SQLE. Among the eleven strains presenting a MIC value under 0.25µg/µl by microdilution method, no substitution was found on SQLE. **Figure 2** shows two examples of results given by the WGS typer from Hedera 22.

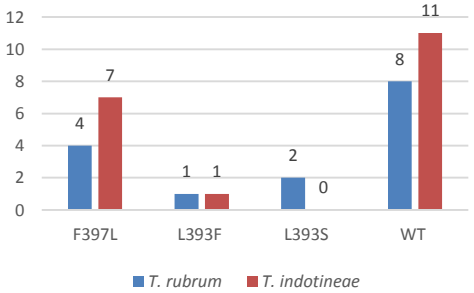
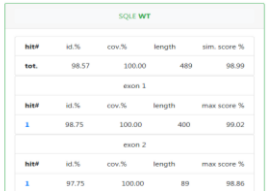


Figure 1: Graph representing the rates of substitutions found in *SQLE* among studied *T. rubrum* and *T. indotineae* strains.



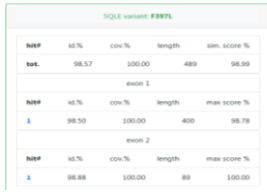


Figure 2: Example of results given by the **WGS typer** after WGS data assembly and analysis. In A: This shows a WT profile for SQLE in a susceptible *T. indotineae* strain for TERB, In B : This shows the presence of F397L AA substitution in SQLE in a resistant *T. indotineae* strain for SQLE.

3) Dendrogram of similarity based on WGS

The study was completed with genetic similarity comparisons of the strains and the generation of a dendrogram. **Figure 3** presents the results obtained with our tool. No clear separation into clusters was observed between resistant/susceptible strains neither in the *T. rubrum* group nor in the *T. indotineae* group . *T. rubrum* and *T. indotineae* species were well separated into two distinct clusters and classified in accordance to reference strains (in orange on fig.3).

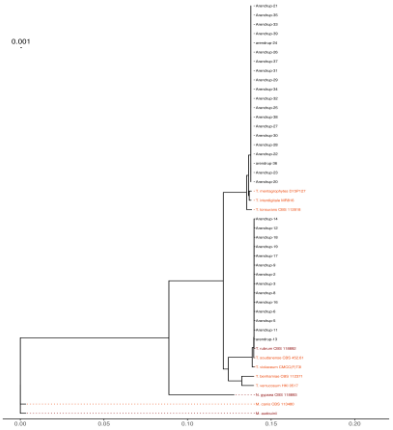


Figure 3: Dendrogram of similarity generated from the whole genome data of the analysed strains. Strains named « Arendrup 2 to 20 » are *T. rubrum* strains with and without resistance profiles for TERB. Strains named « Arendrup 21 to 39 » are *T. indotineae* strains with and without resistance profiles for TERB.

Conclusions

We present here a valuable and innovative tool for the analysis of dermatophytes. The tool permits to easily and accurately detect mutations on the **SQLE gene** responsible for **terbinafine resistance**. A dendrogram of similarity based on WGS data can also be generated and used for genomic comparison between analyzed strains.

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