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A multilocus sequence typing system for *Penicillium marneffei:* an international molecular cyber system for tracking its origin and transmission

Key Messages

1. A multilocus sequence typing (MLST) system was developed for *Penicillium marneffei*, an emerging opportunistic infection in Southeast Asia.
2. A website for *P marneffei* MLST is available at http://mlstdb.hku.hk:14206/MLST_index.html to enable comparison of strains from different localities.
3. Our *P marneffei* MLST system appears to be more discriminating and more suitable for epidemiology studies than other similar systems.
4. Non-housekeeping genes should be incorporated into the MLST system to achieve greater discriminatory power.

Introduction

Penicillium marneffei is the most important dimorphic fungus causing systemic mycosis in Southeast Asia.[1] The global dissemination of the human immunodeficiency virus (HIV) has led to the emergence of this infection as an important opportunistic mycosis in HIV-positive patients. About 10% of acquired immunodeficiency syndrome patients in Hong Kong are infected with *P marneffei*.

A highly reproducible and discriminative typing system enables better understanding of the epidemiology of the fungus. Although multilocus microsatellite typing and pulsed field gel electrophoresis were used to develop typing schemes for *P marneffei*, the results are not suitable for comparison due to experimental variations among different laboratories. We describe a multilocus sequence typing (MLST) scheme of *P marneffei*.

Methods

This study was conducted from January 2006 to December 2007. In order to have a better understanding of the epidemiology of *P marneffei*, we developed an MLST system for *P marneffei* using 44 human isolates.[2] The nucleotide sequences of the 11 housekeeping genes and 14 mannoprotein superfamily homologues in *P marneffei* strains were aligned and compared. The 44 *P marneffei* isolates were sequenced with the five most discriminative loci.

In the first part, DNA extracted from 10 of the 44 strains of *P marneffei* was used as the template for amplification of 11 housekeeping genes (mannose phosphate isomerase, plasma membrane H+ ATPase, pyruvate kinase, glutamate dehydrogenase, phosphoglucomutase, ribonucleoside-diphosphate reductase, glutamate synthase precursor, ribonucleotide reductase, transcription factor PacC, carbon catabolic repressor protein, and DNA topoisomerase II) and 14 lineage-specific genes (including MP1 and its homologues that belong to a novel mannoprotein superfamily).[2]

In the second part, DNA extracted from all 44 strains of *P marneffei* was used as the template for amplification of five of the 14 gene loci of the MP1 homologues (*MP1*, *MPLP4*, *MPLP7*, *MPLP10* and *MPLP13*).[2] The primers were designed by multiple alignments of the 14 homologues so that they were specific to each *MP1* homologue. The nucleotide sequences of the five gene loci in all the *P marneffei* strains were aligned and compared with those of strain PM1 using ClustalX (1.83). The ratio of non-synonymous to synonymous base substitutions (dn/ds) was calculated with START2 (http://pubmlst.org/software/analysis). Construction of dendrograms was performed with the unweighted pair group method with arithmetic mean (UPGMA) using Molecular Evolutionary Genetics Analysis 3.1. Grouping of sequence types (STs) into lineages was performed with BURST (Based Upon Related Sequence Types, http://pubmlst.

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org/analysis/burst/burst.shtml).

Results

Amplification and sequencing of the 11 housekeeping genes from 10 strains of *P marneffei* showed that the nucleotide sequences of all 11 genes were identical.[2]

In the first 10 strains of *P marneffei* sequenced, the sequences of four (*MPLP2*, *MPLP3*, *MPLP6* and *MPLP12*) of the 14 homologues were identical, five (*MP1*, *MPLP4*, *MPLP7*, *MPLP10* and *MPLP13*) showed more variations than others. Therefore, only these five loci were sequenced for the other 34 strains of *P marneffei* and the sequences of these five loci were used for developing the MLST system.[2]

Among the 2201 bp of the five loci, a total of 183 polymorphic sites were observed in the 44 *P marneffei* strains.[2] Allelic profiles were assigned to the 44 *P marneffei* strains. The alleles defined for the MLST system were based on sequence lengths of between 337 bp (*MPLP4*) and 549 bp (*MP1*).[2] The relatively high d_n/d_s ratio for the five genes indicated that a strong positive selective pressure was present at these loci.[2]

A total of 35 different STs were assigned to the 44 *P marneffei* isolates.[2] The overall discriminatory power for the MLST scheme was 0.9884.[2] The UPGMA was used to construct a dendrogram using the concatenated nucleotide sequences of the five gene loci from the 44 isolates.[2] The isolates were grouped into four lineages by BURST, with six STs in group 1, five in group 2, six in group 3 and two in group 4, whereas 16 STs did not belong to any of the four groups.[2]

Discussion

We developed an MLST system for *P marneffei*. The sequences of MP1 homologues are more variable than those of its housekeeping genes. The cloning and characterisation of MP1, which encodes an abundant, secreted and cell wall immunogenic mannoprotein, Mp1p, from *P marneffei* was reported in 1998.[3] Genes with higher lineage specificity in ascomycetes evolve at a much faster rate than those with lower lineage specificity.[4] Therefore, genes with higher lineage specificity are potentially more useful targets than housekeeping genes for typing pathogenic fungi. Furthermore, Mp1p is a cell wall immunogenic protein located on the surface of *P marneffei*, and hence is subject to strong selective pressure by the immune system.[3] Therefore, MP1 homologues are potentially more rapidly evolving than housekeeping genes. According to our complete genome sequence project,[5] there are more than 10 *MP1* homologues in the *P marneffei* genome. We therefore hypothesised that it may be more discriminatory if this set of gene targets were used for MLST in *P marneffei*. We sequenced 11 housekeeping genes and the *MP1* homologues of *P marneffei*. The sequences of the 11 housekeeping genes were identical among the 10 strains of *P marneffei* sequenced, but remarkable variations exist in the sequences of the *MP1* homologues. Therefore, the *MP1* homologues were used as targets for building the MLST system in *P marneffei*.

Our study showed that lineage specific genes may be better candidates than housekeeping genes for sequence based typing. When MLST systems were used in fungal pathogens, the discriminatory power was often unsatisfactory whenever only housekeeping genes were used. When one or two non-housekeeping genes were also included, the discriminatory power was markedly improved. This is in concordance with our previous observation that lineage specific genes were associated with more rapid evolutionary rates.[4] Therefore, lineage specific genes could be better targets for MLST schemes when applied to slowly evolving or recently evolved pathogens.

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