Identification of genetic changes acquired by replicate lineages of *Podospora anserina* during long-term submerged cultivation

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Abstract. Ascomycete fungus *Podospora anserina* is frequently used as a model organism in studies on the mechanisms of ageing due to its limited lifespan with clear signs of senescence on solid media. However, it shows no evidence of senescence when cultured under aeration in liquid media. The mechanisms underlying the unlimited vegetative growth are unknown. We conducted a long-term submerged cultivation experiment of eight replicate lineages of *P. anserina* and analyzed genetic changes that arose and became fixed in these lineages during the cultivation. Several lines of evidence suggest that some of the acquired mutations might be adaptive.

Keywords: *Podospora anserina*, long-term evolutionary experiment, environmental adaptation

1 Introduction

Ascomycete fungus *Podospora anserina* is a model organism used in studies on the mechanisms underlying senescence, as well as in studies on mitochondrial and peroxisomal physiology. Wild strains of *P. anserina* have a limited lifespan when grown on solid media (usually up to 25 days) which might be attributed to the reproductive strategy of the fungus. However, a submerged cultivation (cultivation in liquid media under aeration) results in unlimited vegetative growth of *P. anserina*, as was shown by Turker and Cummings in 1987 [1]. Prolonged submerged cultivation leads to pronounced morphological changes and transition to asexual reproduction. To determine whether there might be a genetic basis for the observed phenotypic changes we established eight independent lineages of *P. anserina* cultivated in liquid aerated media [2] and analyzed genetic changes acquired by these lineages over the course of submerged cultivation.

2 Results and Discussion

After 268 serial passages, eight replicate lineages fixed a total of 142 mutations, including 52 missense and 8 nonsense mutations, and 10 mutations that result in a frameshift. Fractions of both missense and nonsense variants among all fixed mutations were substantially higher than expected by chance. The observed enrichment of the fixed mutations for protein-altering variants suggests that some of the acquired mutations might be adaptive.

Functional annotation of the fixed mutations provides further evidence that some of them might result in the adaptive phenotype. A notable part of the affected genes with available annotation is involved in the growth- and development-associated processes in various ascomycete fungi (e. g., transcription factor *pro-1*, development regulator *FlbA*, sexual reproduction regulator *VeA*, GTPase subunit alpha *FadA*). Moreover, we observed parallel evolution at the gene level with five genes possessing different protein-altering mutations fixed in parallel in more than one independent lineage.

Fixation of distinct missense mutations in seven independent replicate lineages by GTPase subunit alpha *FadA* might be regarded as the most striking example of parallel evolution. Five of these mutations affect Arg and Gly residues (Arg-178 and Gly-183 in mammalian Gαi1) critical for GTP hydrolysis and are likely to result in the constitutive activation of G protein alpha subunit [3], [4]. *FadA* in its active form was shown to promote vegetative growth in *A. nidulans* [5]. An unlimited vegetative growth of *P. anserina* under submerged cultivation is in line with putative constitutive activation of *FadA*. Interestingly, two independent lineages acquired nonsense mutations in *FlbA* gene that encodes a regulator of G protein signaling. *FlbA* is vital for efficient GTP hydrolysis by G-alpha subunit [5]; therefore, the loss-of-function mutations in *FlbA* and missense mutations in *FadA* might have similar phenotypic consequences.

Finally, we analyzed evolutionary conservation of *P. anserina* proteins affected by missense and nonsense mutations. We showed that proteins carrying missense and nonsense mutations are significantly more conserved as compared to genomic background. Furthermore, protein residues affected by missense mutations show high levels of evolutionary constraint, implying functional importance of the affected sites.

Taken together, our results suggest that some of the fixed genetic changes in the replicate lineages of *P. anserina* might be adaptive under the conditions of the experiment.

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