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SPECIFIC AIMS

The goal of this project is to study the evolutionary prospect of microbial eukaryotes, especially the adaptation process of *Saccharomycopsis fibuligera* to different environmental conditions. In this project, we will use *S. fibuligera* as a model organism to study the speciation process of microbial eukaryotes. We believe that microbial eukaryotes should exhibit speciation process similar to that observed in multicellular eukaryotic organisms. We aim to test this hypothesis by comparative genomics analysis and by transcriptomics analysis between different adapted *S. fibuligera* strains. We will use the next generation DNA sequencing techniques, 454 Genome Sequencer and Illumina Genome Analyzer technology, to obtain the whole genome sequence of different adapted *S. fibuligera* strains and to obtain the expression data of these strains for the inferring of genetic variation to the phenotypic variation.

BACKGROUND AND SIGNIFICANCE

It has been accepted that in multicellular eukaryotes, plants and animals, new biological species may arise as a consequence of genetic divergence in allopatric, parapatric or sympatric isolation followed by natural selection. However, despite the large contribution of microorganisms to the world’s biodiversity our understanding of the speciation processes that generate new microbial species remains limited (Martiny et al. 2006). Recently, it was proposed that microbial eukaryotes in general do not show biogeographical divergence due to the large population sizes of the microbes and the global distribution of the microbial eukaryotes (Finlay and Clarke 1999; Finlay 2002; Fenchel and Finlay 2006). This “no microbial biogeography” hypothesis states that “everything is everywhere, and the environment selects” and casts doubt on whether geographic divergence promotes speciation in microbes. This hypothesis’s most important implication is in sexual eukaryotic microbes, where migration and genetic admixture are specifically predicted to inhibit allopatric divergence and speciation. Indeed, the local abundance of microbial eukaryotic species is impressively large and most of them show global distribution. It has been shown that the same genotype of flagellated protozoan was isolated from a shallow inland fjord in Denmark and from hydrothermal vents in the Pacific (Atkins et al 2000). In addition, the same planktonic foraminiferan morphospecies were found to be common to both Arctic and Antarctic waters, and some of these are also genetically identical (Darling et al. 2000). However, recent studies showed that
Saccharomyces paradoxus exhibits occurrence of evolutionary processes for allopatric speciation when comparing the genetic differences among the native S. paradoxus strains isolated in Europe and North America (Kuehne et al. 2007). Moreover, several studies also showed the genetic diversity in Saccharomyces cerevisiae strains isolated from natural and artificial fermentations, tree exudates, and immunocompromised patients (Mortimer et al. 1994; Fay and Benavides 2005; Aa et al. 2006).

S. fibuligera is a food borne dimorphous yeast. It is considered as one of the best producers of amylolytic enzymes in the realm of ascomycetous yeast species (De Mot et al. 1984) and is used in Chinese rice wine production in Asia (Barnett et al., 1990). S. fibuligera has been used in co-cultures with various microorganisms, e.g. with Candida utilis to produce single-cell protein (SCP) (Pasari et al. 1989), with Streptococcus lactis to produce lactic acid (Häggstrom and Dostálek 1981), with Saccharomyces cerevisiae to produce ethanol (Dostálek and Häggstrom 1983; Abouzied and Reddy 1987; Piršelová et al. 1993), and with Rhodosporidium toruloides to produce lipids (Dostálek 1986). Despite S. fibuligera has great potential for application in both agriculture and industry, little is known at the molecular level on this species to date. In this project, we aim to obtain the whole genome sequence of S. fibuligera by the latest DNA sequencing technique, 454 sequencing technology because the 454 technique is more suitable for obtaining accurate sequence data from a novel unsequenced genome. The genome sequence of different adapted S. fibuligera strains will be obtained by using Solexa sequencing technology. The genome sequence obtained by 454 technology will be used as template for Solexa genome sequence assembly. Solexa technology could provide large quantity of sequence data at a much cheaper cost therefore is suitable for intraspecies sequence comparison. We will perform gene annotation to discover the basic molecular information of this species and study the genetic variations between different adapted strains of S. fibuligera.

A major goal of evolutionary biology is to understand the genetic basis of adaptation. Although the use of anonymous DNA sequence markers can be very informative for discriminating the environmental-adapted strains, it is very difficult to make population genetic or functional inferences from the patterns of genetic variations. The fundamental question we could like to address in this project is “What is the relationship between adaptive phenotypic changes and the genetic changes in the genome?” Are adaptive phenotypic changes caused by alterations at many loci of small effect, at a few loci of large effect, or some combination of the two? The results of this research should shed light on
the evolution of microbial eukaryotes, especially the speciation process of microbial fungi.

**Progress**

We have collect and characterized the biological properties of 21 different starch-specialized *S. fibuligera* strains by growth pattern analysis using different starch as sole carbohydrate source (Table 1). These are the strains collected from the breweries except for BCRC2155 which was purchased from Bioresource Collection and Research Center, Hsinchu. We used 18S rDNA sequence to confirm that these strains are *Saccharomycopsis*. Our results indicated that PF43 grew better with rice and potato starch; BCRC21511 grew better with rice, potato and wheat starch; PF53, PF49 and PF51 grew better with corn starch but not other type of starch. In general, we found that BCRC21511 could utilize rice starch, potato starch or wheat starch well compared to other strains. These observations are consistent with our hypothesis that different strains of *S. fibuligera* may adapted to different type of starch.

We used *S. fibuligera* BCRC21511 as our model organism for whole genome sequencing. We have obtained about 329Mb of DNA sequence data which contain at least 3200 contigs that the length of the contig is greater than 1300bp. We are currently annotation the function of genes by blasting the *S. fibuligera* sequencing against known yeast genomes. We are interesting on the carbohydrate metabolism genes. We will use PCR base methods combine with high throughput sequencing by Illumina Genome Analyzer to identify the possible genetic variation for the different starch utility of different strains of *S. fibuligera*.

We are also interested on the reproductive system of *S. fibuligera*. Some of the yeasts exhibit the alternative halploid and diploid state life style which is controlled by the sex determent genes and environmental stress. We are currently working on identifying the sex determent genes in *S. fibuligera*. 
Table 1 Utilization of different starch by different strains of *S. fibuligera*

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<th>Rice</th>
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<th>Wheat</th>
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<td>PF52</td>
<td>PF45</td>
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</tr>
<tr>
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<td>Potato</td>
<td>Wheat</td>
<td>Corn</td>
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<tr>
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<td>PF49</td>
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**Literature cited**


Fenchel, T. and Finlay, B.J. 2006. The diversity of microbes: resurgence of the phenotype.