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Comparison and Analysis of the Genomes of Two *Aspergillus oryzae* Strains

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Supporting Information

ABSTRACT: *A. oryzae* 3.042 (China) and *A. oryzae* RIB40 (Japan) used for soy sauce fermentation show some regional differences. We sequenced the genome of *A. oryzae* 3.042 and compared it to *A. oryzae* RIB40 in an attempt to understand why different features are shown by these two *A. oryzae* strains. We predict 11 399 protein-coding genes in *A. oryzae* 3.042. The genomes of these two *A. oryzae* strains are collinear revealed by MUMmer analysis, indicating that the differences are not obvious between them. Several strain-specific genes of two strains are identified by genome sequences' comparison, and they are classified into some groups, which have the relationship with cell growth, cellular response and regulation, resistance, energy metabolism, salt tolerance, and flavor formation. *A. oryzae* 3.042 showed stronger potential for mycelial growth and environmental stress resistance, such as the genes of chitinase and quinone reductase. Some genes unique to *A. oryzae* RIB40 were related to energy metabolism and salt tolerance, especially genes for Na^+ and K^+ transport, while others were associated with signal transduction and flavor formation. The genome sequence of *A. oryzae* 3.042 will facilitate the identification of the genetic basis of traits in *A. oryzae* 3.042, and accelerate our understanding of the different genetic traits of the two *A. oryzae* strains.

KEYWORDS: *A. oryzae, comparative genomic, mycelial growth, energy, flavor*

INTRODUCTION

Soy sauce, an indispensable condiment used in East Asian countries, is produced by fermenting soybeans and wheat with salty water.[1] *A. oryzae*, a very important filamentous fungus, has been widely used in the soy sauce fermentation industry. *A. oryzae* 3.042 can grow under diverse environmental stress conditions, such as those encountered during soy sauce fermentation. *A. oryzae* RIB40 was isolated from broad bean in the gutter of a soy sauce factory, which indicates that it can resist the high salt press. In addition, the flavors of Chinese and Japanese soy sauces are distinct, and this may be due to the different *A. oryzae* strains.

Proteomics is a powerful platform for analysis of the protein identification. 522 kinds of proteins in *A. oryzae* 3.042 that are expressed specifically during fermentation have been identified using a proteomics approach.[2] However, the prominent features of the strains remain unknown. In addition, *A. oryzae* RIB40, the Japanese strain, has been sequenced.[3] Yet it is so difficult to find the possible explanations of the two strains' features. Comparative genomics has emerged as a powerful tool in genome analysis. Nowadays, there has been increased interest in comparative genomic approaches because these provide a powerful ability to identify multiple genes that are expressed differentially between distinct microbial strains.[4]

Comparative genome analysis, based on the complete genome sequences, can be used to reveal numerous insights into genetics and physiology of *A. oryzae*. To date, the genetic differences between Chinese and Japanese strains remain unclear; thus there exists a strong desire to compare these strains. Here, we present the whole genome sequence of *A. oryzae* 3.042, and the aim of this study is to investigate the genetic differences of *A. oryzae* 3.042 and RIB40 strains by performing a comparison of the genomes.

MATERIALS AND METHODS

Samples and DNA Preparation. *A. oryzae* RIB40 was donated by Dr. Li Pan, South China University of Technology.[5] Strain 3.042 was obtained from the Strain Collection Center, Tianjin University of Science & Technology (Tianjin, China). Strains 3.042 and RIB40 were incubated in rice-juice medium.[6] Genome DNA was extracted using standard protocols[7] after the mycelium of the 3.042 strain was frozen in liquid nitrogen and then crushed with a pestle.

Genome Sequencing, Prediction, Annotation, and Comparison. Roche 454 FLX and the Illumina/Solexa automated machines were used for DNA sequencing.[8] Genomic library construction, sequencing, and finishing were performed at the Tianjin Biochip Corp., China. The sequence data were assembled using Newbler[9] and SOAP[10] assembly software. AUGUSTUS[11] and genBlast were used as predictors. The *A. oryzae* conserved regions were automatically combined into consensus gene structure annotations using the EvidenceModeler (EVM) software.[12] Specific proteins were compared against the NCBI and nonredundant (NR) protein databases by BLAST[13] to forecast their biological functions. tRNA genes were predicted using tRNAscan-SE.[14] The draft genome sequence and the mitochondrion genome complete sequence of *A. oryzae* 3.042 have been deposited at GenBank under the accession codes AKHY00000000 and JX129489, respectively.[15]

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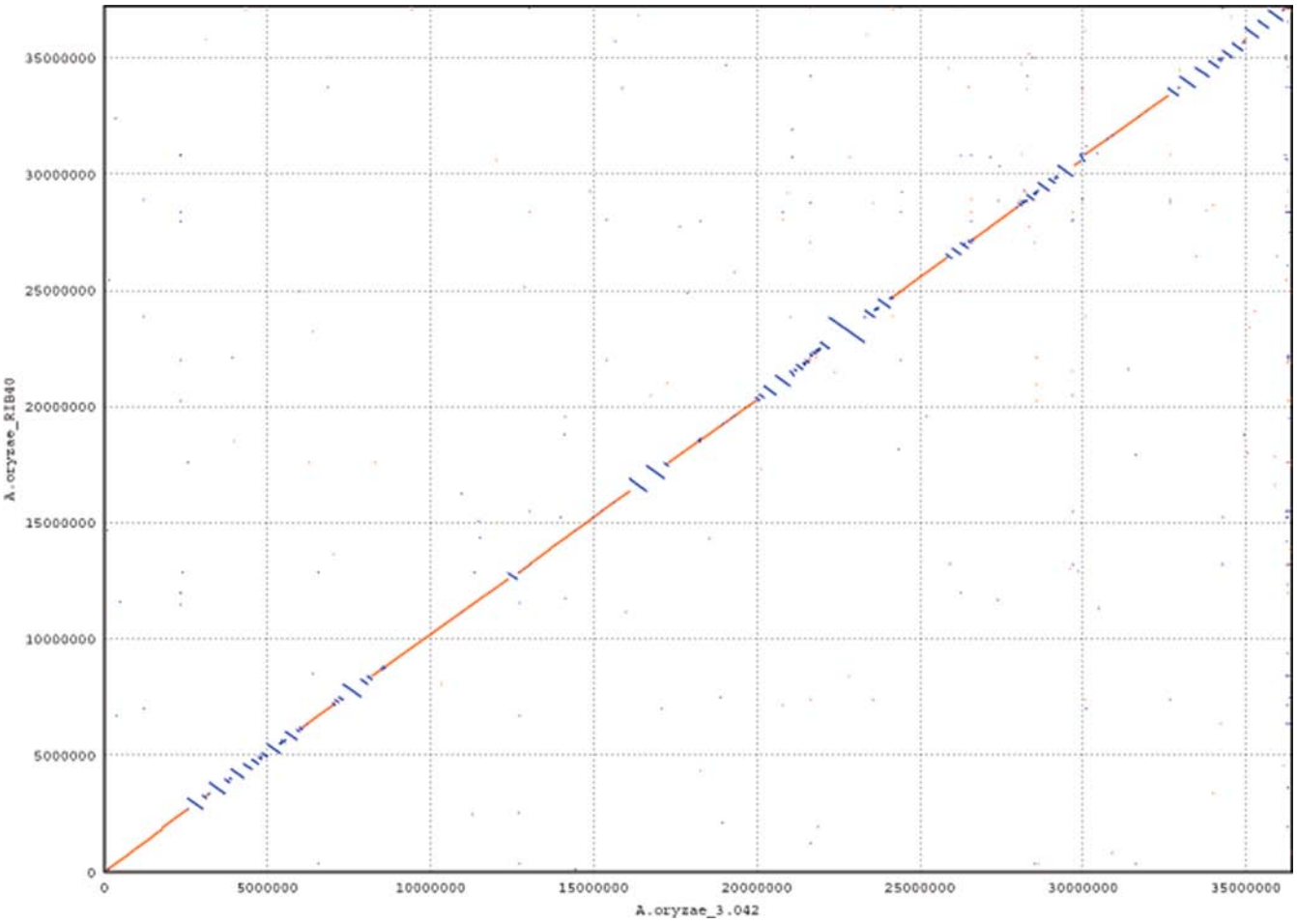

Figure 1. Results of MUMmer-based comparison of *A. oryzae* RIB40 and 3.042 strains. The nucleotide positions are indicated in numbers.

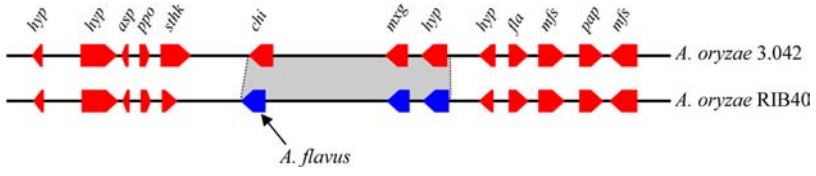

Figure 2. Comparison of the unique gene that encoded chitinase and its flanking regions of *A. oryzae* RIB40 and *A. oryzae* 3.042. The abbreviations of gene annotation names in this figure are listed in Table S3.

Comparative genomics were performed using the MUMmer program,[16] and a synteny plot was generated. The program uses exact matching, clustering, and alignment extension strategies to create a dot plot based on the number of identical alignments between two genomes. OrthoMCL was developed as an alternative approach for automated eukaryotic orthologue group identification.[17] Clusters of proteins where each cluster consists of orthologues or paralogues were generated by OrthoMCL between these two strains. Unique gene acquisitions and losses were found, and the similarity of proteins was encoded by genes at a locus of interest (>50% identity at the predicted protein level).

RESULTS AND DISCUSSION

Genome General Features. The strain *A. oryzae* 3.042 was sequenced using a combination of Illumina-Solexa paired-end strategies and Roche 454 sequencing chemistry. The genome of *A. oryzae* 3.042 was high throughput sequenced to ~100× coverage. Reads were assembled into 226 contigs. The total genome assembly size was found to be 36.5 Mbp with an overall G+C content of 48.3%, which was not expected to exceed the value of *A. oryzae* RIB40 genome size, 37.6 Mbp.

The *A. oryzae* 3.042 genome encodes 11 399 putative coding sequences (CDS), which were carried out on the contigs. After their manual annotation, 6578 CDS (63%) could be assigned to putative biological functions, while 4821 CDS (37%) were annotated as hypothetical proteins of unknown function. A total of 243 tRNAs were predicted by the tRNAscan-SE software.

MUMmer analysis showed that the genomes of the 3.042 and RIB40 strains were mostly collinear, although the genome of *A. oryzae* 3.042 had gaps, translocations, and/or inversions when compared to the RIB40 strain (Figure 1). After examination of the sequence, some of these gaps and/or inversions were associated with integrative and conjugative elements.

Genomes Comparison and Unique Genes Identification. OrthoMCL clustering procedure was used to find unique genes of each strain. OrthoMCL grouped all of the genes into

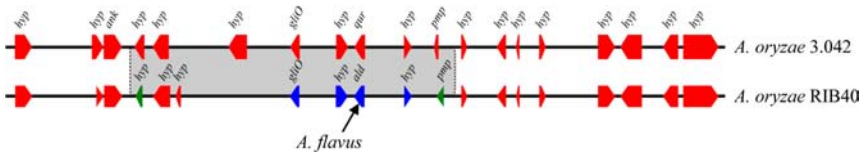

Figure 3. Comparison of the unique gene that encoded quinone reductase and its flanking regions of *A. oryzae* RIB40 and *A. oryzae* 3.042. The abbreviations of gene annotation names in this figure are listed in Table S4.

"orthologue groups" including >11 000 orthologous protein-encoding genes. The genes unique to *A. oryzae* 3.042 were BLASTed against the genome sequences of the other species in NCBI database. Strains 3.042 and RIB40 contained 78 and 102 unique genes other than conserved predicted proteins, respectively (Supporting Information). These remarkable findings may reflect different functions in growth, development, and metabolism. In this present study, attention then focused on the genes related to these aspects.

Cell Growth. In *A. oryzae* 3.042, some genes are directly involved in mycelium growth (Supporting Information). CipC protein and chitinase contribute to hyper-branching and hyphal growth. As shown in Figure 2, gene encode chitinase is similar to the gene in *A. flavus*. Serine/threonine-specific protein phosphatase (PP1), which is also a crucial component of the basic functions, is necessary for cell growth. Phosphatidylserine decarboxylase and fibronectin are essential for cell wall integrity and extracellular matrix and cell−cell interactions. In *A. oryzae* RIB40, the TPR repeat protein promotes cell proliferation, and WD40 repeat protein regulates fungal cell differentiation. Otherwise, different amino acid transporters transport amino acids across the cellular membranes.

Cellular Response and Regulation. Proteins encoded by unique genes are involved in multiple cellular responses, such as cell cycle check-point control, DNA repair, and transcription regulation. In *A. oryzae* 3.042, the NPP1 domain containing protein triggers cell death and the activation of defense signaling reactions.[18] The ankyrin repeat is the amino acid motif in protein databases for protein recognition, and the interaction module is always involved in a diverse set of cellular functions.[19] TBP-interacting protein (TIP49) is a central component in transcriptional regulation.[20] Many regulatory mechanisms might enable the controlled synthesis of polypeptides via stop-condons in cells.[21] The methyltransferase enzyme is critical for the catabolic regulation of many physiological reactions.

In *A. oryzae* RIB40, Ras1 and a putative guanine nucleotide exchange factor perform the crucial steps in signaling.[22] Signal recognition particles play roles in targeting of many functional proteins to relevant locations. In addition, the S−M checkpoint proteins are important in the intracellular signaling pathway.[23]

Resistance. Resistance genes can protect cells from environmental stresses and toxicity agents. Copper amine oxidase and tropomyosin in the 3.042 strain are defense-associated proteins.[24] Aminoglycoside phosphotransferase of *A. oryzae* 3.042 confers resistance to the action of the antibiotics gentamicin and kanamycin.[25] Glutathione-*S*-transferase in the 3.042 strain plays a role in resistances to chemicals, drugs, antibiotics, and insecticides.[26] Meanwhile, the NADPH:quinone reductase of *A. oryzae* 3.042 protects against quinone toxicity.[27] In *A. oryzae* 3.042, the gene that encodes quinone reductase is unique as compared to *A. oryzae* RIB40 (Figure 3).

Secondary metabolites can provide fitness attributes to the producer organism, and these often act to defend against environmental insults.[28] The genome of the 3.042 strain contains several unique genes associated with secondary metabolism. The alpha/beta hydrolases play roles in metabolism,[29] while the C6 zinc finger domain protein[28] and nonribosomal peptide synthetase modules[30] are implicated in the production of secondary metabolites. In addition, the basic region/leucine zipper motif (bZIP) transcription factor regulates various cellular processes, including some pathogen defense mechanisms.[31]

In *A. oryzae* RIB40, the multidrug resistance-associated ABC protein plays a role in defense against toxic natural products, and glycolate oxidase can catalyze a redox reaction to defend against pathogenic infections, which are infected by the pathogenic bacterias such as *Mycobacterium marinum*, *Micrococcus luteus*, and *Escherichia coli*.[32]

Energy Metabolism. Oxidative phosphorylation system is contained in the mitochondrial inner membrane. Various transport proteins and enzymes act as the electron tributaries of the respiratory chain.[33] Ubiquinone is a component of the electron transfer system in the organism. UbiH is known to be involved in ubiquinone biosynthesis in *A. oryzae* 3.042.[34] The unique gene that encodes ferredoxin as a reductant is the electron donor in *A. oryzae* RIB40.[35] F0F1-type ATP synthase catalyzes the synthesis of ATP from ADP and inorganic phosphate.[36]

Salt Tolerance. Cellular osmotic and ionic stresses are closely linked with salt tolerance of strain.[37] Intracellular ion concentration adjustment is necessary for adaptation to high salt concentrations. External Ca^{2+} enhances salt tolerance, which may correlate with alleviating Na^+ toxicity. Calcium-transporting ATPases are essential for Ca^{2+} homeostasis. The Na^+/H^+ antiporter is not a respiratory Na^+ pump, and Na^+ extrusion is sensitive to an H^+ conductor. In addition, six unique genes related to K^+ channels for transporting were found in *A. oryzae* RIB40.

Flavor Formation. Flavor compounds are the main body of soy sauce. Short-chain alcohol dehydrogenase can catalyze aldehydes to alcohols. Predicted hydrolases and peptidases catalyze the removal of kinds of amino acids from proteins. The aldo/keto reductase family proteins found in *A. oryzae* 3.042 catalyze the reduction of aldehydes and ketones to alcohols. Esters are formed by different kinds of alcohols and carboxylic acids in an enzyme-catalyzed reaction. The esterases in the 3.042 strain both hydrolyze and synthesize the ester bonds. PhzC/PhzF protein plays an indispensable role in phenazine biosynthesis.[38] Dienelactone hydrolase promotes the degradation of chloroaromatic compounds.[39] D-Aminoacylase is an attractive candidate for the production of D-amino acids.

In *A. oryzae* RIB40, selenocysteine lyase, serine *O*-acetyltransferase, prephenate dehydratase, and threonine synthase are important for amino acids biosynthesis. Polyamines, which may be degraded to form the precursors of coffee,[40] are cleaved by polyamine oxidase.[41] Geranyl pyrophosphate synthase can supply the precursor for the

production of monoterpene, one of the important flavor compounds.[42]

In our study, the Chinese strain, *A. oryzae* 3.042, was sequenced and compared to the related Japanese strain *A. oryzae* RIB40. These two strains have different phenotypes: the 3.042 strain grows faster, and it can probably grow in more complicated circumstances as compared to the RIB40 strain. However, the salt tolerance of *A. oryzae* RIB40 is higher than the 3.042 strain, which has important implications in industrial application. The soy sauce flavors may be differently fermented by *A. oryzae* 3.042 and RIB40 strains, which has important implications in industrial application.

The diversity of the two strains was explored by comparative genomic, and this approach was supported by multiple types of experimental work. The whole genomic sequences of these two strains were compared in an effective method for the identification of unique genes likely to mediate sequence-specific biological functions. Surprisingly, most unique genes of these two strains were involved in these aspects: cell growth, cellular response and regulation, resistance, energy metabolism, salt tolerance, and flavor formation that highlights the ability of the genome to facilitate future exploration of other biological problems.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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