Analysis of the MAT1-1 and MAT1-2 Gene Ratio in Black Koji Molds Isolated from Meju

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Abstract Aspergillus luchuensis is known as an industrially important fungal species used for making fermented foods such as awamori and shochu in Japan, makgeolli and Meju in Korea, and Pu-erh tea in China. Nonetheless, this species has not yet been widely studied regarding mating-type genes. In this study, we examined the MAT1-1 and MAT1-2 gene ratio in black koji molds (A. luchuensis, Aspergillus niger, and Aspergillus tubingensis) and in Aspergillus welwitschiae isolated from Meju, a fermented soybean starting material for traditional soy sauce and soybean paste in Korea. The number of strains with the MAT1-1 locus was 2 of 23 (A. luchuensis), 6 of 13 (A. tubingensis), 21 of 28 (A. niger), and 5 of 10 (A. welwitschiae). Fungal species A. tubingensis and A. welwitschiae showed a 1 : 1 ratio of MAT1-1 and MAT1-2 mating-type loci. In contrast, A. luchuensis revealed predominance of MAT1-2 (91.3%) and A. niger of MAT1-1 (75%). We isolated and identified 2 A. luchuensis MAT1-1 strains from Meju, although all strains for making shochu in Japan are of the MAT1-2 type. These strains may be a good resource for breeding of A. luchuensis to be used in the Asian fermented-food industry.

Keywords Aspergillus luchuensis, Mating type, Meju

Black aspergilli or Aspergillus section Nigri are an important and diverse group of fungi with several key characteristics including food spoilage, suitability as starters for fermentation, and production of metabolites for use in medicine. Black koji molds are major fungi belonging to this group and are associated with production of distilled alcoholic beverages awamori and shochu in Japan and makgeolli in Korea [1]. Three species Aspergillus luchuensis, Aspergillus niger, and Aspergillus tubingensis mainly constitute black koji molds among which A. luchuensis is an industrially important black aspergillus, especially in the food and fermentation industries of East Asia [2]. Fungus A. luchuensis has been well established as the chief fermentation agent in awamori-koji [3] but is also reported from nuruk and Meju, a Korean traditional fermentation starter for soybean paste and soy sauce [4, 5]. After A. luchuensis, A. niger is another important species of black koji mold that has been widely used in biotechnological processes and is the only one that has been granted the generally regarded as safe (GRAS) status by the U.S. Food and Drug Administration (FDA). With their potential applications, the members of black koji molds are a remarkable fungal resource for industrial research.

Almost all Aspergillus species from section Nigri exclusively propagate through asexual mode of reproduction. Nevertheless, there are reports on sexual reproduction of members of section Nigri that have the so-called mating-type (MAT) genes, which have been identified in other filamentous ascomycetes and found to play a major role in sexual reproduction [6, 7]. These MAT genes have been reported to encode several transcription factors that determine sexual identity and regulate later stages of sexual development. Two distinct classes of MAT genes, MAT1-1 and MAT1-2, are recognized. The MAT1-1 family is known to encode an α-domain protein, and the MAT1-2 gene family codes for a high-mobility group (HMG) box protein. These genes are located either both in a single MAT locus or at 2 loci, called MAT1-1 and MAT1-2, within the genome [8-11]. Homothallic aspergilli are known to possess both MAT genes in the same genome. In case of heterothallic species, only a single MAT locus is found to be present, which can...
contain either MAT1-1 or MAT1-2 family genes.

Sequence analysis has indicated that these MAT1-1 and MAT1-2 loci are vastly divergent, and gene mutation studies in the fungus *Aspergillus fumigatus* have shown MAT genes to be essential for sexual development [12]. Cloning of MAT genes by conventional methods has largely been unsuccessful due to high divergence of the gene sequences among ascomycetes despite evolutionary conservatism of the a box and HMG domains [13]. However, 2 methods, namely, the use of degenerate primers and whole-genome sequence mining, are now available for detection of the mating-type genes in *Aspergillus* species [9, 14-18].

MAT genes are therefore necessary for sexual reproduction among *Aspergillus* species. It has been reported that deletion of MAT1 and MAT2 genes leads to a loss of sexual reproduction in *Aspergillus nidulans*, whereas overexpression suppresses vegetative growth and induces sexual differentiation even under unfavorable conditions [19]. Thus, these genes may have an important function in the breeding of *Aspergillus* fungi. Apart from contributing to the evolution of new species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species. Thus, these genes may have an important function in the breeding of *Aspergillus* fungi. Apart from contributing to the evolution of new species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species, mating in aspergilli may aid survival during abiotic stress conditions and can improve industrially important species.

**Table 1.** The list of black koji mold strains used in this study with their source and mating type

| Species               | KACC No. | Strain information                  | Source               | Mating type |
|-----------------------|----------|-------------------------------------|----------------------|-------------|
| *Aspergillus luchuensis* | 46772    | NBRC 4281 = RIB 2642 = CBS 205.80 (extype of *A. luchuensis*) | Koji, Okinawa, Japan | MAT1-2      |
|                       | 46771    | NBRC 4308 = CBS 117.80 = NRRL 4886 (extype of *A. kawachii*)  | Koji, Kyusyu, Japan | MAT1-2      |
|                       | 46516    | CF 1005 (industrial strain of *A. kawachii* in Korea)         | Koji, Korea          | MAT1-2      |
|                       | 45131    | NBRC 4121 = NRRL 4796 = CBS 564.65 (extype of *A. acida*us)  | Koji, Okinawa, Japan | MAT1-2      |
|                       | 41731    | CBS 119384 = NR 15-1 (extype of *A. coreanus*)                    | Nurrak, Korea        | MAT1-2      |
|                       | 47234    | JCM 2261 = IAM 2112 (extype of *A. awamori*)                     | Koji, Okinawa, Japan | MAT1-2      |
|                       | 47004    | NBRC 4123 = NRRL 4797 = IAM 2397 = ATCC 16884                   | Koji, Okinawa, Japan | MAT1-2      |
|                       |          | = CBS 565.65 = IMI 175963                                    |                      |             |
|                       | 47235    | JCM 22302 = IAM 2255 = CBS 125.52 = R-0436 (extype of *A. inuii*) | Koji, Ryukyu, Japan | MAT1-2      |
|                       | 47005    | K-4133 = NRRL 4750 = CBS 128.52                                 | Unknown, Japan       | MAT1-2      |
|                       | M*29     | This study                                                          | Meju, Yangpyeong, Korea | MAT1-2 |
|                       | M532     | This study                                                          | Meju, Hoengseong, Korea | MAT1-2 |
|                       | M1003    | This study                                                          | Meju, Yeoju, Korea   | MAT1-2      |
|                       | M2093    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2096    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2097    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2098    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2099    | This study                                                          | Meju, Sunchang, Korea | MAT1-2     |
|                       | 46491    | This study                                                          | Meju, Sunchang, Korea | MAT1-2     |
|                       | M2103    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2104    | This study                                                          | Meju, Anseong, Korea | MAT1-2     |
|                       | 46490    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2106    | This study                                                          | Meju, Yongin, Korea  | MAT1-2      |
|                       | M2113    | This study                                                          | Meju, Yangpyeong, Korea | MAT1-2 |

on the ratio of MAT genes in *A. luchuensis*, an industrially important species for fermentation in Asia. We planned to study the MAT1-1 and MAT1-2 gene ratio of *A. luchuensis* and extended our interest to black koji molds isolated from Meju using PCR analysis. The purpose is to expand existing knowledge about MAT genes in *Aspergillus* section Nigri and to use these data for future industrial breeding of *A. luchuensis*.

**MATERIALS AND METHODS**

**Strains and media.** Ex-types of *A. luchuensis*, *A. tubingensis*, *A. niger*, and *A. welwitschiae* were obtained from Korean Agricultural Culture Collection (KACC, Korea), and in addition, *Aspergillus* strains that were isolated from traditional Meju in South Korea were included in this study. Detailed information on the strains is given in Table 1. The strains were inoculated onto malt extract agar to revive the culture and for further experiments.

**Genomic DNA extraction.** Strains of section Nigri species used in this study (Table 1) were grown in 10 mL of malt extract broth at 200 rpm (25~28°C). The mycelium was harvested after 2~3 days of growth by filtration through Miracloth (Merck Millipore, Billerica, MA, USA) and was then placed loosely in microfuge tubes, frozen, and freeze dried. The freeze-dried biomass was ground to a fine powder,
**Table 1. Continued**

| Species          | KACC No. | Strain information                  | Source                      | Mating type |
|------------------|----------|-------------------------------------|-----------------------------|-------------|
| *A. tubingensis* | M100     | This study                          | Meju, Haenam, Korea        | MAT1-2      |
|                  | 46498    | This study                          | Meju, Hoengseong, Korea    | MAT1-1      |
|                  | M1001    | This study                          | Meju, Yeoju, Korea         | MAT1-1      |
|                  | M1004    | This study                          | Meju, Yeoju, Korea         | MAT1-2      |
|                  | M1005    | This study                          | Meju, Pocheon, Korea       | MAT1-2      |
|                  | M1006    | This study                          | Meju, Anseong, Korea       | MAT1-2      |
|                  | M2095    | This study                          | Meju, Yongin, Korea        | MAT1-1      |
|                  | 46499    | This study                          | Meju, Sunchang, Korea      | MAT1-1      |
|                  | M2101    | This study                          | Meju, Sunchang, Korea      | MAT1-1      |
|                  | 46805    | CBS 134.48 = R. Mosseray, No. 726 (type of *A. tubingensis*) | Unknown                     | MAT1-1      |
|                  | 46993    | NRRL 4757 = IAM 2209 = CBS 136.52 = R6711 (type of *A. saitoi*) | Koji, Japan                | MAT1-2      |
|                  | 46994    | NRRL 4758 = IAM 2190 = CBS 137.52 = K-3931 (type of *A. saitoi var. kagoshimaensis*) | Koji, Japan                | MAT1-2      |
|                  | 47137    | This study                          | Meju, Korea                | MAT1-2      |
| *A. niger*       | M44      | This study                          | Meju, Yangpyeong, Korea    | MAT1-2      |
|                  | M224     | This study                          | Meju, Cheongwon, Korea     | MAT1-1      |
|                  | M267     | This study                          | Meju, Goisan, Korea        | MAT1-2      |
|                  | M347     | This study                          | Meju, Korea                | MAT1-2      |
|                  | 46493    | This study                          | Meju, Icheon, Korea        | MAT1-1      |
|                  | M458     | This study                          | Meju, Icheon, Korea        | MAT1-2      |
|                  | M478     | This study                          | Meju, Yangju, Korea        | MAT1-1      |
|                  | 46494    | This study                          | Meju, Hoengseong, Korea    | MAT1-1      |
|                  | M561     | This study                          | Meju, Hoengseong, Korea    | MAT1-1      |
|                  | 46495    | This study                          | Meju, Gimpo, Korea         | MAT1-1      |
|                  | M581     | This study                          | Meju, Gimpo, Korea         | MAT1-1      |
|                  | M602     | This study                          | Meju, Kimcheon, Korea      | MAT1-1      |
|                  | M619     | This study                          | Meju, Iksan, Korea         | MAT1-1      |
|                  | M630     | This study                          | Meju, Gimcheon, Korea      | MAT1-1      |
|                  | M639     | This study                          | Meju, Jcheon, Korea        | MAT1-1      |
|                  | M654     | This study                          | Meju, Sunchang, Korea      | MAT1-1      |
|                  | M701     | This study                          | Meju, Sunchang, Korea      | MAT1-1      |
|                  | M826     | This study                          | Meju, Andong, Korea        | MAT1-1      |
|                  | M2108    | This study                          | Meju, Buan, Korea          | MAT1-1      |
|                  | M2110    | This study                          | Meju, Gongju, Korea        | MAT1-1      |
|                  | M2111    | This study                          | Meju, Gongju, Korea        | MAT1-1      |
|                  | 46497    | This study                          | Meju, Yangpyeong, Korea    | MAT1-1      |
|                  | 45072    | CBS 554.65 = ATCC 16888 = NBRC 33023 = IMI 050566 = NRRL 326 (neotype of *A. niger*) | Tannin-gallic acid fermentation, Connecticut, USA | MAT1-2      |
|                  | 46995    | NRRL 4785 = CBS 115.34 = NBRC 4034 (type of *A. batatae*) | Unknown, Japan             | MAT1-1      |
|                  | 47003    | NBRC 4031 = RIB 2014 = NRRL 4784 = CBS 121.28 (type of *A. aureus*) | Koji, Japan                | MAT1-1      |
|                  | 46998    | NRRL 4859 = CBS 117.51 (type of *A. miyakoenii*) | Koji, Japan                | MAT1-2      |
|                  | 47000    | NRRL 4388 = RIB 2602 = NRRL 4760 = CBS 139.2 = IAM 2185 = R-0635 (type of *A. usami*) | Koji, Japan                | MAT1-1      |
|                  | 47001    | NRRL 4889 (type of *A. usami mut. shirousami*) | Unknown, Japan             | MAT1-1      |
| *A. welwitschiae*| 46492    | This study                          | Meju, Haenam, Korea        | MAT1-1      |
|                  | M268     | This study                          | Meju, Goisan, Korea        | MAT1-1      |
|                  | M504     | This study                          | Meju, Buan, Korea          | MAT1-1      |
|                  | M672     | This study                          | Meju, Sunchang, Korea      | MAT1-2      |
|                  | M792     | This study                          | Meju, Sunchang, Korea      | MAT1-2      |
|                  | M1002    | This study                          | Meju, Yeoju, Korea         | MAT1-2      |
|                  | 46496    | This study                          | Meju, Goisan, Korea        | MAT1-1      |
|                  | M2109    | This study                          | Meju, Buan, Korea          | MAT1-1      |
|                  | 46882    | This study                          | Meju, Haenam, Korea        | MAT1-2      |
|                  | 46996    | NRRL 4948 = CBS 557.65 = ATCC 16877 (neotype of *A. awamori*) | Unknown, Japan             | MAT1-2      |

*M*, Meju.
and total genomic DNA was extracted using the DNeasy Plant Mini-Kit (Qiagen, Valencia, CA, USA) following the manufacturer’s protocol.

Identification of mating types. The primers used to partially amplify the MAT1-1 and MAT1-2 genes in the 74 isolates are presented in Table 2. PCR amplification was conducted in 50-μL reactions, which included 5 μL of 10× PCR buffer, 3 μL of deoxyribonucleotide triphosphates (dNTP; 2.5 mM), 0.4 μL of each primer (100 pmol), 0.3 μL of Taq DNA polymerase (Inclone, Daejeon, Korea), 39.9 μL of sterile deionized water, and 1 μL (approximately 10 ng) of a DNA template. The PCR conditions for primer (MAT1-1F-An, MAT1-1R-An) were as follows: 5 min at 95°C; denaturation for 30 sec at 95°C and annealing and extension for 2 min at 72°C (25 cycles); and a final extension for 5 min at 72°C.

Table 2. Primers used in this study

| Primer       | Sequence                                      | Reference  |
|--------------|-----------------------------------------------|------------|
| MAT1-1F-An   | 5’-GCGGCCACTGAACAGTTTCATTGCT-3’              | [23]       |
| MAT1-1R-An   | 5’-TGATGGAGTATGCGCTTGCTACGATG-3’             | [23]       |
| MAT1-2F      | 5’-TTCCCTGTCGCCAAATGCA-3’                    | This study |
| MAT1-2R      | 5’-GAGATGATGGTCGAGCCTTC-3’                   | This study |
| M1F_Anig     | 5’-GGTCATCCGAATGATGGAG-3’                    | [22]       |
| M1R_Anig     | 5’-CAGCCTGCTTTCAAGCGATCC-3’                  | [22]       |
| MAT5-4       | 5’-AARRTCICCMICCCCAAGC-3’                    | [10]       |
| MAT3-2       | 5’-TTNCCKGGGTTTATGRTARTCNCCG-3’              | [10]       |

Fig. 1. MAT gene amplification from genomic DNA of Aspergillus tubingensis (A) and A. welwitschiae (B). A, Lane M, molecular weight markers; Lanes 1–9, amplicons from Meju isolates (M100, M554, M1001, M1004, M1005, M1006, M2095, M2100, and M2101); lanes 10–13, amplicons from KACC A. tubingensis (46805, 46993, 46994, and 47137); B, Lane M, molecular weight markers; lanes 1–8, amplicons from Meju isolates (M95, M268, M504, M672, M792, M1002, M2107, and M2109); lanes 9 and 10, amplicons from KACC A. welwitschiae (46882 and 46996).
for \textit{MAT1-1}. For \textit{MAT1-2} (\textit{MAT1-2F, MAT1-2R}), we used the following conditions: 5 min at 95°C; denaturation for 1 min at 95°C; annealing for 1 min at 58°C; and extension for 30 sec at 72°C (25 cycles); and final extension for 5 min at 72°C. The PCR conditions for previously reported primers [10, 22] were as follows: Primer (\textit{M1F} \textit{Anig, M1R} \textit{Anig}), the initial denaturation for 5 min at 95°C; followed by 25 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 60°C, and extension for 30 sec at 72°C; and a final extension for 5 min at 72°C for \textit{MAT1-1}. In case of \textit{MAT1-2} (\textit{MAT5-4, MAT3-2}), we used denaturation for 5 min at 95°C; followed by 25 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C, and extension for 30 sec at 72°C; and a final extension for 5 min at 72°C. The reactions were run in a Peltier thermal cycler MJ Research PCT-200 gradient (Waltham, MA, USA). After the PCR, 5 mL of a PCR product was analyzed by electrophoresis and visualized by means of gel documentation equipment.

\textbf{RESULTS AND DISCUSSION}

During our experiments, we initially observed PCR amplification of both \textit{MAT1-1} and \textit{MAT1-2} in the same strains using the primers and protocol reported elsewhere [22]. After optimizing the PCR conditions as described above, we were able to rectify this problem and to obtain either \textit{MAT1-1} or \textit{MAT1-2} for a single strain.

After that, we used PCR to determine the ratio of \textit{MAT} genes in 74 isolates of 4 species of \textit{Aspergillus} section \textit{Nigri} isolated from Meju, a traditional fermented food product of South Korea: namely, \textit{A. luchuensis}, \textit{A. niger}, \textit{A. tubingensis}, and \textit{A. welwitschiae}. PCR analysis revealed amplification of an approximately 200-bp fragment for \textit{MAT1-1} and 300-bp fragment for \textit{MAT1-2} with the primer set designed by Yamada (Figs. 1~3) and the primer set of Horn et al. [22] resulted in a ~500-bp amplicon for \textit{MAT1-1} and a 300-bp amplicon for \textit{MAT1-2} (data not shown). The present PCR results indicate that all isolates yielded either \textit{MAT1-1} or \textit{MAT1-2} but never both \textit{MAT} genes during PCR amplification.

In this study, the \textit{MAT1-1} locus was detected in 2 of 23 strains of \textit{A. luchuensis}, 6 of 13 strains of \textit{A. tubingensis}, 21 of 28 stains of \textit{A. niger}, and 5 of 10 strains of \textit{A. welwitschiae}. Furthermore, in case of \textit{A. tubingensis} and \textit{A. welwitschiae}, distributions of \textit{MAT1-1} and \textit{MAT1-2} were random approaching 50% for each mating type (Fig. 1). In contrast, the \textit{MAT1-1} type is dominant in \textit{A. niger} (75% of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{\textit{MAT1-1} and \textit{MAT1-2} genes amplified from \textit{Aspergillus niger} isolates. A, Lane M, molecular weight markers; lanes 1~15, amplicons from Meju isolates (M22, M224, M267, M347, M450, M458, M478, M557, M561, M579, M581, M602, M619, M630, and M639); B, Lane M, molecular weight markers; lanes, 1~7, amplicons from Meju isolates (M654, M701, M826, M2108, M2110, M2111, and M2112); lanes 8~13, amplicons from KACC \textit{A. niger} (45072, 46995, 47003, 46998, 47000, and 47001).}
\end{figure}
Mageswari et al. studied strains (Fig. 2) and MAT1-2 type is dominant in *A. luchuensis* (91.3% of strains under study) (Fig. 3).

Several research groups have reported the ratio of MAT1-1 and MAT1-2 genes in the species of *Aspergillus* [11, 19, 21, 22]. In the *Aspergillus* section *Flavi*, the MAT1-1 to MAT1-2 gene ratio showed mixed results but to some extent was found to be sample dependent. In *Aspergillus flavus* strains isolated from peanut field soil, this ratio is 1:1 [11]. Nonetheless, *A. flavus* from clinical samples carried only MAT1-1 (100%) [21]. Among other species, e.g., in *Aspergillus oryzae* isolated from soy sauce, 72.5% of strains were found to be of the MAT1-2 type. The preference for MAT1-2 in *A. oryzae* from soybean was hypothesized to be due to its short aerial hyphal phenotype, which can both improve fermentation enzyme efficiency and block growth of contaminating fungi [19].

Other species of *Aspergillus* section *Nigri* except for *A. luchuensis* have been studied regarding the MAT1-1 to MAT1-2 gene ratio [21, 22]. Among 125 isolates from *A. tubingensis*, *A. niger*, and *A. welwitschiae*, the ratio of MAT1-1 and MAT1-2 was found to be 1:1 in isolates belonging to *A. tubingensis* and *A. niger*. Strains of *A. welwitschiae* showed a strong bias toward MAT1-1 [21]. Our present results add to the existing knowledge on the ratio of MAT genes in the *Aspergillus* section *Nigri* with the emphasis on *A. luchuensis*, which has not been widely studied. Most of our strains were isolated from Meju. Although the strains of *A. tubingensis* and *A. welwitschiae* in our results indicated an approximately 1:1 distribution, the majority of *A. niger* strains indicated a bias toward the MAT1-1 type, whereas the strains of *A. luchuensis* showed predominance of the MAT1-2 type (91.3%). The remaining 8.7% of strains (2 strains) were found to be in the MAT1-1 category. The presence of the MAT1-1 mating type in *A. luchuensis* has not been reported until now, and hitherto various researchers have observed MAT1-2 only among 28 strains of *A. luchuensis*, which are used for brewing Japanese traditional spirits such as awamori and shochu [23].

Mating experiments on fungi are useful tools for genetic analysis and are also a good strategy for strain improvement. The mating type in fungi has long been associated with breeding for production of industrially important strains with desired traits. In mushrooms, mating-type genes can be used as marker genes for breeding methods, and their functions that are required for formation of stable dikaryons are essential for breeding [24]. Strain improvement based

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**Fig. 3.** PCR amplification of MAT1-1 and MAT1-2 genes from *Aspergillus luchuensis* isolates. A, Lane M, molecular weight markers; lanes 1~8, amplicons from KACC *A. luchuensis* (46772, 46771, 45131, 47234, 47004, 47235, and 47005); lanes 9~15, amplicons from Meju isolates (M29, M532, M1003, M2099, M2093, M2096, and M2097); B, Lane M, molecular weight markers; lanes 1~8, amplicons from Meju isolates (M2098, M2099, M2102, M2103, M2104, M2105, M2106, and M2113).
on the mating type in penicillin-producing fungus *Penicillium chrysogenum* has been reported to be biotechnologically advantageous because the fungus showed improved penicillin production and fermentor-friendly changes in the morphology of hyphae and conidia upon breeding [25]. Even in ascomycetes, the presence of mating-type genes and their biotechnological potential have been discussed in detail [26].

*A. luchuensis* MAT1-1 mating-type strains were proposed as a valuable source for breeding [23]. Our results therefore should advance the understanding of biology and evolution of *A. luchuensis*. Our findings also have industrial relevance because the sexual cycle can now be utilized for strain improvement for use in the brewing and fermented food industry. In general, natural mating of *A. luchuensis* MAT1-1 and MAT1-2 type strains can have advantages over genetic recombination technologies because recombination tends to occur throughout the genome resulting in excessive genetic variation for screening purposes. Mating is also an inexpensive technique for manipulation of several genes *in vitro* to produce an industrially preferable mutant strain. In addition, the stability of industrial strains remains unaffected when sexual reproduction is used for breeding because no harmful mutations are introduced. Therefore, our identification of MAT1-1 type *A. luchuensis* can be used for selective breeding to produce commercially important strains that are cost-effective and beneficial for industries.

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