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Transfer of Genetic Material between Pathogenic and Food-Borne Yeasts

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Many pathogenic yeast species are asexual and therefore not involved in intra- or interspecies mating. However, high-frequency transfer of plasmid DNA was observed when pathogenic and food-borne yeasts were grown together. This property could play a crucial role in the spread of virulence and drug resistance factors among yeasts.

For millennia, yeasts belonging to the genus *Saccharomyces* have been widely used in preparation of food and beverages. Historically, natural isolates of *Saccharomyces* species have been considered to be harmless, nonpathogenic saprophytes. However, in the past few decades these yeasts have been found to be causative agents of human infections (18, 19). How these originally inoffensive yeasts evolved into pathogenic forms is not understood. Paradoxically, one of the reasons behind the emergence of novel human fungal pathogens is the success of modern medical care, which leads to the survival of immunocompromised patients (23).

Candida glabrata, formerly classified as *Torulopsis glabrata*, can be found as a commensal yeast in healthy individuals, but it is also a human opportunistic pathogen (9). *C. glabrata* is a close relative of *Saccharomyces* species, and recently it was added together with other *Saccharomyces* yeasts to the joint *Saccharomyces* clade (13). *C. glabrata* diverged from the common ancestor of *Saccharomyces* sensu stricto species after whole-genome duplication occurred, and the *C. glabrata* genome subsequently underwent reductive evolution, possibly associated with the emergence of this yeast as a human pathogen (6, 26). The widespread use of immunosuppressive therapy, chemo- and radiotherapy for cancer, and broad-spectrum antimycotic therapy has increased the frequency of both systemic and mucosal infections caused by *C. glabrata* (7). *Candida* species are some of the most common bloodstream pathogens in the United States, and a substantial shift in the epidemiology of hematogenous candidiasis to non-*Candida albicans* species, such as *C. glabrata*, has been observed recently. Fluconazole treatment may have played a role in this shift (1). A nationwide survey of candidemia from 1991 to 2000 in Swiss tertiary care hospitals showed that *C. glabrata* was the major non-*C. albicans* cause of candidiasis, with an incidence of 15% (14). *C. glabrata* was the second most frequently occurring fungus and the dominant non-*C. albicans* species causing candidal vulvovaginitis in pregnant women in China (25). In the Flemish population in Belgium the incidence of infection placed *C. glabrata* third among the organisms identified in symptomatic vulvovaginal candidiasis patients after fluconazole treatment (5). Vulvovaginal candidiasis is an example of a common mucosal infection that also occurs in healthy, immunocompetent women.

Could intra- and interspecies exchange of genetic material be responsible, at least in part, for the spread of virulence factors from pathogenic species to nonpathogenic species? It has been proven previously that in experimental populations *Saccharomyces* species can form hybrids and thereby create novel combinations of genetic material (15). These results suggest that *Saccharomyces* yeasts have the potential to exchange genetic material in nature, and this view is supported by the fact that several natural isolates are indeed hybrids between different species (8, 16). It may well be that some *Saccharomyces* isolates that are human pathogens are naturally occurring interspecific hybrids (4, 17). However, mating per se has not been observed yet for *C. glabrata* (12), and therefore it is not thought that *C. glabrata* can be involved in interspecies mating. To evaluate the possibility of gene transfer between pathogenic and food-borne yeasts species, we studied possible plasmid transfer between *C. glabrata* and the *Saccharomyces* species *S. cerevisiae* and *S. bayanus*.

Plasmid transfer from *S. cerevisiae* to *C. glabrata*. To develop a potential acceptor of plasmids, *C. glabrata* type strain Y475 was mutagenized with ethyl methanesulfonate as described previously (15). Auxotrophic *ura3* mutants were selected on plates containing 5-fluoroorotic acid (5-FOA) at a concentration of 1.00 mg/ml. 5-FOA-resistant mutants were tested for growth on a minimal medium with uracil, on a minimal medium without uracil, and on a synthetic complex medium without uracil, and they were checked for the ability to revert using standard yeast techniques. Only nonreverting strains were used in experiments. Plasmids to be used in the plasmid transfer experiment (P158, P159, and P199) (Table 1) were first transformed into *C. glabrata ura3* mutants (Table 2). Only the *C. glabrata* mutant strains (Y718 and Y719) which were able to grow under selective conditions when they were transformed by plasmids were used as plasmid recipients in the plasmid transfer experiments. *S. cerevisiae* strain Y391 was used as a potential donor of genetic material in the plasmid transfer

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TABLE 1. Shuttle plasmids (*E. coli*-yeast) used in the experiments and their characteristics

Laboratory designation	Designation	Description	Reference
P157	YRp17	*URA3 TRP1 ARS1*, Amp^r Tet^r	3
P158	YEp24	*URA3*, 2μ circle DNA, Amp^r Tet^r	2
P159	YCp50	*URA3 ARS1 CEN4*, Amp^r Tet^r	21
P199	pRS416	*URA3 ARS4 CEN6*, Amp^r	22

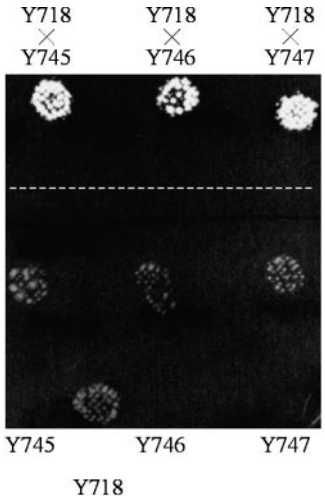

FIG. 1. *C. glabrata* auxotrophic *ura3* strain Y718 was grown together with *S. cerevisiae* strains Y745, Y746, and Y747 containing different plasmids carrying the *URA3* gene on YPD plates. The colonies were replica plated onto the selective minimal medium the following day, and the images show the growth on the minimal medium after 1 week. Four controls (parental strains Y745, Y746, Y747, and Y718) were also grown on the minimal medium.

experiment and was transformed for this purpose with the P158, P159, and P199 plasmids. Overnight cultures of *C. glabrata ura3* mutant strains Y718 and Y719 were mixed with the same volume (300 μl) of overnight cultures of *S. cerevisiae* plasmid donor strains Y745, Y746, and Y747. On YPD plates 5-μl portions of mixed cultures were spotted and replica plated onto the selective minimal medium the following day. Neither the *S. cerevisiae* plasmid donor nor the *C. glabrata* plasmid acceptor could grow on minimal medium plates without uracil (Fig. 1), but after a few days small colonies appeared in the "mixed" spots. The frequencies of plasmid transfer between plasmid donor strains and plasmid recipient strains were calculated by determining the fractions of the plasmid recipient strain clones possessing the plasmids based on all of the plasmid recipient strain colonies plated. In the case of P199 the frequency was 1.2×10^{-4}. Several colonies (referred to below as "resulting strains") (Table 2) were checked for their karyotype patterns, the presence of exchanged plasmid, and the ability to lose the acquired plasmid. Chromosomes were prepared as described by Petersen et al. (20) and were separated by pulsed-field gel electrophoresis using a five-step program, as follows: step 1, 240-s pulse for 6 h; step 2, 160-s pulse for 13 h; step 3, 120-s pulse for 10 h; step 4, 90-s pulse for 10 h; and step 5, 60-s pulse for 3 h. The angle was 60°, and the potential was 150 V (4.5 V/cm). All but one of the resulting strains exhibited the same karyotype pattern as the *C. glabrata* plasmid recipient (Fig. 2A). Thus, *C. glabrata* cells acquired the plasmid from the

TABLE 2. Strains used in this study and their characteristics

Strain	Species	Genotype or origin	Reference or comment[a]
Y244	*S. bayanus*	*MAT*α *ura3*	15
Y391	*S. cerevisiae*	*MAT*α *ura3 trp1 gal2*	M1-2B from T. Nilsson-Tillgren
Y475	*C. glabrata*	Asexual prototroph	Type strain NRRL Y-65
Y718	*C. glabrata*	*ura3*	EMS mutagenesis of Y475
Y719	*C. glabrata*	*ura3*	EMS mutagenesis of Y475
Y727	*C. glabrata*	Y718 with P158 obtained by plasmid transfer (from Y745)	
Y728	*C. glabrata*	Y718 with P159 obtained by plasmid transfer (from Y746)	
Y729	*C. glabrata*	Y718 with P199 obtained by plasmid transfer (from Y747)	
Y730	*C. glabrata*	Y719 with P158 obtained by plasmid transfer (from Y745)	
Y731	*C. glabrata*	Y719 with P159 obtained by plasmid transfer (from Y746)	
Y732	*C. glabrata*	Y719 with P199 obtained by plasmid transfer (from Y747)	
Y745	*S. cerevisiae*	Y391 transformed with P158	Plasmid donor
Y746	*S. cerevisiae*	Y391 transformed with P159	Plasmid donor
Y747	*S. cerevisiae*	Y391 transformed with P199	Plasmid donor
Y760	*C. glabrata*	*his3*	From type strain BG98, from B. Cormack
Y765	*C. glabrata*	*his3 ura3*	EMS mutagenesis of Y760
Y781	*C. glabrata*	Y765 transformed with P159	Plasmid donor
Y782	*C. glabrata*	Y765 transformed with P159	Plasmid donor
Y784	*C. glabrata*	Y765 transformed with P157	Plasmid donor
Y832	*S. bayanus*	Y244 with P159 obtained by plasmid transfer (from Y782)	
Y833	*S. bayanus*	Y244 with P159 obtained by plasmid transfer (from Y782)	
Y834	*S. bayanus*	Y244 with P159 obtained by plasmid transfer (from Y782)	
Y835	*S. bayanus*	Y244 with P159 obtained by plasmid transfer (from Y782)	
Y836	*S. bayanus*	Y244 with P159 obtained by plasmid transfer (from Y782)	
Y837	*S. bayanus*	Y244 with P159 obtained by plasmid transfer (from Y781)	
Y840	*S. bayanus*	Y244 with P157 obtained by plasmid transfer (from Y784)	
Y841	*S. bayanus*	Y244 with P157 obtained by plasmid transfer (from Y784)	

[a] EMS, ethyl methanesulfonate.

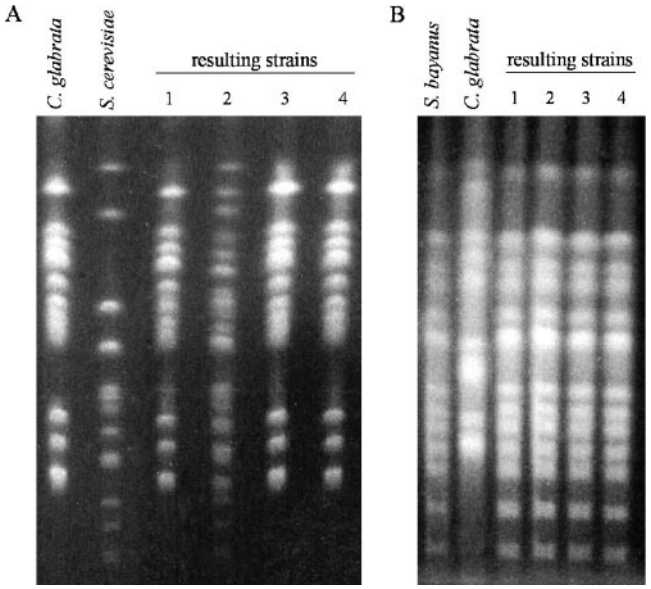

FIG. 2. (A) Karyotypes of *C. glabrata* recipient strains Y718 and Y719, *S. cerevisiae* donor strains Y745, Y746, and Y747, and the four resulting strains (lane 1, Y729; lane 2, Y732; lane 3, Y719/Y606 no. 3; lane 4, Y719/Y748 no. 4). All but one strain had the same karyotype pattern as the *C. glabrata* plasmid recipient. The karyotype of resulting strain Y732 (lane 2) had both *S. cerevisiae* and *C. glabrata* chromosome bands. (B) Karyotypes of *S. bayanus* recipient strain Y244, *C. glabrata* donor strains Y781, Y782, and Y784, and the four resulting prototrophs (lane 1, Y837; lane 2, Y832; lane 3, Y840; lane 4, Y841). All four resulting strains had the same karyotype pattern as the recipient strain.

donor *S. cerevisiae* strain. The karyotype of Y732 displayed both *S. cerevisiae* and *C. glabrata* sets of chromosome bands, but this strain was later shown to consist of cells of both parents. The presence of a plasmid in *C. glabrata* recipient strains Y718 and Y719 was confirmed by PCR with total DNA isolated from strains resulting from plasmid transfer (designated strains Y727 to Y732) using primers Amp^{r}12.01 (5′-CAA ATA TGT ATC CGC TCA TGA GAC A-3′) and Amp^{r}12.02 (5′-GTA AAC TTG GTC TGA CAG TTA CC-3′). For amplification of the plasmid ampicillin resistance-encoding gene, the following conditions were used: 94°C of initial denaturation for 3 min and then 35 cycles of 30 s at 94°C, 1 min at 55°C, and 1.5 min at 72°C, followed by 72°C for 3 min. Total DNA from Y718 and Y719 served as a negative control. The possibility of plasmid loss from strains resulting from plasmid transfer was tested by growing these strains under nonselective conditions. The resulting strains were grown overnight in liquid YPD medium, and then the cells were spread on solid YPD medium. After 3 days colonies were replica plated onto minimal medium without uracil. Only the colonies which had not lost the plasmid could grow under selective conditions. The percentage of the P199 plasmid lost from *C. glabrata* strain Y729 was 84%. In conclusion, *C. glabrata* indeed acquired the plasmid at a relatively high frequency when it was grown in the presence of *S. cerevisiae* cells.

Plasmid transfer from *C. glabrata* to *S. bayanus*. *C. glabrata* strain Y760 was mutagenized with ethyl methanesulfonate and selected with 5-FOA. Double mutant Y765 was transformed with *URA3* gene-containing plasmid P157 (Y784) or P159 (Y781 and Y782) and was used as a *C. glabrata* donor of genetic material (Table 2). A food-borne yeast species belonging to the *Saccharomyces* sensu stricto group, *S. bayanus* (Y244), was chosen as the potential plasmid acceptor. Plasmid transfer experiments were carried out as described above, and prototrophic strains were isolated. Plasmid transfer frequencies were calculated; for plasmid P157 the transfer frequency was 2.2×10^{-4}, and for plasmid P159 the transfer frequency was 1.6×10^{-4}. For several strains chromosomes were separated by pulsed-field gel electrophoresis using the program described above, and all of the strains tested exhibited the *S. bayanus* chromosomal pattern (Fig. 2B). Thus, *S. bayanus* cells acquired the plasmid from the *C. glabrata* donor strain. The presence of the plasmid in *S. bayanus* plasmid recipient strain Y244 was also confirmed by PCR when plasmids were first isolated from the strains and rescued in *Escherichia coli* strain XL-1 Blue (laboratory designation, P311; Stratagene) (24), and then the plasmid tetracycline resistance-encoding gene was amplified with gene-specific primers Tet^{r}11.27 (5′-AGT GCC ACC TGA CGT CTA AGA-3′) and Tet^{r}11.28 (5′-GTT TGC GCA TTC ACA GTT CTC C-3′). The following conditions were used for the PCR: 94°C of initial denaturation for 4 min and then 35 cycles of 40 s at 94°C, 45 s at 54°C, and 2.5 min at 72°C, followed by 72°C for 5 min. In conclusion, *S. bayanus* also acquired the plasmid when it was grown in the presence of *C. glabrata* cells.

Concluding remarks. Although sexual activity (zygote, G_1 arrest) between *C. glabrata* and *S. cerevisiae* cells has not been observed previously, we observed a relatively high frequency of transfer of genetic material between these yeast species. The mechanism(s) for this transfer is not clear. Previously, DNA transfer between bacteria (*E. coli*) and yeasts (*S. cerevisiae* and *S. kluyveri*) was reported as transkingdom conjugation (10, 11). In our experiments recipient species could have picked up plasmid molecules released by the donor strains through cell lysis, or the plasmid transfer could have resulted from cytoduction. In addition, despite the "asexual" nature of *C. glabrata*, a potential cryptic sexual life cycle (27) could promote rare interspecies mating. The transfer observed could allow the spread of virulence factors and resistance to medical drugs even between distantly related yeast species and could probably help in the "transformation" of harmless saprophytes into potential causative agents of human infections.

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REFERENCES

1. **Abi-Said, D., E. Anaissie, O. Uzun, I. Raad, H. Pinzcowski, and S. Vartivarian.** 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin. Infect. Dis. **24:**1122–1128.
2. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8:**17–24.
3. **Botstein, D., and R. W. Davis.** 1982. Principles and practice of recombinant DNA research with yeast, p. 607–636. *In* J. N. Strathan, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Clemons, K. V., P. Park, J. H. McCusker, M. J. McCullough, R. W. Davis, and D. A. Stevens.** 1997. Application of DNA typing methods and genetic analysis to epidemiology and taxonomy of *Saccharomyces* isolates. J. Clin. Microbiol. **35:**1822–1828.
5. **De Vos, M. M., M. Cuenca-Estrella, T. Boekhout, B. Theelen, N. Matthijs, T. Bauters, H. Nailis, M. A. Dhont, J. L. Rodriguez-Tudela, and H. J. Nelis.**

2005. Vulvovaginal candidiasis in a Flemish patient population. Clin. Microbiol. Infect. **11:**1005–1011.

6. **Dujon, B., D. Sherman, G. Fischer, P. Durrens, S. Casaregola, I. Lafontaine, J. De Montigny, C. Marck, C. Neuveglise, E. Talla, N. Goffard, L. Frangeul, M. Aigle, V. Anthouard, A. Babour, V. Barbe, S. Barnay, S. Blanchin, J. M. Beckerich, E. Beyne, C. Bleykasten, A. Boisrame, J. Boyer, L. Cattolico, F. Confanioleri, A. De Daruvar, L. Despons, E. Fabre, C. Fairhead, H. Ferry-Dumazet, A. Groppi, F. Hantraye, C. Hennequin, N. Jauniaux, P. Joyet, R. Kachouri, A. Kerrest, R. Koszul, M. Lemaire, I. Lesur, L. Ma, H. Muller, J. M. Nicaud, M. Nikolski, S. Oztas, O. Ozier-Kalogeropoulos, S. Pellenz, S. Potier, G. F. Richard, M. L. Straub, A. Suleau, D. Swennen, F. Tekaia, M. Wesolowski-Louvel, E. Westhof, B. Wirth, M. Zeniou-Meyer, I. Zivanovic, M. Bolotin-Fukuhara, A. Thierry, C. Bouchier, B. Caudron, C. Scarpelli, C. Gaillardin, J. Weissenbach, P. Wincker, and J. L. Souciet.** 2004. Genome evolution in yeasts. Nature **430:**35–44.
7. **Fidel, P. L., Jr., J. A. Vazquez, and J. D. Sobel.** 1999. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin. Microbiol. Rev. **12:**80–96.
8. **Groth, C., J. Hansen, and J. Piskur.** 1999. A natural chimeric yeast containing genetic material from three species. Int. J. Syst. Bacteriol. **49:**1933–1938.
9. **Haley, L. D.** 1961. Yeasts of medical importance. Am. J. Clin. Pathol. **36:** 227–234.
10. **Heinemann, J. A., and G. F. Sprague, Jr.** 1989. Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. Nature **340:**205–209.
11. **Inomata, K., M. Nishikawa, and K. Yoshida.** 1994. The yeast *Saccharomyces kluyveri* as a recipient eukaryote in transkingdom conjugation: behavior of transmitted plasmids in transconjugants. J. Bacteriol. **176:**4770–4773.
12. **Kaur, R., R. Domergue, M. L. Zupancic, and B. P. Cormack.** 2005. A yeast by any other name: *Candida glabrata* and its interaction with the host. Curr. Opin. Microbiol. **8:**378–384.
13. **Kurtzman, C. P., and C. J. Robnett.** 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Leeuwenhoek **73:**331–371.
14. **Marchetti, O., J. Bille, U. Fluckiger, P. Eggimann, C. Ruef, J. Garbino, T. Calandra, M. P. Glauser, M. G. Tauber, and D. Pittet.** 2004. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin. Infect. Dis. **38:**311–320.
15. **Marinoni, G., M. Manuel, R. F. Petersen, J. Hvidtfeldt, P. Sulo, and J. Piskur.** 1999. Horizontal transfer of genetic material among *Saccharomyces* yeasts. J. Bacteriol. **181:**6488–6496.
16. **Masneuf, I., J. Hansen, C. Groth, J. Piskur, and D. Dubourdieu.** 1998. New hybrids between *Saccharomyces* sensu stricto yeast species found among wine and cider production strains. Appl. Environ. Microbiol. **64:**3887–3892.
17. **McCullough, M. J., K. V. Clemons, J. H. McCusker, and D. A. Stevens.** 1998. Intergenic transcribed spacer PCR ribotyping for differentiation of *Saccharomyces* species and interspecific hybrids. J. Clin. Microbiol. **36:**1035–1038.
18. **McCusker, J. H., K. V. Clemons, D. A. Stevens, and R. W. Davis.** 1994. Genetic characterization of pathogenic *Saccharomyces cerevisiae* isolates. Genetics **136:**1261–1269.
19. **Nielsen, H., J. Stenderup, and B. Bruun.** 1990. Fungemia with *Saccharomycetaceae*. Report of four cases and review of the literature. Scand. J. Infect. Dis. **22:**581–584.
20. **Petersen, R. F., T. Nilsson-Tillgren, and J. Piskur.** 1999. Karyotypes of *Saccharomyces* sensu lato species. Int. J. Syst. Bacteriol. **49:**1925–1931.
21. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:**237–243.
22. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
23. **van Burik, J. A., and P. T. Magee.** 2001. Aspects of fungal pathogenesis in humans. Annu. Rev. Microbiol. **55:**743–772.
24. **Ward, A. C.** 1990. Single-step purification of shuttle vectors from yeast for high frequency back-transformation into *E. coli*. Nucleic Acids Res. **18:**5319.
25. **Wenjin, Q., and S. Yifu.** 2006. Epidemiological study on vaginal *Candida glabrata* isolated from pregnant women. Scand. J. Infect. Dis. **38:**49–54.
26. **Wolfe, K.** 2004. Evolutionary genomics: yeasts accelerate beyond BLAST. Curr. Biol. **14:**R392–R394.
27. **Wong, S., M. A. Fares, W. Zimmermann, G. Butler, and K. H. Wolfe.** 2003. Evidence from comparative genomics for a complete sexual cycle in the 'asexual' pathogenic yeast *Candida glabrata*. Genome. Biol. **4:**R10–R19.