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Med Mycol. Author manuscript; available in PMC 2009 July 13.

Published in final edited form as:
Med Mycol. 2007 August ; 45(5): 429–434. doi:10.1080/13693780701377162.

Deletion of *ALS5, ALS6* or *ALS7* increases adhesion of *Candida albicans* to human vascular endothelial and buccal epithelial cells

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Summary

C. albicans yeast forms deleted for *ALS5, ALS6* or *ALS7* are more adherent than a relevant control strain to human vascular endothelial cell monolayers and buccal epithelial cells. In the buccal and vaginal reconstituted human epithelium (RHE) disease models, however, mutant and control strains caused a similar degree of tissue destruction. Deletion of *ALS5* or *ALS6* significantly slowed growth of the mutant strain; this phenotype was not affected by addition of excess uridine to the culture medium. These studies demonstrate similar phenotypic characteristics for the *als5Δ/als5Δ, als6Δ/als6Δ* and *als7Δ/als7Δ* strains that are not observed in any of the other *C. albicans alsΔ/alsΔ* strains.

Keywords

Candida albicans; ALS gene family; adhesion; host-pathogen interaction

Introduction

The *Candida albicans* ALS family includes eight genes that encode large cell-surface glycoproteins [1]. Initially, it was hypothesized that the Als proteins function in adhesion of *C. albicans* to host surfaces [1]. Deletion of single ALS genes showed contributions to adhesion for Als1p, Als2p, Als3p, Als4p and Als9p [2-6]. This work focuses on the remaining ALS genes: *ALS5, ALS6* and *ALS7*. The phenotype of *als5Δ/als5Δ, als6Δ/als6Δ*, and *als7Δ/als7Δ* strains was examined in two different adhesion assays and an in vitro model of epithelial tissue destruction.

Materials and methods

Strains and construction of alsΔ/alsΔ mutants

Strains and methods for mutant construction and validation were published previously [3]. Table 1 details the construction of *C. albicans* strains deleted for *ALS5, ALS6* or *ALS7*. Strain CAI12 (*iro1-ura3Δ::λimm*^{434}*/IRO1 URA3*) [11] was used as a control in all phenotypic assays.

Culture conditions

Strains for phenotypic testing were streaked from -80°C glycerol stocks to YPD plates (1% yeast extract, 2% peptone, 2% glucose with 2% agar for plates). Plates were incubated 24 h at 37°C and stored at 4°C for no more than one week. A single *C. albicans* colony was resuspended in 1 ml YPD and 10 μl used to inoculate a 10 ml YPD culture. The culture was incubated 16

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h at 37°C with 200 rpm shaking. Typical cell counts following this procedure are approximately 2 to 3 × 10^8 cells ml^{-1} with approximately 20% budding cells.

Growth rate and cellular aggregation measurements

Growth rate measurement methods were published previously [3]. Cellular aggregation measurements were made using a published method [3]. *C. albicans* cells were grown in YPD as described above and washed in DPBS (Cambrex catalog number 17-512Q) prior to the assay. The number of yeast cells in an aggregate was recorded for 100 independent cellular units. Budding cells were counted as one cell, since bud attachment is not due to aggregation. Differences in cellular aggregation were judged by mixed model analysis of variance (PROC MIXED in SAS; SAS Institute) of the means of duplicate measurements from three separate days [3].

Adhesion assays

Yeast forms for adhesion assays were grown in YPD and washed in DPBS as described above. Human umbilical vein endothelial cell assays were conducted in a 6-well plate format [3]. Buccal epithelial cell adhesion assays also followed a published method [5].

Reconstituted human epithelium (RHE) model

Methods for assessing *C. albicans* destruction of buccal and vaginal RHE are published [3, 12,13].

Results

Deletion of *ALS5, ALS6* or *ALS7* resulted in significantly increased adhesion of the mutant strain to human vascular endothelial cell monolayers and buccal epithelial cells (Fig. 1A and B). This observation is opposite of the result expected from deletion of a putative adhesin and suggests the potential for an anti-adhesive role for Als5p, Als6p and Als7p. *C. albicans* mutant strains cultured in the presence of exogenous uridine also showed increased adhesion relative to a control strain suggesting that the observed phenotype is not due to placement of the *URA3* marker (Fig. 1C). Reintegration of a single wild-type ALS allele did not restore wild-type adhesion levels except for the *ALS6* strains in the endothelial cell assay (Fig. 1A). Further investigation is required to determine if these results reflect allelic functional differences or a requirement for the presence of both alleles for wild-type function. The need to reintegrate both wild-type alleles to restore wild-type function was reported for another *C. albicans* cell wall protein-encoding gene, *ECM33* [14]. Cellular aggregation was evaluated for the mutant strains since this property can affect adhesion assay results. *ALS5* or *ALS6* deletion produced a slight, but statistically significant, increase in cellular aggregation (Table 3). However, this aggregation increase is not sufficient to account for the adhesion assay results (Fig. 1) suggesting that an alternative explanation is required. Potential explanations for the increased adhesion of the mutant strains include up-regulation of another adhesin-encoding gene in response to ALS deletion or alteration of cell wall structure that exposes other adhesive moieties on the *C. albicans* surface. Additional investigation is necessary to distinguish between these possibilities.

Despite the large increase in adhesion associated with deletion of *ALS5, ALS6* or *ALS7*, destruction of epithelial cells in the buccal RHE disease model showed no difference between a control strain and the *als5Δ/als5Δ, als6Δ/als6Δ* and *als7Δ/als7Δ* mutant strains (Fig. 2). Testing of vaginal RHE produced similar results (data not shown). Inoculation of the vaginal RHE model with other previously published *alsΔ/alsΔ* strains [3-5] produced results similar to those observed for buccal RHE (data not shown). The greatest decrease in epithelial layer damage was observed for the *als3Δ/als3Δ* strain with lesser reductions observed for the

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als1Δ/als1Δ and *als2Δ/PMAL2-ALS2* strains. No changes from control strain activity were observed for the *als4Δ/als4Δ* and *als9Δ/als9Δ* strains.

Another notable phenotype of the *C. albicans* strains in which *ALS5, ALS6* or *ALS7* was deleted was decreased growth rate compared to the control strain (Table 3). While all three mutant strains consistently grew more slowly than the control, only the growth rate for strains lacking *ALS5* or *ALS6* was statistically significantly slower (Table 3). Reintegration of the *ALS5SA* allele (Table 1) restored the growth rate, but reintegration of a single *ALS6* allele did not (Table 3). Addition of 100 μg ml^{-1} uridine to the culture medium did not change the growth kinetics of the mutant strains suggesting that placement of the *URA3* marker is not responsible for this phenotype (data not shown). The slowed growth rate phenotype prompted repeated construction of the *als5Δ/als5Δ* strain. Each independently constructed *als5Δ/als5Δ* strain had a slowed growth rate. It is formally possible that these ALS genes play a role in maintaining wild-type growth rate in *C. albicans*.

Discussion

Data presented here complete an initial phenotypic analysis of adhesion of *C. albicans* mutant strains in which wild-type expression of one of the ALS genes was disrupted. Growth conditions for adhesion assays were selected based on knowledge of ALS gene expression patterns in vitro. Adhesion assays used germ tubes grown in RPMI 1640 medium to assess the adhesive contribution of Als1p, Als2p, Als3p and Als4p [3,4] while yeast forms grown overnight in YPD were assayed for Als5p, Als6p, and Als7p in this manuscript, and for Als9p [5]. As more is learned about in vitro Als protein production, additional culture conditions can be identified and used to better understand the adhesive contribution of Als proteins.

The phenotypes of increased adhesion and slightly slowed growth rate are common to *C. albicans* strains lacking *ALS5, ALS6* or *ALS7*. These genes are also consistently transcribed at a lower level than for other ALS genes, even in *C. albicans* cells recovered from human clinical specimens and animal models of disease [reviewed in 1]. These observations suggest that a sparse presence of Als5p, Als6p and Als7p is sufficient for function. Cellular localization of Als5p, Als6p and Als7p has not been determined, leaving open the possibility that the proteins are membrane-bound, rather than crosslinked to cell wall glucan like other Als proteins [reviewed in 1]. Similar phenotypic data for *ALS5, ALS6* and *ALS7* could indicate that their encoded proteins have functional redundancy or even that they function in a multiprotein complex. The intriguing preliminary results presented here highlight the numerous features of these genes and proteins that remain to be explored in *C. albicans*.

Acknowledgements

This research was funded by grant DE14158 from the National Institute of Dental and Craniofacial Research, National Institutes of Health. This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number C06 RR16515-01 from the National Center for Research Resources, National Institutes of Health.

References

1. Hoyer LL, Green CB, Oh S-H, Zhao X. Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family—a sticky pursuit. Med Mycol. 2007(Under revision)
2. Fu Y, Ibrahim AS, Sheppard DC, et al. *Candida albicans* Als1p: an adhesin that is a downstream effector of the *EFG1* filamentation pathway. Mol Microbiol 2002;44:61–72. [PubMed: 11967069]
3. Zhao X, Oh S-H, Cheng G, et al. *ALS3* and *ALS8* represent a single locus that encodes a *Candida albicans* adhesin; functional comparison between Als3p and Als1p. Microbiology 2004;150:2415–2428. [PubMed: 15256583]

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4. Zhao X, Oh S-H, Yeater KM, Hoyer LL. Analysis of the *Candida albicans* Als2p and Als4p adhesins suggests the potential for compensatory function within the Als family. Microbiology 2005;151:1619–1630. [PubMed: 15870470]

5. Zhao X, Oh S-H, Hoyer LL. Unequal contribution of *ALS9* alleles to adhesion between *Candida albicans* and vascular endotheial cells. Microbiology. 2007(In press)

6. Phan QT, Myers CL, Fu Y, Sheppard DC, Yeaman MR, Welch WH, Ibrahim AS, Edwards JE, Filler SG. Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol 5:e64. [PubMed: 17311474]

7. Fonzi WA, Irwin MY. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 1993;134:717–728. [PubMed: 8349105]

8. Wilson RB, Davis D, Enloe BM, Mitchell AP. A recyclable *Candida albicans URA3* cassette for PCR product-directed gene disruption. Yeast 2000;16:65–70. [PubMed: 10620776]

9. Zhao X, Oh S-H, Jajko R, et al. Analysis of *ALS5* and *ALS6* allelic variability in a geographically diverse collection of *Candida albicans* isolates. Fungal Genet Biol. 2007(Under revision)

10. Zhang N, Harrex AL, Holland BR, et al. Sixty alleles of the *ALS7* open reading frame in *Candida albicans: ALS7* is a hypermutable contingency locus. Genome Res 2003;13:2005–2017. [PubMed: 12952872]

11. Porta A, Ramon AM, Fonzi WA. *PRR1*, a homolog of *Aspergillus nidulans palF*, control pH-dependent gene expression and filamentation in *Candida albicans*. J Bacteriol 1999;181:7516–7523. [PubMed: 10601209]

12. Green CB, Cheng G, Chandra J, et al. RT-PCR detection of *Candida albicans* ALS gene expression in the reconstituted human epithelium (RHE) model of oral candidiasis and in model biofilms. Microbiology 2004;150:267–275. [PubMed: 14766904]

13. Cheng G, Wozniak K, Wallig MA, et al. Comparison between *Candida albicans* agglutinin-like sequence gene expression patterns in human clinical specimens and models of vaginal candidiasis. Infect Immun 2005;73:1656–1663. [PubMed: 15731066]

14. Martinez-Lopez RL, Monteoliva R, Diez-Orejas C, Nombela C, Gil C. The GPI-anchored protein CaEcm33p is required for cell wall integrity, morphogenesis and virulence in *Candida albicans*. Microbiology 2004;150:3341–3354. [PubMed: 15470113]

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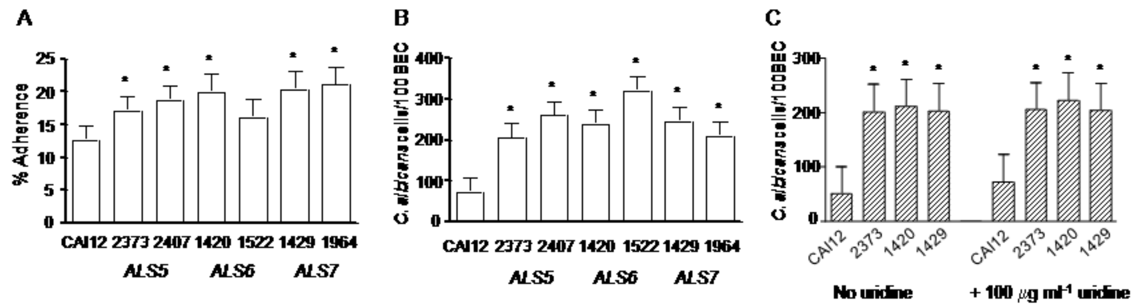

Fig. 1.

Adhesion assay data. Histogram showing the adherence of *C. albicans* strains to monolayers of human umbilical vascular endothelial cells (A), and buccal epithelial cells (B and C). Asterisks mark results that are significantly different from the control strain CAI12 ($P < 0.05$). Deletion of *ALS5, ALS6* or *ALS7* results in increased adhesion of *C. albicans* yeast forms to both cell types. Reintegration of a wild-type allele complemented the *als6Δ/als6Δ* strain in the endothelial cell assay. In general, however, reintegration of a single ALS allele did not complement the mutant phenotype suggesting the potential for allelic effects or a gene dosage requirement for full activity. Increased adhesive effects were also observed for *C. albicans* yeast forms cultured in the presence of excess uridine (C), suggesting that the results observed in (A) and (B) are not due to placement of the *URA3* marker.

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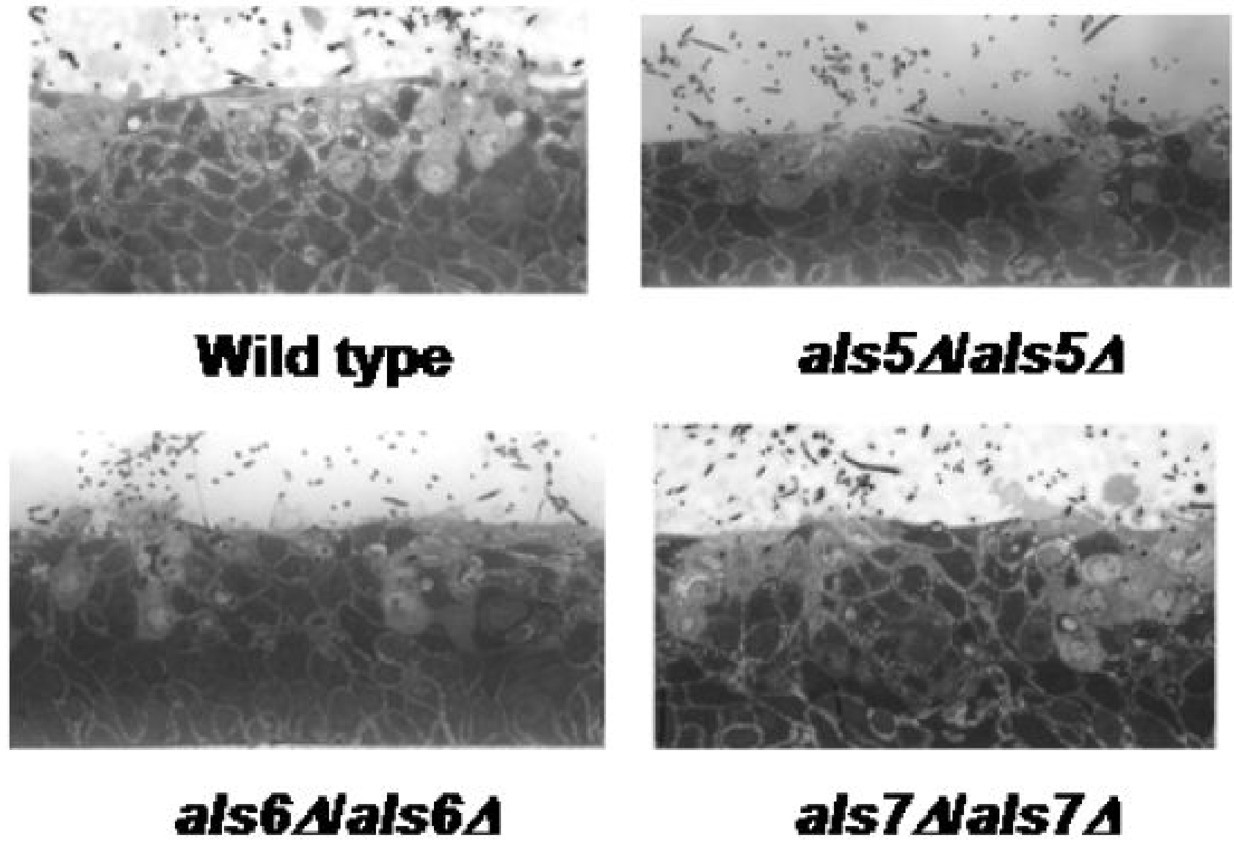

Fig. 2.
Light micrographs of buccal RHE inoculated with various *C. albicans* strains for 8 h. The ability of strains lacking *ALS5, ALS6* or *ALS7* to destroy epithelial cells was similar to that of the control strain CAI12.

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Table 1

Summary of methods for disruption of *ALS5, ALS6* or *ALS7* in *C. albicans*[a]

Gene	PCR Deletion Cassette[b]			*hisG-URA3-hisG* Deletion Cassette[c]				Reintegration of Wild-Type Allele[d]				Southern Blot Probe
			alsΔ/ALS				alsΔ/alsΔ				alsΔ/alsΔ::□□□	
	Allele[e]	Primer Names[f]	Strain Name	Allele	Upstream Primers[f]	Downstream Primers[f]	Strain Name	Allele	Upstream and Coding Region Primers[f]	Downstream Primers[f]	Strain Name	Primer Names[f]
ALS5	N/A[g]			*ALS5SA*	ALS5upF ALS5upR	ALS5dnF ALS5dnR	1447					ALS5gfpF ALS5gfpR
				ALS5LA			2373	*ALS5SA*	ALS5upF ALS5R	ALS5dnF ALS5dnF	2407	
ALS6	*ALS6-1*	ALS6-5DR ALS6-3DR	1268	*ALS6-2*	ALS6upF ALS6upR	ALS6dnF ALS6dnR	1420	*ALS6*	ALS6upF ALS6R	ALS6dnF ALS6dnR	1522	ALS6gfpF ALS6gfpR
ALS7	*ALS7-1*	ALS7-5DR ALS7-3DR	1275	*ALS7-2*	ALS7upF ALS7upR	ALS7dnF ALS7dnR	1429	*ALS7*	ALS7upF ALS7R	ALS7dnF ALS7dnR	1964	ALS7gfpF ALS7gfpR

[a] All deletions were constructed in strain CAI4 (*iro1-ura3Δ::λimm*^{434}/ *iro1-ura3Δ::λimm*^{434}) [7], which was a generous gift from William Fonzi (Georgetown University).

[b] Amplification of a deletion cassette using plasmid pDDB57 as template was described by Wilson et al. [8]. Plasmid pBBD57, which contains a PCR-amplifiable copy of a deletion cassette that encodes a *URA3* selectable marker and direct-repeat flanking sequences to promote excision of the cassette, was provided by Aaron Mitchell (Columbia University).

[c] This method for gene deletion was described by Fonzi and Irwin [6]. Deletion of ALS genes uses plasmid pHUL [3], which is a modified version of pMB7 [7] that contains *Hin*dIII-*Avr*II-*Xho*I-*Spe*I restriction sites 5′ of the *hisG-URA3-hisG* cassette, and *Kpn*I-*Sst*II-*Ngo*MIV-*Sst*I sites downstream of the cassette. To make a disruption cassette, sequences upstream and downstream of the target gene are cloned into pHUL using the *Avr*II-*Xho*I and *Sst*II-*Ngo*MIV restriction sites, respectively. The final cassette is excised by *Avr*II-*Ngo*MIV digestion prior to transformation into a Uri$^-$ strain.

[d] Reintegration of a wild-type ALS allele was accomplished using plasmid pUL [3]. Plasmid pUL differs from pHUL in that the *hisG-URA3-hisG* cassette in pHUL is replaced by *URA3* in pUL.

[e] In SC5314, the sequence of the *ALS5* alleles is nearly identical except that the small allele (*ALS5SA*) has 4 tandem copies of the repeated sequence within the central domain while the large allele (*ALS5LA*) has 5 tandem copies [8]. *ALS6* alleles each encode 4 tandem repeat copies and are likely to have only minor sequence differences [9]. SC5314 *ALS7* alleles were described by Zhang et al. [10] and have the same number of tandem repeat copies and same configuration of repeated sequences within the 3′ domain.

[f] Primer names correspond to sequences shown in Table 2.

[g] N/A = not applicable. This method was not used to disrupt this gene.

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Table 2

Oligonucleotide primers used in this study

Primer Name	Primer Orientation	Primer Sequence (5′–3′)
ALS5upF	F	CCCCCTAGGACCAGCATTGTCAATCGAACCA
ALS5upR	R	CCCCTCGAGTATGAGGCTCCGGCAAAAGC
ALS5R	R	CCCCTCGAGTGTCAACGTTTGAGAATGACG
ALS5dnF	R	CCCCCGCGGCGTCATTCTCAAACGTTGACA
ALS5dnR	R	CCCGCCGGCATATCCACTTATTAGCTGTCA
ALS5F13	F	CCCGGTACCCAACTAAAACTTTATCATCAAATCAC
ALS5gfpR	R	CCCCTCGAGCTGGTGTTAGCAGTTGGTAGTTGTTTG
ALS6–5DR	F	AAGTTTAAAAGAAAGCATGTTTCCTGTAGGAAATTTCATTCA TTGACTTGAATAAACATCGTTTTCCCAGTCACGACGTT
ALS6–3DR	R	TAAATAAACTCTAGAAATTGAAATATCTATAATAACGAAAAT AACAAAGTCAACGTTTGATGTGGAATTGTGAGCGGATA
ALS6upF	F	CCCCCTAGGAATCCCTGCGTATTATGGTATGG
ALS6upR	R	CCCCTCGAGTGAAATTTCCTACAGGAAACATGC
ALS6dnF	F	CCCCCGCGGCAAACGGGATTGTACCAAATC
ALS6dnR	R	CCCGCCGGCTGCTTCAGATCCAACACGTAA
ALS6R	R	CCCCTCGAGAAAAACAGAACAAAAAAAACGACACC
ALS6gfpF	F	CCCGGTACCTGTTTCAATCAATTGCCTATC
ALS6gfpR	R	CCCCTCGAGTGTCGGTGAATGGTGATGCTG
ALS7–5DR	F	TTTGAAAATAAGAATTTTTCATCAATCTAACAATCTACAATT TTCAACAGTCTAATACCTGTTTTCCCAGTCACGACGTT
ALS7–3DR	R	CATATAAATAATACATAAAACCTGGGTTTAAAAAACTGAAA ATTCATAACGAAAATCTTGTGTGGAATTGTGAGCGGATA
ALS7upF	F	CCCCCTAGGACCCGCCACAAAGTCACAGAA
ALS7—upR	R	CCCCTCGAGGTATAGTAATTGTAAGGTAACC
ALS7dnF	F	CCCCCGCGGCAAGATTTTCGTTATGAATTTTCAG
ALS7dnR	R	CCCGCCGGCAACCAGTGCTTTAGTATTGTG
ALS7R	R	CCCCTCGAGATCTTAGTCTCGATATAGTGTATC
ALS7gfpF	F	CCCGGTACCCCCAATAAATAATAATGAACACAAAAA
ALS7gfpR	R	CCCCTCGAGAGGTATTAGACTGTTGAAAATTGTAGAT

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Table 3

Cellular aggregation and growth rate data[a]

Strain	ALS Gene Genotype	Cellular Aggregation (cells/aggregate)	Doubling Time (h)
CAI12	Control	1.002 ± 0.005	1.67 ± 0.05[1], 1.75 ± 0.06[2] 1.85 ± 0.06[3]
2373	*als5Δ/als5Δ*	1.032 ± 0.005*	1.60 ± 0.05[1]*
2407	*als5Δ/als5Δ::ALS5SA*	1.013 ± 0.005	1.68 ± 0.08[1]
1420	*als6Δ/als6Δ*	1.023 ± 0.005*	1.66 ± 0.07[2]*
1522	*als6Δ/als6Δ::ALS6*	1.017 ± 0.005*	1.69 ± 0.03[2]*
1429	*als7Δ/als7Δ*	1.010 ± 0.005	1.67 ± 0.07[3]
1964	*als7Δ/als7Δ::ALS7*	1.013 ± 0.005	1.64 ± 0.08[3]

[a]Means and standard errors are reported. Asterisks indicate means that are significantly different from the CAI12 control ($P < 0.05$).

[b]Growth rate was measured separately for *ALS5-, ALS6-* and *ALS7*-related strains. The superscript 1, 2 or 3 for the CAI12 data matches the superscripts for the different experimental groups in the growth rate column.