Table S1. Oligonucleotide used for sequencing and amplification of sgRNAs.

Primer name	Sequences 5′ – 3′
TEF-F	GGTCTAGAACCTGGACAAATCGTTAAAC
CYC-R	GCGGCCGCCTTCGAGCGTCCCAAAACCT
sgURA3-1	GGTGGGCTTCAGCTCTCGTCGTTTTAGAGCTAGAA
sgURA3-2	GTCCGTGAGGACGAAACGAGTAAGCTCGTCGGTGGGCTTCAGCTC
sgURA3-3	TTCGTCCTCACGGACTCATCAGGGTGGGTATGAATTATATTAA
sgAOXS-1	GATCAATGTAGACAAGCTTGGTTTTAGAGCTAGAA
sgAOXS-2	GTCCGTGAGGACGAAACGAGTAAGCTCGTCGATCAATGTAGACAA
sgAOXS-3	TTCGTCCTCACGGACTCATCAGGATCAATATGAATTATATTAA
sgAOX2-1	CTGCATTTCCGGCATTCCCAGTTTTAGAGCTAGAA
sgAOX2-2	GTCCGTGAGGACGAAACGAGTAAGCTCGTCCTGCATTTCCGGCAT
sgAOX2-3	TTCGTCCTCACGGACTCATCAGCTGCATTATGAATTATATTAA

Table S2. Plasmid used in this study.

Name	Backbone	Marker	Promoter	Gene	Terminator	origin
P414 *CAS9(TEF1)*	P414	*TRP1*	*ScTEF1p*	*CAS9*	*ScCYC1t*	Reference 28
pNTA	pNTA	*NTC*	*CaGAPp*	*NTA*	*SaGAPt*	This study
pAN-ARS	pAN	*NTC*	*CaURA3p*	*NTA*	*CaURA3t*	This study
pAN-CAS9	pAN	*NTC*	*CaGAPp*	*CAS9*	*CaGAPt*	This study
pAN-CAS9gURA3	pAN-CAS9	*NTC*	*CaTEF1p*	*sgURA3*	*ScCYC1t*	This study
pAN-CAS9gAOXS	pAN-CAS9	*NTC*	*CaTEF1p*	*sgAOXS*	*ScCYC1t*	This study
pAN-gURA3	pAN	*NTC*	*CaTEF1p*	*sgURA3*	*ScCYC1t*	This study
pAN-gAOXS	pAN	*NTC*	*CaTEF1p*	*sgAOXS*	*ScCYC1t*	This study
pAN-gAOX2	pAN	*NTC*	*CaTEF1p*	*sgAOX2*	*ScCYC1t*	This study

Ca : *Candida aaseri, Sc* : *Saccharomyces cerevisiae,* sg: single guide



Strain number	Yeast species	Accession No.	Similarity (%)	Lipase activity
1	*Candida sp.*	JQ247717	94.0	+
2	*Cryptococcus laurentii*	FN428921	97.1	+++
3	*Yamadazyma mexicana*	AB365477	97.0	+
4	*Rhodotorula mucilaginosa*	HE660061	95.0	+
5	*Pichia caribbica*	KC111450	94.4	-
6	*Candida sp.*	JQ247716	91.0	+
7	*Pichia caribbica*	KC111450	95.3	+
8	*Pichia caribbica*	KC111450	96.0	+
9	*Rhodotorula mucilaginosa*	KC205168	96.2	++
10	*Sphingobium yanoikuyae*	JN700070	99.0	++
11	*Pantoea agglomerans*	FJ593002	99.2	-
12	*Ochrobactrum intermedium*	AM490610	98.5	-
13	*Uncultured Verticillium*	GU055566	96.2	++
14	***Candida aaseri***	**JQ247716**	**94.1**	+++++
15	*Rhodotorula sp.*	JX310560	96.7	++
16	*Candida sp.*	JQ247716	93.4	+

Figure S1. Lipase activity of 16 yeasts isolated from compost of empty fruit bunches of palm oil. Cells were spotted on YNB plate containing 2% glucose and 1% of tributyrin and grown for 5 days.

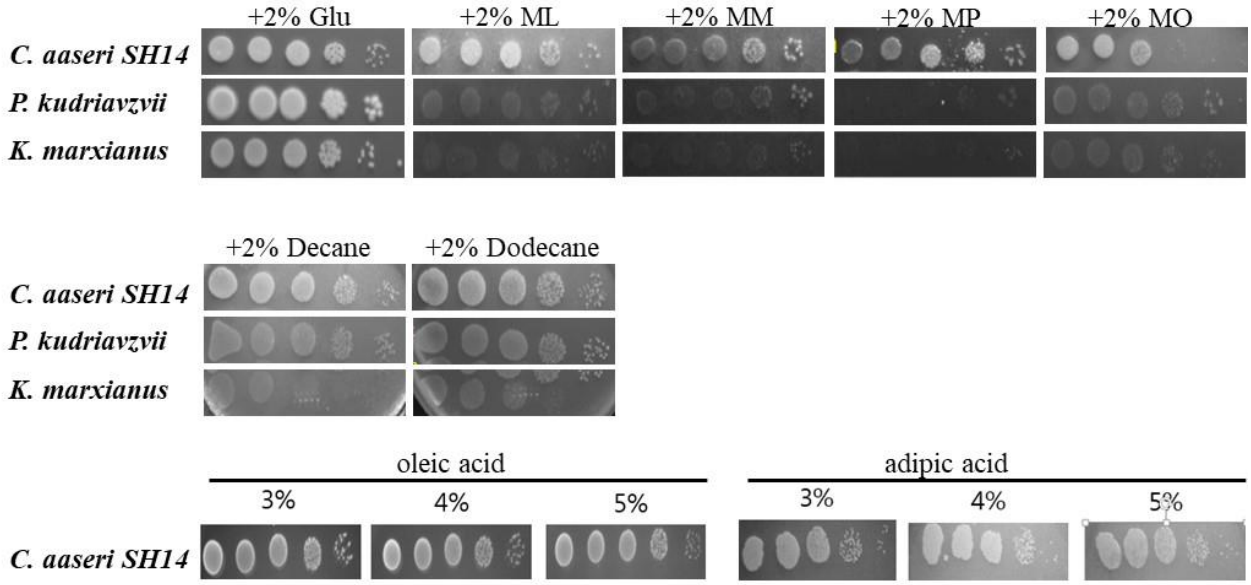


Figure S2. Utilization of long-chain fatty acids and alkanes by different yeasts and resistance to organic acids. Serially diluted yeasts were spotted on YNB plate containing 2% of the indicated carbon sources and grown for 5 days. ML; methyl lauric acid, MM; methyl myristic acid, MP; methyl palmitic acid, MO; methyl oleic acid.

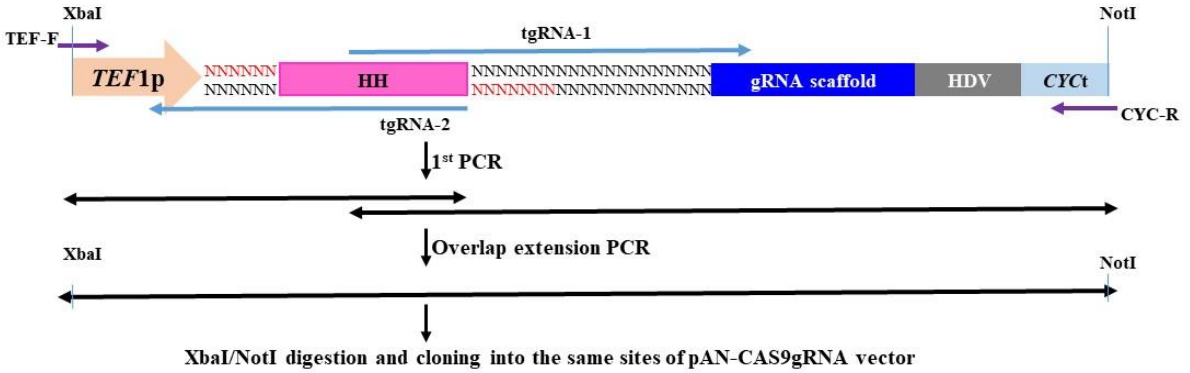


Figure S3. Construction of sgRNA expression vector. NNNNNN indicate 6 bp complementary sequence required for release of HH from sgRNA. Two fragment were amplified using TEF-F/tgRNA2 and tgRNA1/CYC-R primers, respectively and annealed into a single fragment by overlap extension PCR using TEF-F and CYC-R primers.

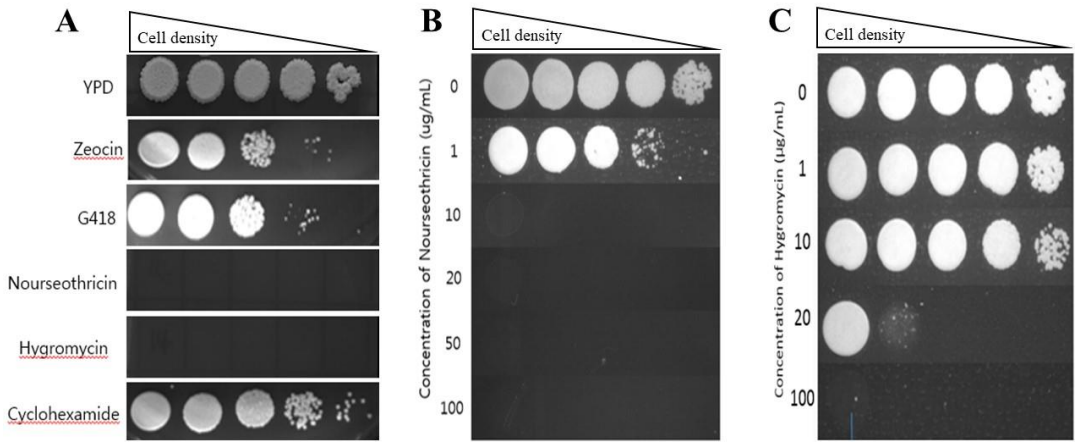


Figure S4. Antibiotic sensitivity test of *C. aaseri* SH14. Serially diluted *C. aaseri* SH14 was spotted on YPD plate containing 100 μg/mL of antibiotics and grown for 5 days **(A)**. Determination of minimum inhibitory concentration for NTC **(B)** and hygromycin **(C)**.

>ARS1 (1265 bp)
CTGCAGCCCGGGGGATCAATCCAACTTAAAGTTTCTCTTTGTTGATATGGGTGGTAAAGTTAGT
GAATTTAAAGAAGAGTTCAAGAAGTTAGATTTGTACGCCCCTAATGGTGTGGATATTGTCATTC
ATAATGCTGGTATCAGTGGTCCTAACTTCTTTGTTAAATCTACTGAATACGATGTAGAATCTGCA
CTTCCAGTAGTTGCAATCAACTATCTTGGTACTGTGAAATTGTATCAAGCTGTTTACCCATACCT
TTTCAAAGGAAATGGAACAAAAAAGCTCATTTTAACAAGCTCACTTGCTAGTTCTATGGGACA
AATGCCTTTTGGAAGTAATACTTATGGAGCTTCTAAAGCTGCTGTCAACCATTTTGGTGTCCAA
ATTGCTACTGAACATCAAAATTCAGACAATCCTCTTATTAAAAATTCTATCACTGTATTATTACA
TCCCGGTCTTGTTCTTACTGATATGGCTGCCATGGAAGGAGACTCTGGCTCTTCGGATACTACA
CCTTCTTGTGAATTAATTACACCAGATGTATCTGCAAAAGGCACTTTAGATTTGGTTGCACGTCT
TACCTCAAAAGACAATGGTAAGTTTTTCGATTACCAAGGAAACAACATAAGTTTGTAAGTCCA
CTAGTGGAGGTCATATTCCATTTTTGTTTAAGGTATTTCACTTGTTGAAATCATTTGTATGATAAA
TGAAACATCAAGAGTCGTGTATGGTCATTTGAATAATATCTAGTAATAATCCACTAAAAGACCA
ATTTTCCTTCTTCATAGTTTTCTAATAGATTCCAATTACTTTATTTATAGCATGAGTTTTTTTTCTG
TATTTTTAAAGCTCTTTGAAATGAATTGTTAAAACTTCTTGATATCTTTACTCTTTATTTTCTCTGT
TGTGAAAATCACAATTTAAAAAATTTTCTACTCTCATACTGTTACTGAATTCTTCACTCATCAAT
GAAAATTCCATTTCTCTATAAAATAATCAGACCTCTCACACCATATCAATAAATAGACGTACCT
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GACTTACTACCCTATATTATGGTTCCTCGCGAAAAATCTGGGGAAATTATCACACCATGTTCAG
TACAAGAACCATTGCCTCGAGATTGTTTGGAACTAGATTCGTTTCCCATTCTCTTTCAGTGGTCA
AATATGATAAGAGTTCCACCTCATTGAAGATCCACT

>ARS2 (789 bp)
AGATATCATGTGGAAGGAAAACATGATGTTGTTTATATTTCAGAACCCTTTGAAATCAAAGTTA
GAGAGTTGGATGTCCCTACATCATCAGATGAAGTTACACTGTTTTGCAAATCAATTGAAATACT
TAACTGATAAAGTATTGGAATTAGATCTGATTTATCACAAAATCGAATCAATGAACTCTAACAT
TTTAGTTATTTTTGAAAGATTAACTAAATTGATTTCATTATCAACCAATATTAAGATTAATGAAA
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AGATAGAATACATTATACAAATAACTAGAAGAAATATAAAAAGTATAACAAAACATGTTGAAA
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CTCGCgTGCCCCAAACATTATTCATTGACTAAACATTCTCTAATAAATAACTATTTACATTACTCC
TTATCTTCTTTGCCATTTCACTGCAATGTTCAATAGTGTTATCCAAATCCTTTAAGTCACCATTAA
CCCAGATACCAACAATTTCATTCTTCAAGCACATGAAACTAACTAAACAATCGATGTCTAATTG
CAGATCCCCCGGGCTGCAG

Figure. S5. Sequences of ARS1 and ARS2. Yellow boxes indicate the core regions of *ARS1* and *ARS2* for the replication and stability of plasmid.

ATGGATAAGAAATACTCAATAGGCTTAGATATTGGCACAAATAGCGTCGGATGGGCGGTGATC
ACTGATGAATATAAGGTTCCGTCTAAGAAGTTCAAGGTTTTGGGAAACACAGACCGCCACAGT
ATCAAGAAGAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACTCGT
CTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAA
ATCTTCTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTCT
TGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATCTTTGGAAATATAGTAGATGAAGTTG
CTTATCATGAGAAATATCCAACTATCTATCATTTGCGAAAGAAATTGGTAGATTCTACTGATAAA
GCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGTCATTTCTTGATT
GAGGGTGATCTAAATCCTGATAATAGTGATGTGGACAAACTATTCATCCAGTTGGTACAAACCT
ACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTC
TGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAA
AAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTTTGACCCCTAATTTCAAATCAAATT
TTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGATTTAGATAA
CTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGTTCTTGGCAGCTAAGAATTTATCAGATG
CTATCTTACTTTCAGATATTCTAAGAGTAAATACTGAAATAACTAAGGCTCCCCTATCAGCTTCA
ATGATTAAACGCTACGATGAACATCATCAAGACTTGACTCTTTTAAAAGCGTTAGTTCGACAA
CAACTTCCAGAAAAGTATAAAGAAATCTTTTTTGATCAATCAAAGAACGGATATGCAGGTTAT
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GTCTGAAGAAACAATTACCCCTTGGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCA
ATCATTTATTGAACGCATGACAAACTTTGATAAGAATCTTCCAAATGAGAAAGTTCTACCAAA
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GGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATT
AGATTTCTTGAAATCAGATGGTTTTGCCAATCGCAATTTCATGCAGTTGATCCATGATGATAGTT
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CATATTGCAAACTTAGCTGGTAGCCCTGCTATCAAGAAAGGTATTTTACAGACTGTAAAAGTTG
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GCAAAATGAAAAGCTTTATCTCTATTATCTCCAAAATGGAAGAGACATGTATGTGGACCAAGA
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CAAATTTTGGATAGTCGCATGAATACTAAGTACGATGAAAATGATAAACTTATTCGAGAGGTTA
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CTCTAATATCATGAACTTCTTCAAGACAGAAATTACACTTGCAAATGGAGAGATTCGCAAACG
CCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTTTGC
CACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGAAGACAGAAGTACAGA
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AGAAAGACTGGGACCCAAAGAAGTATGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCC
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AGAAGATAACGAACAAAAACAATTGTTTGTGGAGCAGCATAAGCATTATTTAGATGAGATTAT
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AACGATATACGTCTACAAAAGAAGTTTTAGATGCCACTCTTATCCATCAATCCATCACTGGTCT
TTATGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGAC

Figure S6. Sequence of *C. aaseri* codon optimised Cas9 gene.

AGTATCAAATTTCATTAATGAATAAGTTTCAACTTGTAAATCACATAAAATCTCTACCGCTTTAT
CCAAATCATTTTTCTTTGTTAAATAAATATCACTCAAATTACGTGATACAATTGCCCTTTCTACTT
CAATAAAGATTTTTTTATCACTAACAGTTCGAATAGTTTCAATCAATTTAATCTTTAATTCTAATT
CTTTATGATTATTCTCATCTAAACTGGCCATTTTCTCAATTATATGTGCAATAAACACTTGGATAC
TGCTTTTCATTTGTCCATGCTTCTTACATAAGATGGGAATCAATTCATTCATGTATTCCCAGTCTG
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CCGGGAGTTGTTTGTCCAAGATGGACGTGTAGTCCTTCTCTGCTTTTAAAGGATCTTCTCTAGA
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ACACAAGAAACATGAGTAGGACACGCCTTTTTTATTATTTAAAGATTCTAAATAGTTAATAAAG
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TGGTGATTTCTTGAAAAGCGCGATATTTGAAGCCAAATTTCAACACAAGAAACATGAGTAGGA
CACGCCTTTTTTATTATTTAAAGATTCTAAATAGTTAATAAAGGCAAATACAATTGATATTAGTTA
TGGTGGAAATTGGTACCGATGGGAAGTTGAAAGTAATTACAAAAAGAGTACCCGCGGATTTG
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CTGAGCCATGAATAGACTGATAATCAATTGGTTATCTCTTTCTTACCAAGCTAGAACTTCACTATACT
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AAACTTGAACACTTTGGTCTAAACCTTGTCTATTTATTCTATATAATTCTATTATTAAAATTTCCATAC
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*AATTAATATAATTCATAGGATCC*ATGATCTGTTCATTCTTTAGACTATTGACCATTGTAACTTTGGT
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CATGGTACATTGGATTCCATTAGAGGTGCTTTATCCAGTAGTAACATCACTGGTGTTAAAAAGG
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CAACCAACTTATGCACCAGAATTGGCTTCCAACTTACTTGGTGTTGCTTTAGGTGGATGGGTTA
CCAATATTACTGCTACTATAACAAGTGTTAGTGGTACCATATTCTCTGGATTGGGTGCTATGGGA
ATGGCTGGTTTAAGTAATGAGTACACCGATTTATACGGTTACCTTAAGACTGCTATGCCAGCAG
ATAAATATGAAGAATTCACTAAAGCTTATTCAATATGTGCTGCTGAAGCTCTTATTGAATATAAT
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AACCGACATATTCCATCATTCGTAACAATACATTGGGTTTAATTGCTGGTCAGATGCCAGAAAT
TCCTGTTTTCGTTTACCATGGAACTCTCGACCAGATCGTACCATATGATCAAGCTGAAAGGGTT

TATGATATTTGGTGTGATGCTGGTATTAAATCTTTTGAATTTGCTACTGATTTAACTGCTGGTCAT
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GACTCACTAATTACCGAGAATTAGAATCTATAGATGATTTTTTCACTCGATTAAATCTTATCACC
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AGGTAATGGAACTGCTAGTCAGGTGGAATATTGGTGTAATCGCAAGGAGGCACTGATTTTAAA
GCAAATTTATGGATGTTTTGCCATGACAGAACTAGGACATGGTTCTAATGTACCACGGACTAAA
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GGGAATTGACGGCTTTGAGAATAGATAGACTCACTAATTACCGAGAATTAGAATCTATAGATG
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ATTTAGGCTTGTTTTTAAACTGTATTAAAGGTAATGGAACTGCTAGTCAGGTGGAATATTGGTGT
AATCGCAAGGAGGCACTGATTTTAAAGCAAATTTATGGATGTTTTGCCATGACAGAACTAGGA
CATGGTTCTAATGTA

Figure S7. Integration of CaLIP2 overexpression cassette at sgAOX2 via NHEJ. The blue box indicates the 5′ repetitive sequence and the yellow box indicates the 3′ repetitive sequence. The red box is a PAM sequence and gray box is a recognition site for DNA cleavage by CRISPR-Cas9. Underlined sequences indicate *Ca*LIP2 gene sequence. *TEF1* promoter is an italic character before *CaLIP2* sequence and *CYC1* terminator sequences located downstream of *CaLIP2* with bold character.

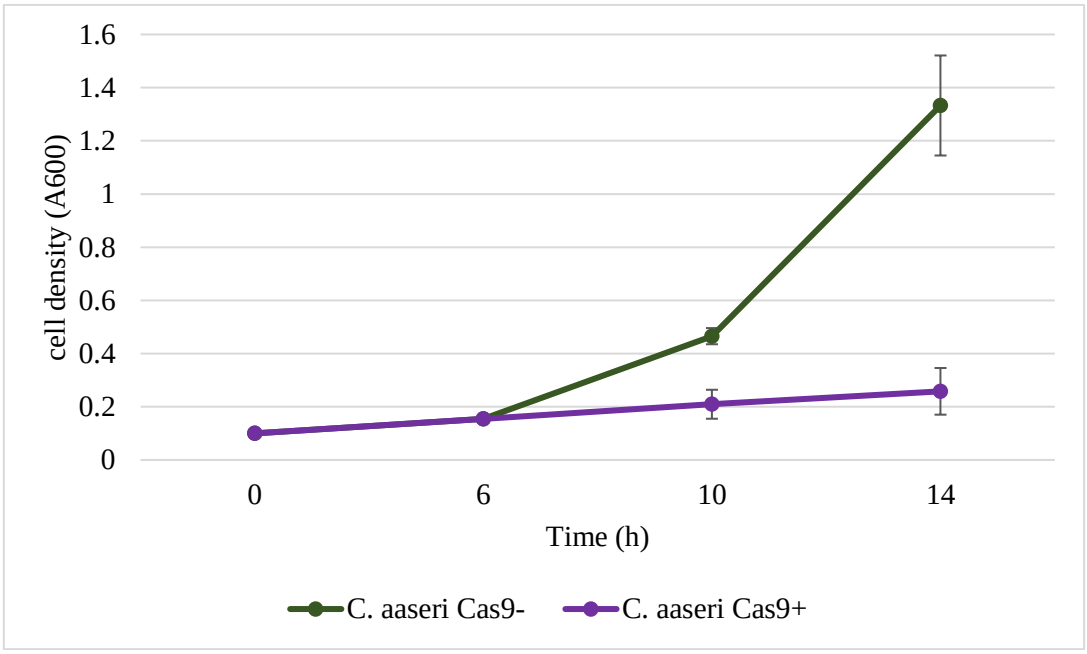


Figure S8. Effect of Cas9 expression on the growth of *C. aaseri* SH14. *C. aaseri* SH14 harboring a plasmid free of Cas9 (Cas9-) and a plasmid with Cas9 (Cas9+).