

Escherichia coli*, but Not *Staphylococcus aureus*, Functions as a Chelating Agent that Exhibits Antifungal Activity against the Pathogenic Yeast *Candida albicans

Swagata Bose ^{1,2}, Durg Vijai Singh ³, Tapan Kumar Adhya ² and Narottam Acharya^{1,*}

¹Department of Infectious Disease Biology, Institute of Life Sciences, Bhubaneswar-751023, India.

²KIIT School of Biotechnology, Bhubaneswar-751021, India.

³Department of Biotechnology, School of Earth, Biological and Environmental Sciences, Central University of South Bihar, Gaya- 824236, India.

*Correspondence to:

Narottam Acharya, Phone: 91-674-2304278, Fax: 91-674-230 0728

E-mail: narottam_acharya@ils.res.in; narottam74@gmail.com

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Figure S1 Bacterial biofilm estimation and CLSM. (A) Biofilm of *Sa* pure culture and co-culture with 5% of *Ca* and *Ec* culture filtrates; (B) quantification of *Sa* biofilm in the presence of 5%, 25%, and 50% of *Ca* and *Ec* culture filtrates. Similarly, (C) Biofilm of *Ec* pure culture and co-culture with 5% of *Ca* and *Sa* culture filtrates. (D) *Ec* biofilm estimation in the presence of 5%, 25%, and 50% of *Ca* and *Sa* filtrates. Graph plotted using Graph Pad Prism 8.0. (E, F) For CLSM, the bacterial biofilm matrices were grown in the 8-well chambered slides and visualized under 40x Leica TCS SP5 confocal scanning microscope. Mean values from three independent experiments are shown, and error bars represent the SEM. P-value <0.05; ****: P-value <0.0001 (significant) and ns: >0.05 (non-significant).

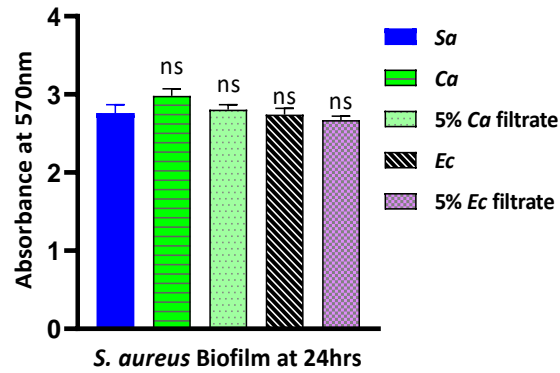
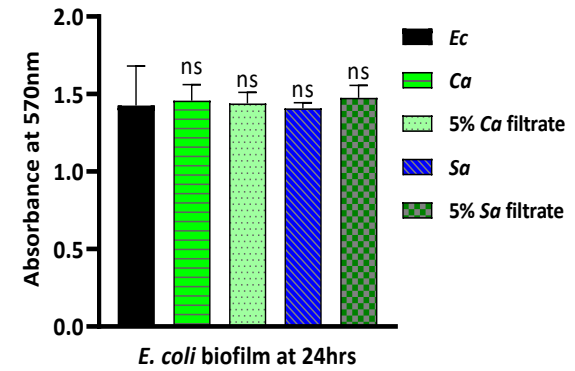
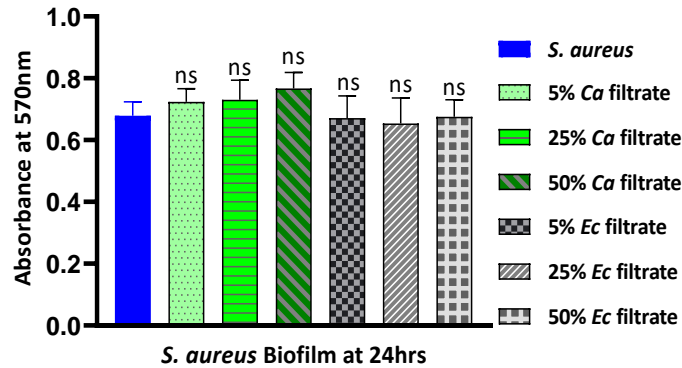
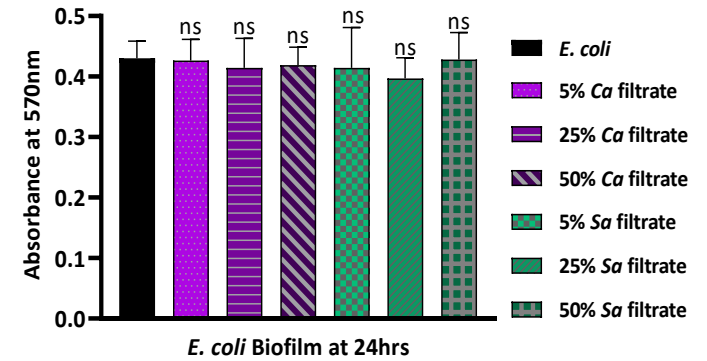
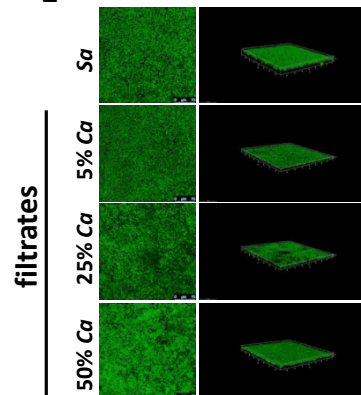
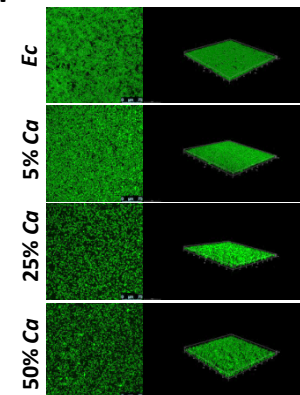
Figure S2 Quantification of Mg²⁺ ions. Threshold concentration of divalent ions estimated using different range of Mg²⁺ ion concentrations ranging between 62.5μM to 0.24μM on pre-formed *Ca* biofilm. The graph was plotted using Graph Pad Prism 8.0. Mean values from three independent experiments are shown, and error bars represent the SEM. P-value <0.05; ****: P-value <0.0001 (significant) and ns: >0.05 (non-significant).

Figure S3 Morphology assessment of *C. albicans* filamentation-defective mutants in the presence of serum. *C. albicans* strains (WT, HLC52, HLC54, and JKC19) were grown in YPD media without and with 10% FBS serum at 30°C with continuous shaking, the morphology imaged at 30mins (A), and at 3hrs time interval (B). The imaging was carried under 40x microscope (Leica DM500).

Table S1: Primers used during RT-PCR and Semi-quantitative analyses. Sequences of primers and cyclic conditions used for amplification of *C. albicans* genes used in this study were given.

Targets	Sequences (5'-3')	Cyclic conditions	Amplicon size (bp)
GAPDH	(Fp) GACCGTTGACGGTCCATCC (Rp) CATCGGTGGTTGGGACTC	D at 95°C for 15" A at 52°C for 30" E at 72°C for 30" } 30cycles	161
ALS3	(Fp) CGGTTGCGACTGCAAAGA C (Rp) GACCAACCCAAAACAGCATTCC	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	127
HWPI	(Fp) CAGTTCCACTCATGCAACCATC (Rp) GCAATACCAATAATAGCAGCACCG	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	115
ERG11	(Fp) TTACCTCATTATTGGAGACGTGATG (Rp) CACGTTCTCTTCTCAGTTTAATTTCTTTC	D at 95°C for 15" A at 52°C for 30" E at 72°C for 30" } 30cycles	85
ERG3	(Fp) TCCAGTTGATGGGTTCTTCC (Rp) GGACAGTGTGACAAGCGG	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	179
SAP3	(Fp) GTTACTGGTCCCCAAGGTG (Rp) CTTGTCCTTGACCAGCTTGAC	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	208
EFG1	(Fp) CCAGGGTGGTGCTGCTAATAG (Rp) GGGTGAAGGGTGAAGTGAACC	D at 95°C for 15" A at 55°C for 30" } 30cycles	241

		E at 72°C for 30"	
<i>CPHI</i>	(Fp) GACGCTTCTGGGTTT CC (Rp) ATCCCATGGCAATTTGTTG	D at 95°C for 15" A at 54°C for 30" E at 72°C for 30" } 30cycles	160
<i>NRG1</i>	(Fp) CACCTCACTTGCAACCCC (Rp) GCCCTGGAGATGGTCTGA	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	198
<i>TUPI</i>	(Fp) CTCTTGGCGACAGGTGCAG (Rp) GTGGTGACGCCGTCTTCGA	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	224
<i>ECE1</i>	(Fp) GAGATGGCGTTCCAGATGTT (Rp) CCGGCATCTCTTTTAACTGG	D at 95°C for 15" A at 55°C for 30" E at 72°C for 30" } 30cycles	214

A**C****B****D****E****F****Figure S1**

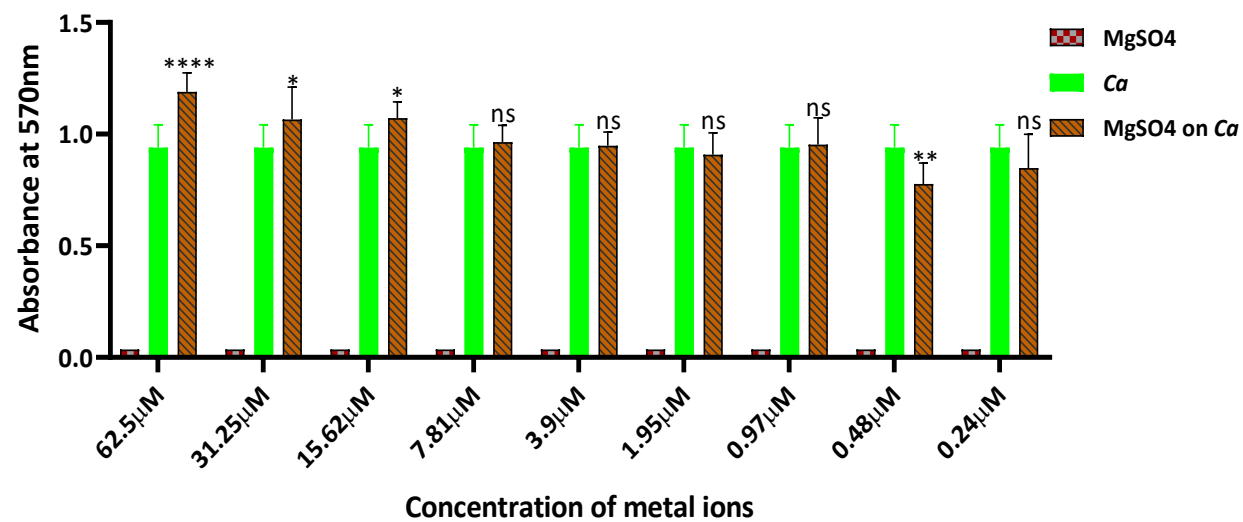


Figure S2

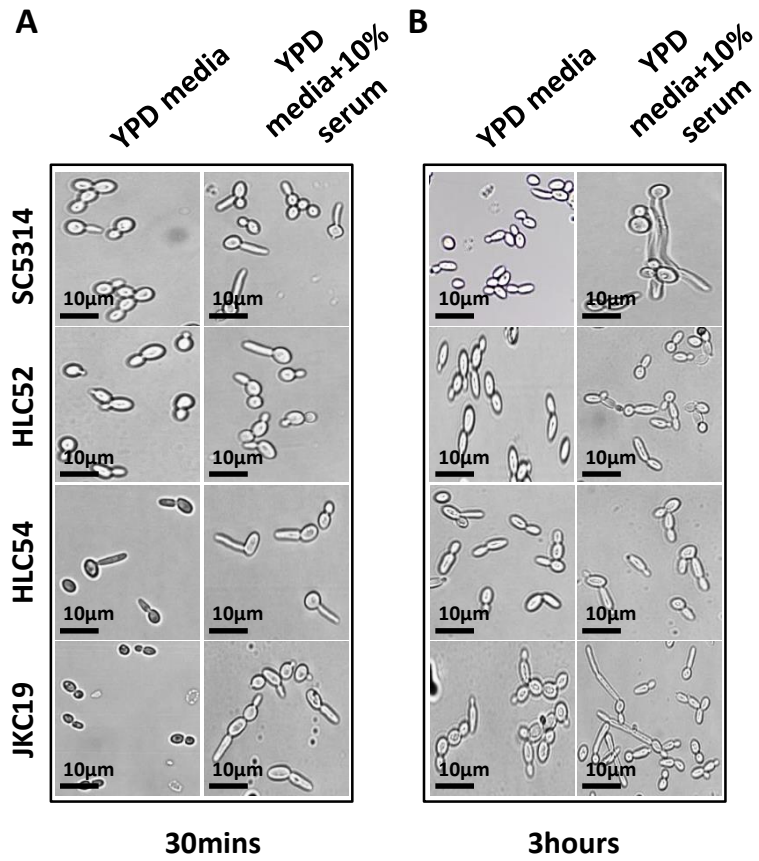


Figure S3