

Supplementary Materials

Phytopigment alizarin inhibits multispecies biofilm development by *Cutibacterium acnes*, *Staphylococcus aureus*, and *Candida albicans*

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Table S1. qRT-PCR primer sequences used for *C. acnes*.

Gene	Description	Primer
PPA1761	Lipase	Forward 5'-CCT CAA GGT GTC TGG TCA TC-3'
		Reverse 5'-GAC TTC GTC GTT TTT CCA GA-3'
PPA1796	Lipase	Forward 5'-CTC AAG GTT CGT GAA CGA GT-3'
		Reverse 5'-GCC ATA GAG CTC CTT GTT GA-3'
PPA2105	Lipase	Forward 5'-GAT TTC CTT AGC ACG TGG AG-3'
		Reverse 5'-GAT GAC GGT GTA GGC GAT AC-3'
PPA1035	Alpha/beta hydrolase, lipase	Forward 5'-TAT CGA TCA TGG GTG ATT CC-3'
		Reverse 5'-ATA GAG GGC GAG GAC TGA CT-3'
PPA380	Hyaluronate lyase	Forward 5'-CGC TCT GAA GGA TTC GTC-3'
		Reverse 5'-GTC GTG CAG GAT ACA CAT GA-3'
<i>hly</i>	Hyaluronate lyase	Forward 5'-CAA CAT CGC CGT GTT TAT TG-3'
		Reverse 5'-CCC ATG ACG ACG TAG AGG AT-3'
PPA0721	Invasion-associated protein	Forward 5'-AGA AGA AGG AAC AGG AGG CC-3'
		Reverse 5'-TCA GGA ATC GGC ACT TGA CT-3'
PPA1962	Invasion-associated protein	Forward 5'-TTC AGT AAA GGC CCG AGT GT-3'
		Reverse 5'-ACA GGT AGT GGT GGT CGA TG-3'
PPA1983	Adhesins (Thrombospondin type 3 repeats, PKD, SEST domains)	Forward 5'-AAG GGG ATT ACG GCT GTC AA-3'
		Reverse 5'-CAG CCT GGT CTT TGT GGT TC-3'
PPA1906		Forward 5'-AAG GGG ATT ACG GCT GTC AA-3'

	Adhesins (Thrombospondin type 3 repeats, PKD, SEST domains), PTRPs/host-interacting factors	Reverse 5'-CAG CCT GGT CTT TGT GGT TC-3'
PPA1715	PTRPs (Proline-Threonine Repetitive Protein, recognizing human fibrinogen) /host-interacting factors	Forward 5'-GTT CAA GCT GTC CCA CAA GG-3'
		Reverse 5'-TTG CTA TCG GTG AGG GTC TC-3'
PPA2127		Forward 5'-TCA CTG ATG CGA AGA TCC AG-3'
		Reverse 5'-TTG CCA ACT TCT TGT CAA CG-3'
<i>luxS</i>	S-ribosylhomocysteine lyase	Forward 5'-AGC CCA ACA AGG AAC ATC TG-3'
		Reverse 5'-TGT TGA GCT TGA GGA TGT CG-3'
PPA0149	Glycosyltransferase	Forward 5'-AGT ACA TGG CTT CCC GAG TG-3'
		Reverse 5'-CTT GGG ACT CGA AGT TGA GC-3'
<i>btuR</i>	Cobinamide/cobalamin adenosyltransferase	Forward 5'-GGA AGA TGC TCT TCG GGC GCT-3'
		Reverse 5'-GCC TCA GGG TTC TCC GCA GC-3'
<i>cbiL</i>	Precorrin-2 C(20)-methyltransferase	Forward 5'-GCG CGA GGC AGA CGT GAT CC-3'
		Reverse 5'-GAC ACC GGA CCT CTC CCG CA-3'
<i>roxP</i>	Radical oxygenase	Forward 5'-GCA TCT AGC CCT CTC ACC AT-3'
		Reverse 5'-CTG AGA GTC CGG TAG GTG GT-3'
<i>tly</i>	Lysis of red blood cells	Forward 5'-CAG GAC GTG ATG GCA ATG CGA-3'
		Reverse 5'-TCG TTC ACA AGA CCA CAG TAG C-3'
PPA0349	Polysaccharide capsule biosynthesis	Forward 5'-CTT CTT CGT CGA CCA GTT CC-3'
		Reverse 5'-TCA GCT GTC TCG TCA ACA CC-3'
PPA1098	DnaK, Hsp70 family protein	Forward 5'-TGG ATC TGG GAG TCG ACC TA-3'
		Reverse 5'-TTA GCA CCG TTG AGC AGA GA-3'
PPA2139	Cutinase	Forward 5'-CGA GAA TGG ACT GTG CTT CG-3'
		Reverse 5'-GTT GAT GGC ATC GTG GAG TC-3'
PPA1222	Polyprenol monophosphomannose synthase	Forward 5'-ATG ACG CCC TAG TCG AAA TG-3'
		Reverse 5'-ATG AGT TCG CGG TAC TTT GG-3'
<i>16s rRNA</i>	16S ribosomal RNA (House keeping gene)	Forward 5'-GGG GCT TAA CCC TGA GCG TGC -3'
		Reverse 5'-TTC GCT CCC CAC GCT TTC GC-3'

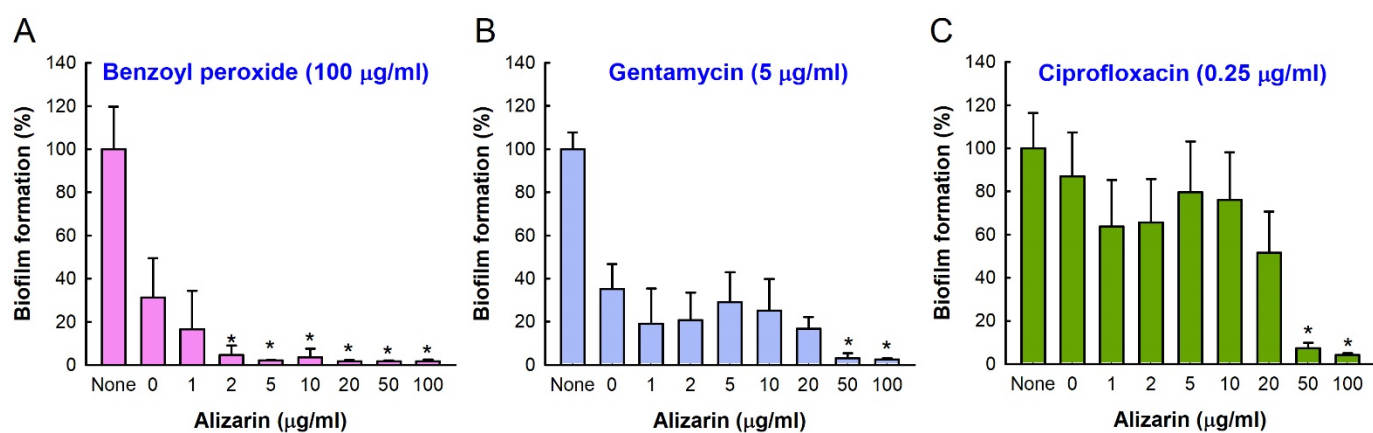


Figure S1. Combinatory antibiofilm activity of alizarin along with antibiotics against *C. acnes*. Biofilm inhibitions by benzoyl peroxide at 100 µg/ml (A), gentamycin at 5 µg/ml (B), and ciprofloxacin 0.25 µg/ml (C) with alizarin (0, 1, 2, 5, 10, 20, 50, and 100 µg/ml) were measured after incubation for 6 days in 96-well plates at 37°C under anaerobic conditions. Error bars indicate standard deviations. *, $P < 0.05$ vs. non-treated controls.