

```

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P6 597 AHLDDGGVGFVATDSDLADVATRKGTWIDVNP SRKVKGADEVIERAYASLHVTHHDPVAMALLPTASRSHTMALATRPGVEPPTVLRNDATQAVRSAGALLTKDPTVVTTLAFWEPA
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12.1.L1 601 AHLDDGGVGFVATDSDLADVATRKGTWIDVNP SRKVKGADEVIERAYASLHVTHHDPVAMALLPTASRSHTMALATRPGVEPPTVLRNDATQAVRSAGALLTKDPTVVTTLAFWEPA
consensus 601 *****

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P6 717 TCGGVAVNRPALVQTRRESANQMEVVIVEPTQKRKGLTIVTIGSWKVKTADSHVDVSCENAAGTLHEVDTAGLGGQSVRVTLARQVQTPTSGGGRHRA -----
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CCUG33950 699 TCGGVAVNRPALVQTRRESANQMEVVIVEPTQKRKGLTIVTIGSWKVKTADSHVDVSCENAAGTLHEVDTAGLGGQSVRVTLARQVQTPTSGGGRHRA -----
266 721 TCAQMTADAPALIQQAQGSRVVEVMSPEPTQKRPBLTVAIRGVTWVNSSDRESVRSKDKTTLRINADLGGQSVRVTLSPALPKPTKPSLRASSYPPLGLPHTSS
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consensus 721 *****

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Figure S1. Sequence alignment of HYL variants of the *P. acnes* phylotypes IA, IB, II.
 HYL sequences of the following strains were selected (in bracket their phylotypes): KPA171202 (IB); 6609 (IB); P6 (IB); HL001PA1 (II); CCUG33950 (II); 266 (IA); 15.1.R1 (IA); 12.1.L1 (IA).

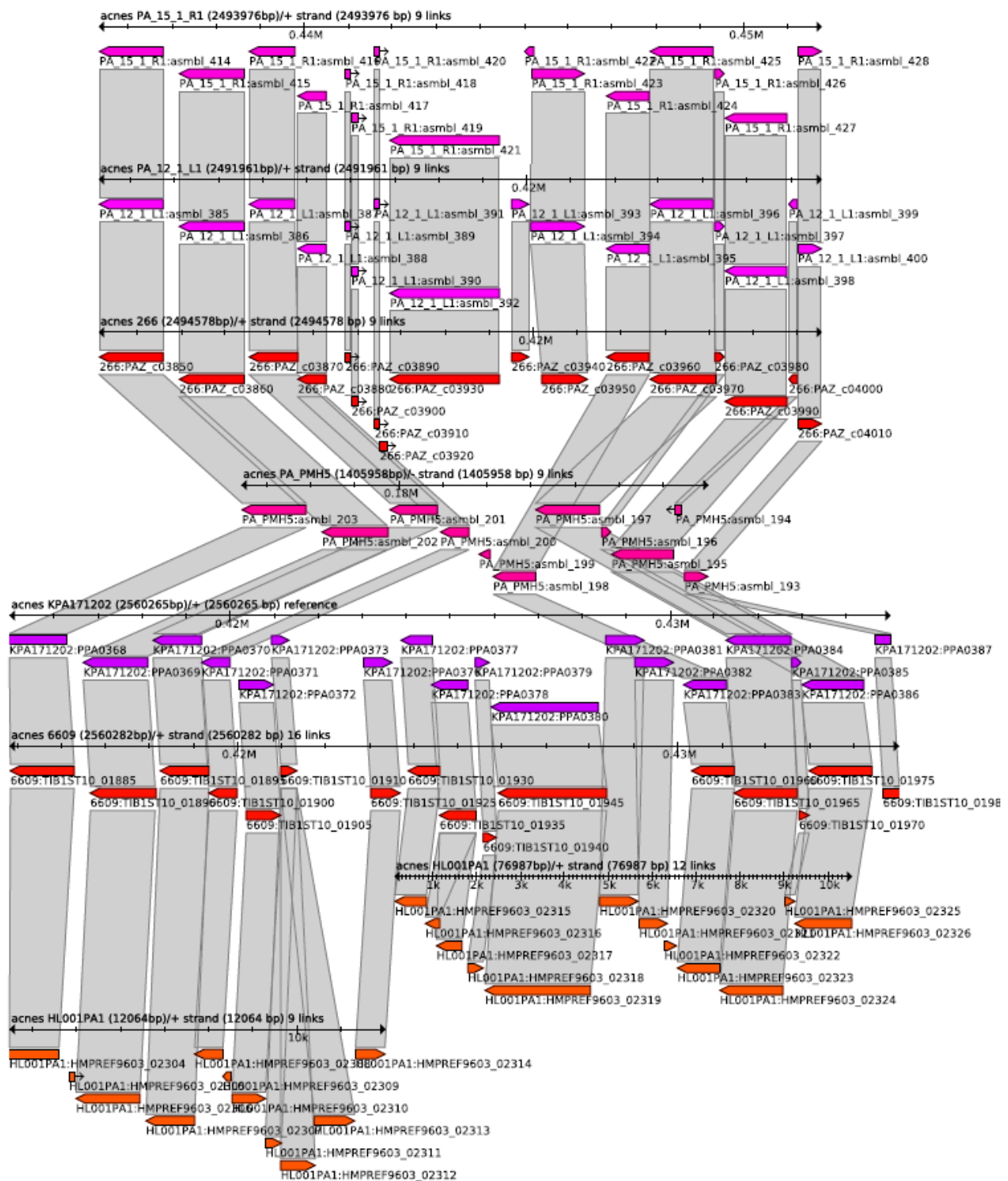


Figure S2. Extended genome context comparison of the region encoding *hyl* in *P. acnes*. The genomic region around *hyl* from strains 15.1.R1 (IA), 12.1.L1 (IA), 266 (IA), PMH5 (III), KPA171202 (IB), 6609 (IB), HL001PA1 (II) was compared using Sybil. The grey connecting blocks indicate amino acid sequences that share over 75% identity. Note that the genome of HL001PA1 is not closed.

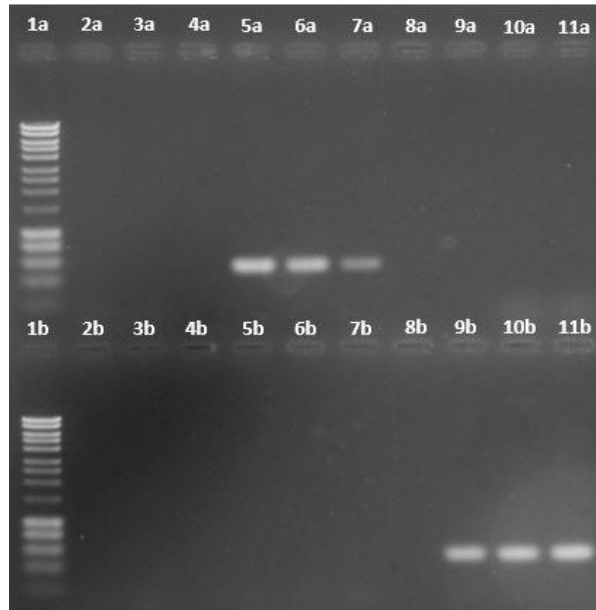
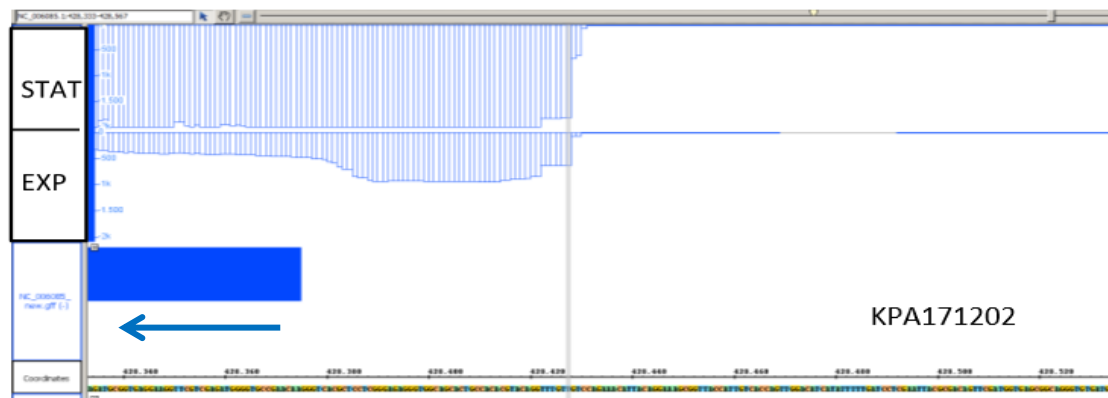


Figure S3. PCR assay to differentiate the two HYL versions of *P. acnes*. The detection of *hyl* in *P. acnes* strains was done by using HYL-IA- and HYL-IB/II-specific primers. PCR results with the primer pair for HYL-IA is shown in lanes 2a-11a, and with the primer pair for HYL-IB/II in lanes 2b-11b. Lanes: 1, DNA marker; 2, negative control (PCR water); Δhyl mutant; 4, empty; 5, 266 (IA); 6, 12.1.L1 (IA); 7, 15.1.R1 (IA); 8, PMH5 (III); 9, KPA171202 (IB); 10, P6 (IB); 11, CCUG33950 (II).

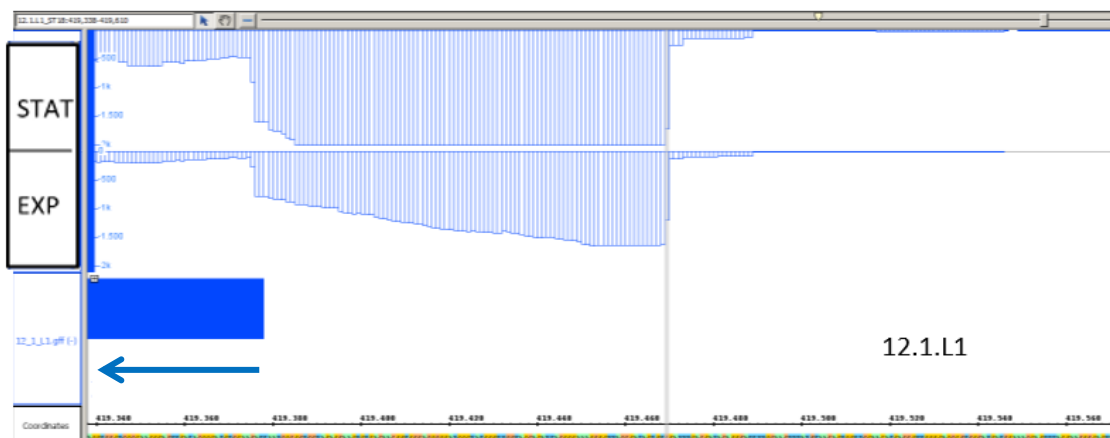
A



-35
-10
TSS

AATATGATGTCCAACTGGTGACAATGGTAACCGCTTTCCGTAATGTTTCTGGACA**A**ACAAA
 CCTGTACGTGTGGCAGTGCTGCCACCCTCTCCCGAGGAGCGTGACCCTTG

B



-35
-10
TSS

CCAACGGTCATCTTCGAACCACTGTTAGATAAAGTTGCAAATGCTGTATCGTCAAATC**G**GAACTAT
 CGGTAACCGCTTTCCCGTAATCTGCTAGGAACCCATACCCATCCGCGTGGCACCGTTGTCACACTTGC
 TCTAGGAGCGGATTAATG

Figure S4. Transcriptional start sites (TSS), putative -10 and -35 promoter regions and 5'-UTR of the *hly* gene in *P. acnes*. A: strain KPA171201 (IB); B: strain 12.1.L1 (IA).

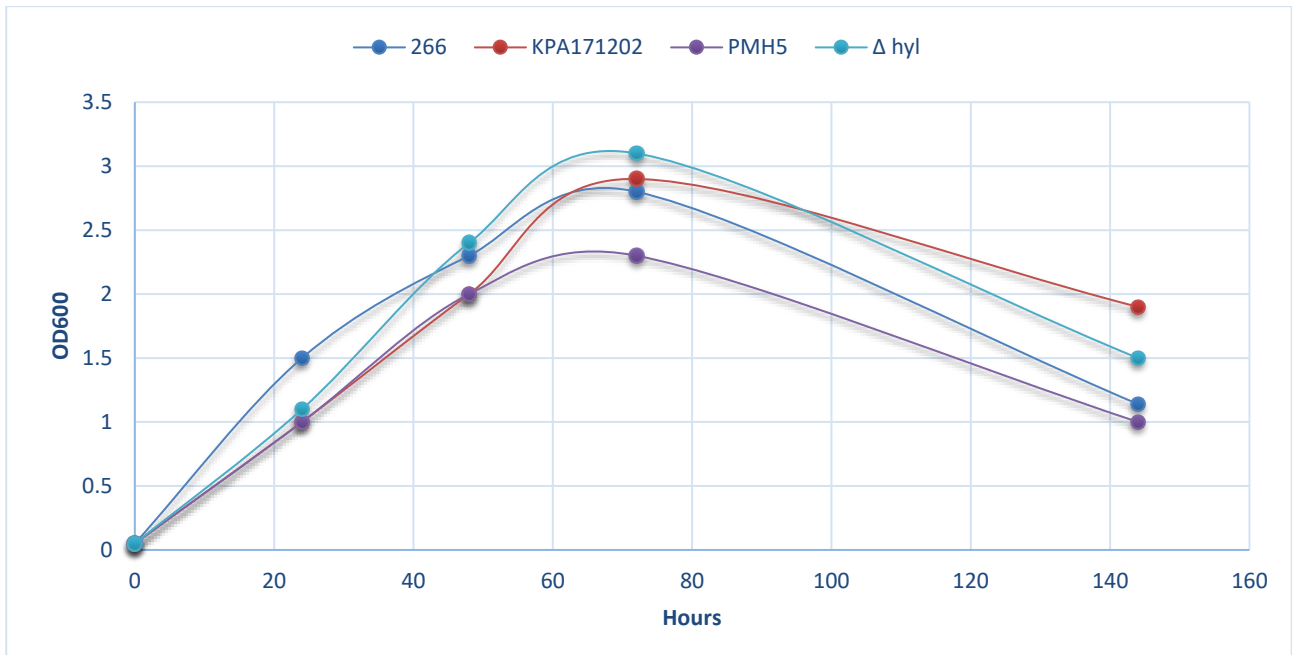


Figure S5. Growth curves for investigated *P. acnes* strains.

Strains were grown in BHI medium under anaerobic conditions at 37°C. OD was measured photometrically at 600 nm.

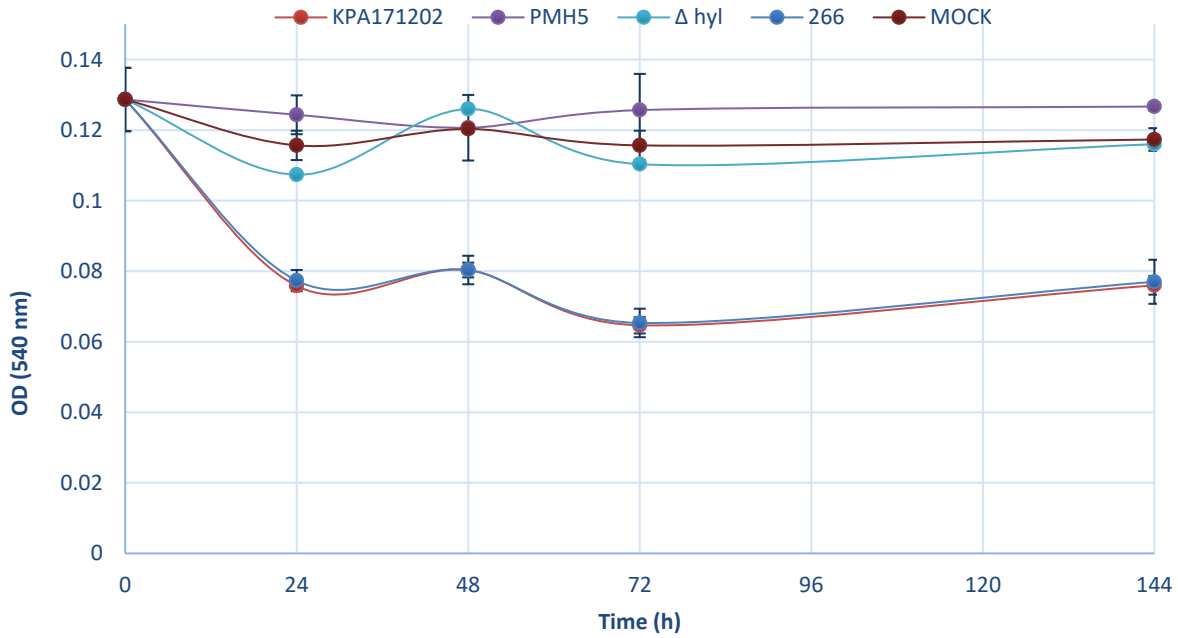


Figure S6. Turbidimetric assay to determine HA-degrading activity in *P. acnes*. Supernatants from strains 266, KPA171202, PMH5 and the Δhyl mutant were harvested from cultures grown to different time points (24 h, 48 h, 72 h, 144 h) and incubated with HA for 10 min. The turbidity of HA was determined after reaction with acidic albumin reagent at OD_{540 nm}. The experiment was repeated twice, and each sample was done in triplicates. Bars represent the mean SD.

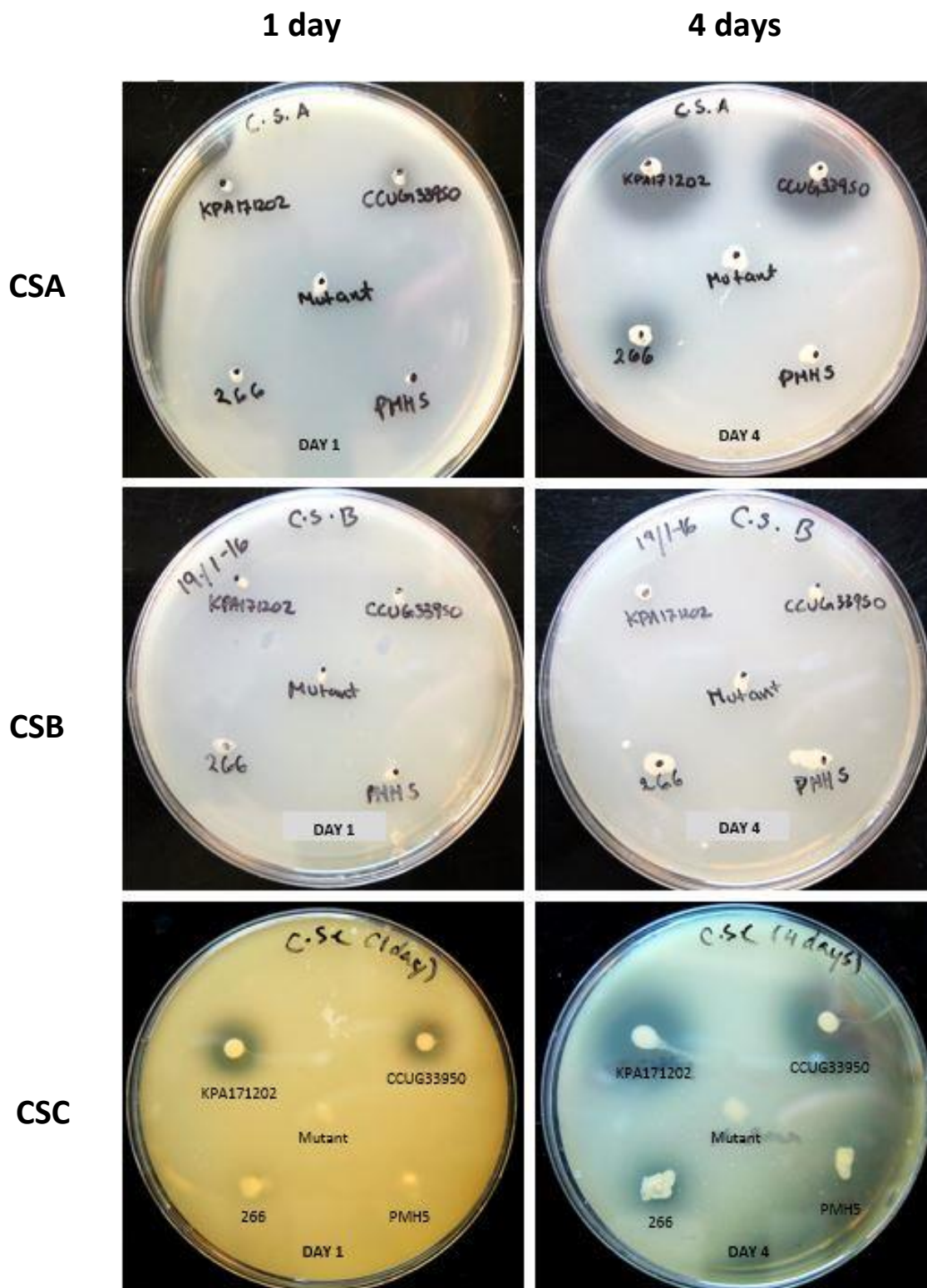


Figure S7. Chondroitin sulfate (CS) degradation by HYL activity of *P. acnes* strains after 1 and 4 days of incubation. Colonies of *P. acnes* strains KPA171202 (IB, upper left), CCUG33950 (II, upper right), 266 (IA, lower left), PMH5 (III, lower right) and the Δhyl mutant (center) were point-inoculated onto CS-containing plates and incubated for 1 and 4 days. Plates were flushed with 2N acetic acid for 15 min to detect CS-degrading activity. None of the investigated strains showed any CSB (dermatan sulfate)-degrading activity, but type IB/II strains were able to degrade CSA and CSC. The type IA strain showed only weak CSA and CSC degradation after 4 days incubation.

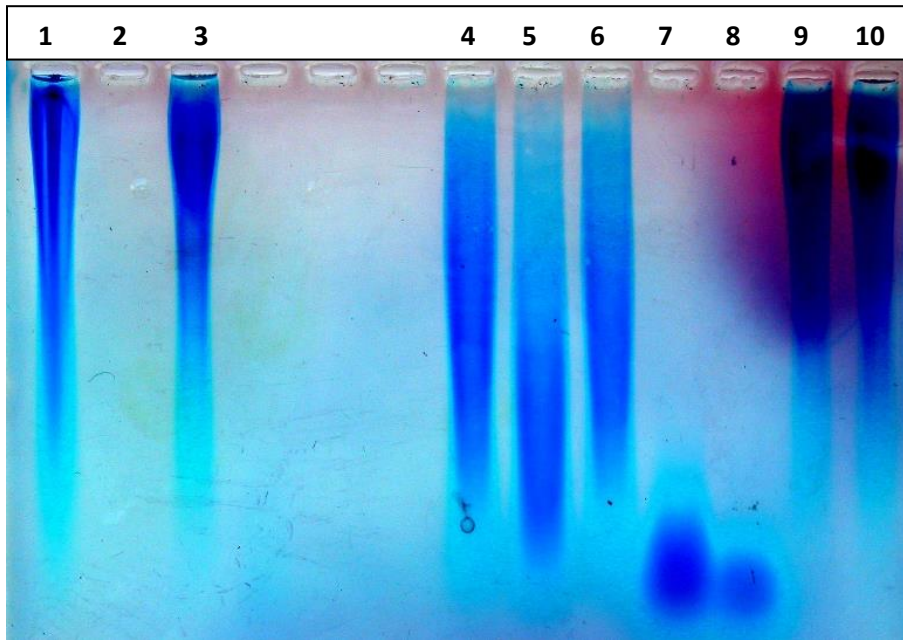


Figure S8. Hyaluronic acid fragments produced by the activity of HYLs of *P. acnes*.

HA was incubated with culture supernatants of *P. acnes* strains grown to the stationary phase for extended times (30 min instead of 10 min). Visualization of HA fragments on a stained 1% agarose gel. Extended incubation time did not resolve in complete degradation of HA by HYL-IA. Lanes: 1, HA input; 2, positive control (HYL from *S. pyogenes*); 3, culture medium (control); 4; strain 266 (IA); 5, strain 12.1.L1 (IA); 6, strain 15.1.R1 (IA); 7, strain KPA171202 (IB); 8, strain CCUG33950 (II); 9, strain PMH5 (III); 10, strain Δhly mutant.

Table S1. Information about the strains used in this study.

Strain	Origin/reference	Phylotype	MLST/SLST *	<i>hyl</i> gene
KPA171202	skin/culture contaminant [41]	IB	CC36/H2	+ (HYL-IB/II)
Δ<i>hyl</i> (KPA171202)	this study	IB	CC36/H2	-
P6	prostatic tissue [66]	IB	CC36/H2	+ (HYL-IB/II)
CCUG33950	cerebrospinal fluid (CCUG strain collection) [7]	II	CC53/K5	+ (HYL-IB/II)
12.1.L1	skin/acne lesion [7,21]	IA	CC18/A1	+ (HYL-IA)
15.1.R1	skin/acne lesion [67]	IA	CC3/C1	+ (HYL-IA)
266	pleuropulmonary infection [48]	IA	CC18/A1	+ (HYL-IA)
3.6.A1	skin/healthy [7,21,67]	IA	singleton/E3	+ (HYL-IA)
PMH5	skin/progressive macular hypomelanosis lesion [68]	III	singleton/L1	-

* MLST/SLST schemes: references [7,11]

Additional references:

66. Fassi Fehri, L.; Mak, T.N.; Laube, B.; Brinkmann, V.; Ogilvie, L.A.; Mollenkopf, H.; Lein, M.; Schmidt, T.; Meyer, T.F.; Brüggemann, H. Prevalence of *Propionibacterium acnes* in diseased prostates and its inflammatory and transforming activity on prostate epithelial cells. *Int. J. Med. Microbiol.* **2011**, *301*, 69-78.

67. Brüggemann, H.; Lomholt, H.B.; Tettelin, H.; Kilian, M. CRISPR/cas loci of type II *Propionibacterium acnes* confer immunity against acquisition of mobile elements present in type I *P. acnes*. *PLoS One* **2012**, *7*, e34171.

68. Petersen, R.; Lomholt, H.B.; Scholz, C.F.; Brüggemann, H. Draft genome sequences of two *Propionibacterium acnes* strains isolated from progressive macular hypomelanosis lesions of human skin. *Genome Announc.* **2015**, *3*, pii: e01250-15.

Table S2. Primers used in this study.

Purpose	Primer sequence 5'-3'	
Amplification of <i>hyl</i> of types IB/II	HYL_IB/II_for	CTGCCGACTTCGGTACAACC
	HYL_IB/II_rev	TTACGAGCCGATTCGACGAT
Amplification of <i>hyl</i> of type IA	HYL_IA_for	CGCCATCAAGGCACCAACAA
	HYL_IA_rev	GTGATGGTAATGCCGTCGACTA
Amplification of <i>hyl</i> upstream region	PPA0380_1	CTCCTGGGTCCGCTGAGAGA
	PPA0380_2	GCGGTACCGGGTCACGCTCCTCGGGAGA
Amplification of <i>hyl</i> downstream region	PPA0380_3	GCGGTACCTGTGCCGCTCACCTAACTA
	PPA0380_4	GCAGTGGGCTTCTCGGTGTA
Amplification of erythromycin resistance gene	ermE_for	GAGCGCACCGACCCGGTCTCGT
	ermE_rev	TGCTGCGCCAGCGTTGTGCG

Table S1: Identified HYL in the culture supernatant of *P. acnes* strains.

Highlighted are the identified peptides (in red). Note that HYL-IA was the best match for strains 266 and 12.1.L1 and HYL-IB/II for strain KPA171202

Strain	Protein Name	Mass kDa	Sequence	Score	Matches	Sequences	Coverage %	Unique Peptides
strain 266 (IA)	Hyaluronate lyase (HYL-IA)	89,998	MFDIPYQVPSRRTFLSLSAIAIAASPEMPDAFASPDPIWSALCEK WTDIITGR NAAKTADPRARAIIAKT DKR VATILTDLASSSSR TTVLLSANLQK EESFFITTA RAISSIACAWATPGSAYHAEPHVLSVICIDALKDFCRL RYHPSQDEYGNWWDWEDGASRAIGDVMCILHDALPTDVMAAAAAGIDHFVDPWPYQQPESVKPTAHP TQPVISTGANRMDLTRAVICRSIATGDESKLRHAVQGLPDSWR VAEGDGFR ADGGFIQHSHPYTGSGYD VLLSGLAMLLPLVAGTRFDITDSAQANLLSQVERGIVPVMYGGQILDCVGRSISR DEPAAMHGMSIARSM LLMANAIPAHR AELWR GTVHGWMTNRNTFDHLEPASLR DIDLFDTAANVRPIESSTPTYFASIDRLVHRT PNWLIAVSNCSNRISWYEGNSENEWASRTSQGMRYLMLPEDMGQYEDGFWATVDYSAPTGTVDSTPL KR AVGTAWAER TPDNEWSSGLASGEWSAAASQITSQDSTLKARR LWVGLK DALLELTTDSTDASKATTV VEHRKVKG TPPELLVDGITITSKTSFDNPHWAHLR GVGGYVFATDVLTAQLEKR KGSWIDVNPARTVKGF NEAIER NYASLHVTHHNRPVAVAVLPTASRSQTMALARPVDNLFIVLSNDR MVQAVR STGCLLTKAPT VTTYAFWKPATCAGMTADAPAIITQQAQGSR VEVIMSEPTQK RPSLTVAIIEGVWTVENSSDRISVRSKDTT TLR INTADLGGQSIRVTLSPALPKTPKPSLR ASSYPLGLPHTSS	3397	85	19	23,8	13
	Hyaluronate lyase (HYL-IB/II)	88,13	MFGTPSRRTFLTASALSAMALAASPTVTDIAAAPGPDWSALCERWIDIITGRRAARTSDPRARAIIAKTDRK VAEILTDLVSGSSRQTVLISADLRK EQSPFITK TARAIESMACAWATPGSSYHKDPEILSACIEGLRDFCRLRYN PSQDEYGNWWDWEDGASRAVADVMCILHDVLPPEVMSAAAAGIDHFIPDPWFQQPASVKPTANPVQP VVSTGANRMDLTRAVMCRSIATGDEKRLRHAVDGLPDAWR VTTEGDGFR ADGGFIQHSHPYTGSGYDVL FSGLAMLPVLSGMR FDIVESAR KAFHDQVERGFIPVMYNGQILDDVGRSISRINESAAMHGISIARAML MMADALPTHRAEQWRGIVHGWMARNTFDHLEPSTLVDISLFDAAAKARVPESSTPSYFASMDRLVHRT ADWLITVSNCSDRIAWYEGNGENEWASRTSQGMRYLPPGDMGQYEDGYWATVDYSAPTGTVDSTPL KRAVGASWAAKTPTNEWSSGLASGSWSAAASHITSQDSALKARR LWVGLK DAMVELTTDVTDDASRAITV VEHRKVASSSTK LLVDGNRVSSATSFNPR WAHLDGVGGYVFATDLDLADVATRKGTWIDVNPVRKVKG ADEVIERAYASLHVTHHNRPVAVAVLPTASRSHTMALATRPGEVPEFTVLR NDATVQAVRSAGALLTK DPTV VTTLAFWKPATCGGVAVNRPALVQTRESANQMEVVIVEPTQKRGSLVTIEGSWKVKTADSHVDVSCENA AGTLHVDTAGLGGQSVRVTLARQVTQTPSGGGRHDRA	374	13	8	8,1	7

strain 12.1.L1 (IA)	Hyaluronate lyase (HYL-IA)	89,998	MFDIPYQVPSRRFTLSLSALSALSAIAIASPEMPDAFASPDPIWSALCEK WTDIITGR NAAKTADPRARAIIAKT DKR VATILTDLASSSR TTVLLSANLQK ESSFITTAR AISSIACAWATPGSAYHAEPHVLVSCIDALKDFCRL RYHPSQDEYGNWWDWEDGASRAIGDVMCILHDALPTDVMMAAAAAGIDHFVDPWPYQQPESVKPTAHP TQPVISTGANRMDLTRAVICR SIATGDESKLR HAVQGLPDSWR TVAEGDGR ADGGFIQHSHPYTGSGY DVLLSGLAMLLPLVAGTRFDITDSAQANLLSQVERGIVPVMYGGQILDVCRGRSISR IDEPAAMHGMSIARS MLLMANAIPADR AELWR GTVHGWMTNRTFDHLSEPA SLRDIDLFTAANVRPIESSTPTYFASIDRLVHR TPNWLIAVSNCSNRISWYEGNSENEWASRTSQGMRYLMLPEDMGQYEDGFWATVDYSAPTGTVDSTP LKR AVGTAWAER TPDNEWSGGLASGEWSAAAQITSQDSTLKARR LWVGLK DALLELTDDVSTDASKATT VVEHRKV TPPELLVDGITITSKTSFDNPHWAHLR GVGGYVFATDVLTAQLEKR KGSWIDVNPARTVK GFNEAIER NYASLHVTHHNRPVAVLPTASRSQTMALAQRPVDNLFIVLSNDR MVQAVR STGCLLTKAP TVVTYAFWKPATCAGMTADAPAIQQAQGSR VEVIMSEPTQKR PSLTVAIEGVWTVENSDDRISVSRSD KTTTLR INTADLGGQSIRVTLSPALPKPTKPSLRASSYPLGLPHTSS	3446	86	21	26,8	15
	Hyaluronate lyase (HYL-IB/II)	88,13	MFGTPSRRTFLTASALSAMALAASPTVTDAIAAPGPDWSALCERWIDIITGRRAARTSDPRARAIIAKTDRK VAEILTDLVSGSSRQTVLISADLRK EQSPFITK TARAIESMACAWATPGSSYHKDPEILSACIEGLRDFCRLRYN PSQDEYGNWWDWEDGASRAVADVMCILHDVLPPEVMSAAAAGIDHFIPDPWFQQPASVKPTANPVQP VVSTGANRMDLTRAVMCRSIATGDEKRLRHAVDGLPDWR VTEGDGR ADGGFIQHSHPYTGSGYDVL FSGLAMLPFLVSGMR FDIVESARKAFHDQVER GFIPVMYNGQILDVDRGRSISR INESAAMHGISIARAML MMADALPTHR AEQWRGIVHGWMMARNTFDHLSEPTLVDISLFDAAA ARPVESSTPSYFASMDR LVHR TADWLTIVSNCSDRIAWYEGNGENEWASRTSQGMRYLLPGDMGQYEDGYWATVDYSAPTGTVDSTP LKR AVGASWAAK TPTNEWSGGLASGSWSAAASHITSQDSALKARR LWVGLK DAMVELTDDVTTDASR AI TVVEHRK VASSSTK LLVDGNRVSSATSFNPR WAHLDGVGGYVFATDLDLADVATRKGTWIDVNP SRKV KGADEVIER AYASLHVTHHDPVAVALLPTASRSHTMALATRPGEVPTVLR NDATVQAVRSAGALLTKD PTVVTTLAFWKPATCGGVAVNRPALVQTR ESANQMEVVIVEPTQKR GSLTVTIEGSWKVKTADSHVDVSC ENAAGTLHVDTAGLGGQSVRVTLAR QVTQTPSGGGR HDRA	2343	70	19	21,3	18
strain KPA (IB)	Hyaluronate lyase (HYL-IB/II)	88,13	MFGTPSRRTFLTASALSAMALAASPTVTDAIAAPGPDWSALCER WIDIITGR RAARTSDPRARAIIAKTDRK VAEILTDLVSGSSRQTVLISADLRKEQSPFITK TARAIESMACAWATPGSSYHKDPEILSACIEGLRDFCRLRYN PSQDEYGNWWDWEDGASRAVADVMCILHDVLPPEVMSAAAAGIDHFIPDPWFQQPASVKPTANPVQP VVSTGANRMDLTRAVMCRSIATGDEKRLRH AVDGLPDWRVTEGDGR ADGGFIQHSHPYTGSGYDVL LFSGLAMLPFLVSGMR FDIVESARKAFHDQVER GFIPVMYNGQILDVDRGRSISR INESAAMHGISIARAM LMMADALPTHR AEQWR GIVHGWMMAR NTFDHLSEPTLVDISLFDAAA ARPVESSTPSYFASMDR LVH RTADWLTIVSNCSDRIAWYEGNGENEWASRTSQGMRYLLPGDMGQYEDGYWATVDYSAPTGTVDST PLKR AVGASWAAK TPTNEWSGGLASGSWSAAASHITSQDSALKARR LWVGLK DAMVELTDDVTTDASR AI TVVEHRK VASSSTK LLVDGNRVSSATSFNPR WAHLDGVGGYVFATDLDLADVATRKGTWIDVNP SRKV KGADEVIER AYASLHVTHHDPVAVALLPTASR SHTMALATRPGEVPTVLRNDATVQAVRSAGALLTK DPTVVTTLAFWKPATCGGVAVNRPALVQTR ESANQMEVVIVEPTQKR GSLTVTIEGSWKVKTADSHVDVSC ENAAGTLHVDTAGLGGQSVRVTLAR QVTQTPSGGGR HDRA	6017	171	34	32,1	34