

Supplementary Figure 1. Minimum inhibitory concentration of actifensin peptide against Gram positive pathogens determined by well diffusion assay.

Phylum	Species	Per. Identity	Description	
Actinobacteria	A. ruminicola		AfnA	GFGCNLITSNPYQCSNHCKSV-GYRGGYCKLRTVCTCY
Ascomycota	Helicocarpus griseus	52.26		MRFSTVFAVVSALSMTALALPSPVTEDVNLAEREAAPEPMPEELVAAFTKLGERSLEGEEDNVIAKR <mark>GFGC</mark> TIWGG <mark>N</mark> DKP <mark>C</mark> HR <mark>HC</mark> KSIK <mark>G</mark> YK <mark>GGYCK</mark> VGGV <mark>C</mark> KC <mark>Y</mark>
Ascomycota	Blastomyces percursus	57.89	hypothetical protein	MRLSAVFAIISALSMTALAIPAPAPEDLDIAEATADLATRDAPVEAIPDDFVGDLAGLNDDDDDDDDDDEDERPAHALQKR <mark>GWGC</mark> NIFGG <mark>N</mark> DYR <mark>C</mark> HR <mark>HC</mark> KSIR <mark>G</mark> YK <mark>GGYCK</mark> LGGI <mark>C</mark> KC <mark>Y</mark>
Ascomycota	Blastomyces silverae	57.89	hypothetical protein	MRFSAVFAIISALSMTALAIPAPAPEDLDIAEATADLAARDARMGAIPDDFAGDLAGLDDDDDDDDDDDDDENPARTLQKR <mark>GWGC</mark> NIFGG <mark>N</mark> DYR <mark>C</mark> HR <mark>HC</mark> KSIK <mark>G</mark> YK <mark>GGYCK</mark> LGGI <mark>C</mark> KCY
Ascomycota	Blastomyces gilchristii	57.89		MRFSAVFAIISALSMTALAIPAPAPEDLDIAEATADLAARDAPVEAIPDDFAGDVSGLDDEDDENSAGALQKR <mark>GWGC</mark> NIFGG <mark>N</mark> DYR <mark>C</mark> HR <mark>HC</mark> KSIS <mark>G</mark> YK <mark>GGYCK</mark> LGGI <mark>C</mark> KC <mark>Y</mark>
Ascomycota	Blastomyces dermatitidis	57.89	hypothetical protein	MRFSAVFAIISALSMTALAIPVPAPEDLDIAEATADLAARDAPVEAIPDDFAGDVSGLDDDDDDDDDDDDEDENSAGALQKR <mark>GWGCNIFGGN</mark> DYR <mark>CHRHC</mark> KSIS <mark>G</mark> YK <mark>GGYCK</mark> LGGI <mark>CKCY</mark>
Ascomycota	Blastomyces dermatitidis	57.89	hypothetical protein	MRFSAVFAIISALSMTALAIPAPAPEDLDIAEATADLAARDAPVEAIPDDFAGDVSGLDDDDDDDDDDEDENSAGALQKR <mark>GWGC</mark> NIFGG <mark>N</mark> DYR <mark>C</mark> HR <mark>HC</mark> KSIS <mark>G</mark> YK <mark>GGYCK</mark> LGGI <mark>C</mark> KCY
Ascomycota	Emmonsia crescens	55.26	hypothetical protein	MRFSAIFAIISALSMTALAMPAPAPEDFGIAEAAADLAARNAPADAIPDDFAGDLAGMDDDDDDDDY-ENSVGSLQKR <mark>GWGCTIFGGN</mark> DSR <mark>CHRHC</mark> KSIR <mark>GYRGGYCK</mark> LGGI <mark>CKCY</mark>
Ascomycota	Emmonsia sp.	55.26	hypothetical protein	MRASAIFAIISALSMTTLAMPAAAPEDFDIAAATADLAARGAPAEAIPDDFAGDLAGLDDDDDDDDDDDD-SAGVLQKR <mark>GWGC</mark> TIFGG <mark>N</mark> DSR <mark>C</mark> HR <mark>HC</mark> KSIR <mark>G</mark> YR <mark>GGYCK</mark> LGGI <mark>C</mark> KCY
Ascomycota	Emergomyces pasteurianus	55.26	hypothetical protein	MRVSAILAIISALSMTALAIPAPAPEDFDIAEATADLAARDAPAEAVPDDFAGDLAGLDDDDDDDDY-ENSAGVLQKR <mark>GWGC</mark> TIFGG <mark>N</mark> DSR <mark>CHKHC</mark> KSIR <mark>GYRGGYCK</mark> LGGI <mark>CKCY</mark>
Mollusca	Ruditapes philippinarum	60.53	defensin	MKMMIVFTVLFLAAMILPDVDA

Supplementary Figure 2. Eukaryotic sequences with homology to afnA identified using BLASTp. Residues with 100% identity are highlighted.

Genus_species_strain_peptide		1	2	3	4	5	6	7	8	9	10	11 1	2 1	3 14	15	16	17	18	19	20	21 2	22 23	3 24	25	26	27 2	8 29	30	31	32 3	34	35	36	37 3	8 39	40	41	42	43	44	45 4	6 47	48	49
Corynebacterium_sp_HMSC06D04_afnA	1	100	33	30	32	32	32	32	32	31	42	42 4	2 4	1 41	34	40	40	34	34	34	35 3	34 3	35	39	42	45 4	5 45	41	41	33 4	7 44	53	45	45 4	5 40	40	48	42	48	48	48 4	8 39	44	42
Actinomyces_oris_MMRCO6_1_C75_afnA2	2	33	100	94	96	96	87	88	88	87	44	44 4	4 5	1 51	35	43	43	43	43	43	43 4	43 43	2 42	38	46	40 3	9 39	36	38	46 4	3 45	42	42	42 4	4 39	39	40	37	39	39	41 4	1 34	38	44
Actinomyces oris CCUG 34286 afnA7	3	30	94	100	99	99	87	85	85	86	47	47 4	7 5	3 53	36	43	43	45	45	45	45 4	45 43	3 43	38	47	42 4	2 42	38	42	48 4	16 46	44	45	45 4	7 41	41	43	40	42	42	14 4	4 34	41	47
Actinomyces_oris_S64C_afnA5	4	32	96	99	100	100	88	84	84	84	47	47 4	7 5	3 53	36	43	43	45	45	45	45 4	45 43	3 43	38	47	42 4	2 42	38	42	48 4	16 46	44	45	45 4	7 41	41	43	40	42	42	14 4	4 34	41	47
Actinomyces_oris_S24V_afnA5	5	32	96	99	100	100	88	84	84	84	47	47 4	7 5	3 53	36	43	43	45	45	45	45 4	45 43	3 43	38	47	42 4	2 42	38	42	48 4	16 46	44	45	45 4	7 41	41	43	40	42	42	14 4	4 34	41	47
Actinomyces_sp_oral_taxon_171_F0337_afnA4	6	32	87	87	88	88	100	88	88	86	48	48 4	8 5	3 53	35	45	45	43	43	43	43 4	43 42	42	38	46	42 3	9 39	38	40	45 4	15 43	42	45	45 4	5 41	41	42	38	40	40	43 4	3 35	41	47
Actinomyces_oris_MMRCO6_1_C50_afnA4	7	32	88	85	84	84	88	100	100	100	47	47 4	7 5	3 53	35	43	43	43	43	43	43 4	43 43	2 42	38	46	42 3	6 36	38	38	45 4	15 43	42	44	44 4	4 39	39	40	38	40	40	13 4	3 34	40	46
Actinomyces_oris_MMRCO6_1_C50_afnA3	8	32	88	85	84	84	88	100	100	100	47	47 4	7 5	3 53	35	43	43	43	43	43	43 4	13 42	2 42	38	46	42 3	6 36	38	38	45 4	15 43	42	44	44 4	4 39	39	40	38	40	40	43 4	3 34	40	46
Actinomyces_sp_HMSC075C01_afnA3	9	31	87	86	84	84	86	100	100	100	46	46 4	6 5	2 52	34	40	40	42	42	42	42 4	12 4	2 42	37	44	42 3	5 35	38	38	45 4	14 44	43	44	44 4	4 39	39	40	38	40	40	43 4	3 33	40	44
Actinomyces_oris_MMRCO6_1_C50_afnA1	10	42	44	47	47	47	48	47	47	46 1	00 1	00 9	8 6	4 64	45	49	49	49	49	49	51 4	19 5	2 52	53	58	51 4	8 48	50	52	58 5	57 57	58	56	56 5	2 56	56	55	56	63	63 (61 6	1 42	48	59
Actinomyces_sp_HMSC075C01_afnA1	11	42	44	47	47	47	48	47	47	46 1	00 1	00 9	8 6	4 64	45	49	49	49	49	49	51 4	19 5	2 52	53	58	51 4	8 48	50	52	58 5	57 57	58	56	56 5	2 56	5 56	55	56	63	63 (61 6	1 42	48	59
Actinomyces_sp_oral_taxon_171_F0337_afnA1	12	42	44	47	47	47	48	47	47	46	98	98 10	0 6	4 64	45	49	49	49	49	49	51 4	19 5	2 52	53	58	51 4	8 48	50	52	58 5	57 57	58	56	56 5	2 56	5 56	55	56	63	63 (51 6	1 42	48	58
Actinomyces_sp_Chiba101_afnA1	13	41	51	53	53	53	53	53	53	52	64	64 6	4 10	0 100	41	45	45	42	42	42	40 4	10 40	40	42	54	40 4	0 40	42	48	46 5	4 51	. 56	52	52 5	3 45	45	51	47	54	54 !	51 5	1 38	45	54
Actinomyces_denticolens_PA_afnA1	14	41	51					53					4 10																					52 5										
Actinomyces_sp_2119_afnA1	15							35					_		100															49 5				47 4			52			49			47	
Actinomyces_sp_Chiba101_afnA2	16	40						43				49 4					100									61 4				53 5				55 5			53			48 !				49
Actinomyces_denticolens_PA_afnA2	17			43	43	43	45	43	43	40	49	49 4	9 4	5 45			100													53 5				55 5			53			48 !				
Actinomyces oris MMRCO6_1_C50_afnA2	18											49 4				_					94 9													52 5									49	
Actinomyces_sp_HMSC075C01_afnA2	19							43				49 4									94 9													52 5			55			45				
	20											49 4									96 9													52 5					45					
Actinomyces_oris_CCUG_34286_afnA5	21	35	43	45	45	45	43	43	43	42	51	51 5			52	63	63	94	94	96 1	.00 9	96 90	96											54 5										
Actinomyces_oris_CCUG_34286_afnA2	22			45	45	45	43	43	43	42	49	49 4									96 10					55 4								52 4			53			45				
Actinomyces_oris_S64C_afnA2	23	35	42	43	43	43	42	42	42	42	52	52 5	2 4								96 9				60	57 4	9 49	62	58	56 5	3 56	59	54	54 4	9 49	49	55	52	47	47 !	52 5	2 46	49	60
Actinomyces_oris_S24-V_afnA2	24		42	43	43	43		42						0 40							96 9						9 49			56 5							55	52		47 !			49	
Actinomyces_sp_2119_afnA2	25																									63 4	8 48							52 4	7 51	51						1 45		
Actinomyces_sp_2119_afnA3	26				47																61 5				100									52 5					51					
Actinomyces_sp_2129_afnA		45			42			42																										55 5									5 52	
Actinomyces_sp_oral_taxon_849_str_F0330_afn		45						36													51 4					66 10								52 4									51	
Actinomyces_johnsonii_F0542_afnA	29	45						36) 45						51 4					66 10								52 4					47				51	
Actinomyces_naeslundii_S44D_afnA	30			38				38											57				2 62			52 4		100						48 5										
Actinomyces sp_oral_taxon_414_F0588_afnA	31	41	38	42	42	42	40	38	38	38	52	52 5	2 4	8 48	3 47	53	53	56	56	56	58 5	56 58	3 58	48	52	53 5	5 55	64	100	52 5	52 55	59	58	58 6	1 45	45	61	54	52	52 !	55 5	5 48	54	60
Actinomyces_sp_oral_taxon_171_F0337_afnA3	32	33	46	48	48	48																						_						63 5										
Actinomyces_oris_CCUG_34286_afnA3	33																									58 5				68 10									76				5 51	
Actinomyces_oris_MMRCO6_1_C75_afnA1	34				46	46	43																			61 5				74 7									67					
Actinomyces_sp_HMSC075C01_afnA4_		53																												61 8									67				60	
Actinomyces_oris_S64C_afnA3	36																													63 8									70				53	
Actinomyces_oris_S24V_afnA3	37	45		45		45																								63 8									70					
Actinomyces ruminicola DPC7226 afnA	38	45		47		47																				54 4								57 10					58				64	
Actinomyces_oris_S64C_afnA1	39			41	41	41	41	39																		51 4				60 6					6 100				72				55	
Actinomyces_oris_S24V_afnA1	40	40						39							50											51 4			45						6 100				72				55	
Actinomyces_sp_CtC_72_afnA	41	48																																62 7			$\overline{}$							
Actinomyces_oris_CCUG_34286_afnA6	42																																	72 5				_						
Actinomyces_oris_S64C_afnA4																																		70 5									46	
Actinomyces_oris_S24V_afnA4	44		39									63 G		4 54																				70 5									46	
Actinomyces_oris_CCUG_34286_afnA4	45							0					-																					72 6									50	
Actinomyces_oris_CCOG_34286_afnA1																																		72 6									50	
Actinomyces_ons_ccod_s4266_amA1 Actinomyces_naeslundii_Pn6N_afnA																																		48 4										
Actinomyces_naesiundii_Pn6N_arnA Actinomyces_succiniciruminis_AM4_afnA2																																		53 6										
																																		53 6										
Actinomyces_succiniciruminis_AM4_afnA1	49	42	44						80			59 5	0 5	+ 54	> 53	49	49	28	58	28	01 5	00 00	60	54	01	55 5	2 52	61	60	48	02 51	. 56	52	52 6	3 45	, 49	69	49	21	31	5 5	5 51	65	100

Supplementary Figure 3. Percent-identity matrix of actifensin

(Actifensin_ruminicola_DPC7226_afnA) mature peptide amino acid sequence and homologous afnA sequences.

Supplementary Table 1. Actinomyces genomes used in in silico screen.

Supplmentary Table 2. afnA mature sequences and source genomes.

Supplementary Table 3. Growth conditions of indicator species used in this study.

References

1. Dash TS, Shafee T, Harvey PJ, Zhang C, Peigneur S, Deuis JR, Vetter I, Tytgat J, Anderson MA, Craik DJ. 2019. A centipede toxin family defines an ancient class of $CS\alpha\beta$ defensins. Structure 27:315-326. e7.