



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

Phylum	Species	Per. Identity	Description	
Actinobacteria	<i>A. ruminicola</i>	-	AfnA	-----GFGCNLITSNPKCSNHCKSV-GRGGYCKLRTVCTCY-----
Ascomycota	<i>Helicocarpus griseus</i>	52.26	hypothetical protein	MRFSTVFAVVSALSMTALALPSPVTEDVNLAE-----REAAPEPMPEELVAAFTKLGERSLEGEEDN----VI AKRFGGCTIWGGNDKPCRHRCKSIKGYKGGYCKVGGVCKCY-----
Ascomycota	<i>Blastomyces percursus</i>	57.89	hypothetical protein	MRLSAVFFAIISALSMTALAIPAPAPEDLDIAEATADLATRDAPVEAIPDDFVGDLAGLNDDDDDDDEDERPAHALQKRWGCGNI FGGNDYRCHRCKSIKGYKGGYCKLGGI CKCY-----
Ascomycota	<i>Blastomyces silverae</i>	57.89	hypothetical protein	MRFSAVFAIISALSMTALAIPAPAPEDLDIAEATADLAARDARMGAI PDDFAGDLAGLDDDDDDDEDENPARTLQKRWGCGNI FGGNDYRCHRCKSIKGYKGGYCKLGGI CKCY-----
Ascomycota	<i>Blastomyces gilchristii</i>	57.89	conserved hypothetical protein	MRFSAVFAIISALSMTALAIPAPAPEDLDIAEATADLAARDAPVEAIPDDFAGDVSGLDDEDD----ENSAGALQKRWGCGNI FGGNDYRCHRCKSIKGYKGGYCKLGGI CKCY-----
Ascomycota	<i>Blastomyces dermatitidis</i>	57.89	hypothetical protein	MRFSAVFAIISALSMTALAIPVPAPEDLDIAEATADLAARDAPVEAIPDDFAGDVSGLDDEDDDDDEDENSAGALQKRWGCGNI FGGNDYRCHRCKSIKGYKGGYCKLGGI CKCY-----
Ascomycota	<i>Blastomyces dermatitidis</i>	57.89	hypothetical protein	MRFSAVFAIISALSMTALAIPAPAPEDLDIAEATADLAARDAPVEAIPDDFAGDVSGLDDEDDDDDEDENSAGALQKRWGCGNI FGGNDYRCHRCKSIKGYKGGYCKLGGI CKCY-----
Ascomycota	<i>Emmonsia crescens</i>	55.26	hypothetical protein	MRFSAFIAIISALSMTALAMPAPAPEDFDIAEAAADLAARNAPADAIPDDFAGDLAGMDDDDDDDDY-ENSVGSLQKRWGCGTI FGGNDSRCHRCKSIKGYRGGYCKLGGI CKCY-----
Ascomycota	<i>Emmonsia sp.</i>	55.26	hypothetical protein	MRSAIFAIISALSMTTLAMPAAAPEDFDIAAATADLAARGAPAEAIPDDFAGDLAGLDDDDDDDDEND-SAGVLQKRWGCGTI FGGNDSRCHRCKSIKGYRGGYCKLGGI CKCY-----
Ascomycota	<i>Emergomycetes pasteurianus</i>	55.26	hypothetical protein	MRVSAI LAIISALSMTALAIPAPAPEDFDIAEATADLAARDAPAEAVPDDFAGDLAGLDDDDDDDDY-ENSAGVLQKRWGCGTI FGGNDSRCHRCKSIKGYRGGYCKLGGI CKCY-----
Mollusca	<i>Ruditapes philippinarum</i>	60.53	defensin	MKMMIVFTV---LFLAAMILP-----DVDA-----GFGCP-----NDYSCSNHCRDSIGCRGGYCKYHVICTCYGCKKRRSIQE

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

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.

Supplimentary 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.