Supplementary material:

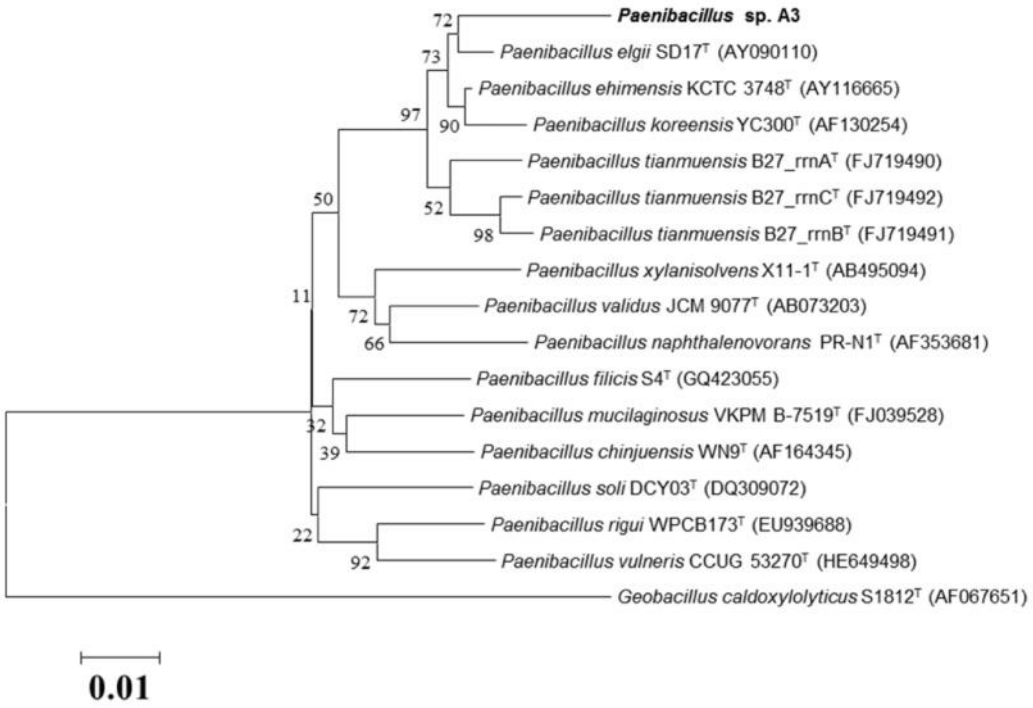
Characterization of antimicrobial peptide, Penisin, a Class Ia Noval Lantibiotic from a *Paenibacillus* sp. Strain A3

Piyush Baindara[1], Vasvi Chaudhray[1], Garima Mittal[1], Luciano M. Liao[2], Carolina O. Matos[2], Neeraj Khathri[1], Octavio L. Franco[3,4], Prabhu B. Patil[1] and Suresh Korpole[1‡]

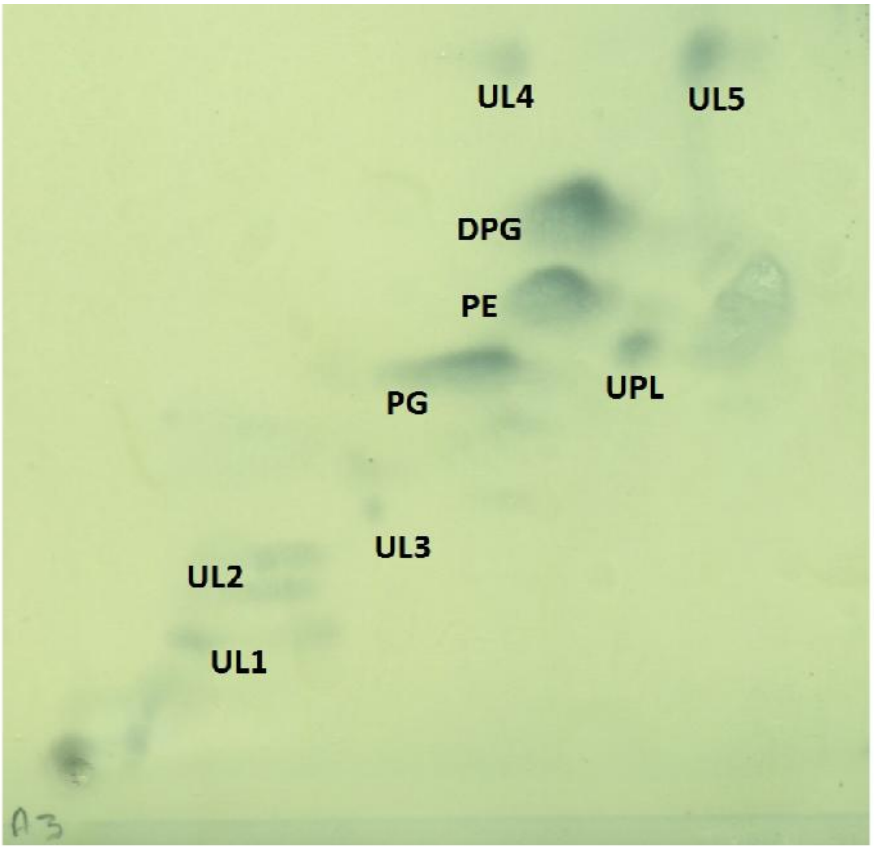
1. CSIR-Institute of Microbial Technology, Chandigarh, India. 2. Institute of Chemistry, Federal University of Goiás, Goiânia, GO, Brazil 3. Centro de Analises Proteomicas e Bioquimicas, Pós-graduacão em Ciências Genomicas e Biotecnologia, Brasília, Brazil 4. S-Inova, Programa de Pós-Graduacão em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, MS, Brazil

Supplementary Table 1: Identity of putative homologous genes involved in biosynthesis of penisin (9.8 Kb region).

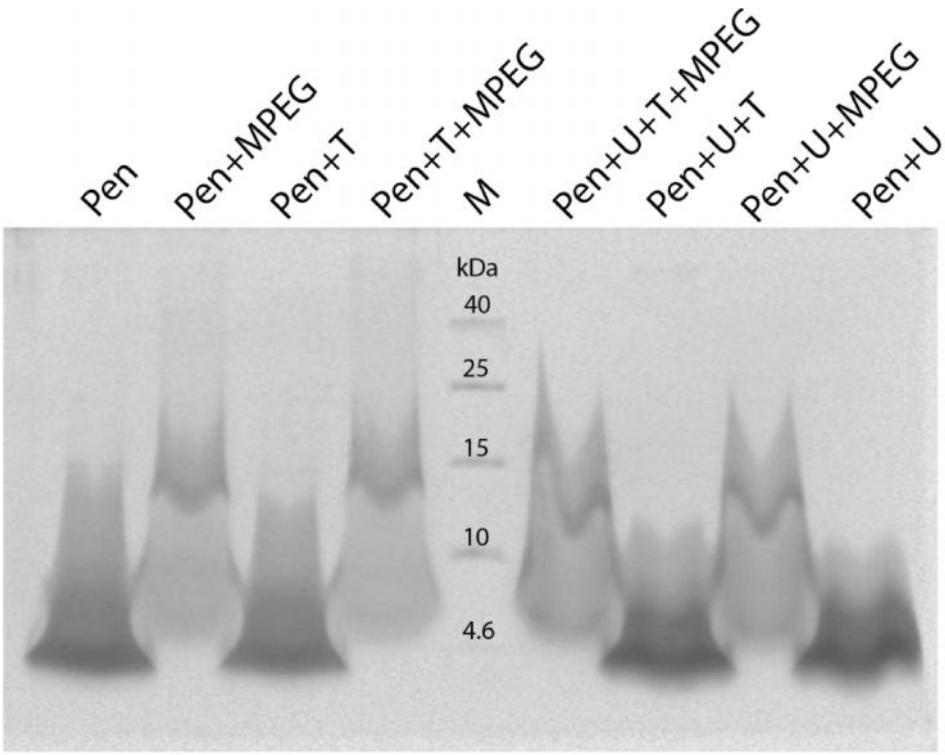
ORF	Size of putative protein (aa)	Putative function	Sequence homolog (Genbank accession)	Identities (%)
penR	269	Transcriptional regulator	transcriptional regulator [Paenibacillus elgii], WP_010497942.1	92%
penD	561	Dehydratase	dihydroxy-acid dehydratase [Paenibacillus ehimensis], WP_025851100.1	99%
penC	454	Cyclase	lanthionine synthetase C-like protein [Paenibacillus elgii], WP_010497958.1	92%
penT	625	ABC transporter (ATP-binding protein)	ABC transporter ATP-binding protein [Paenibacillus sp. MSt1], WP_036689879.1	96%
penB	1040	Biosynthesis	ElgB [Paenibacillus elgii B69], AFI99858.1	94%
penA2	63	penisin	elgicins [Paenibacillus elgii B69], AFI99859.1	74%
penA1	64	penisin	elgicins [Paenibacillus elgii B69], AFI99859.1	94%



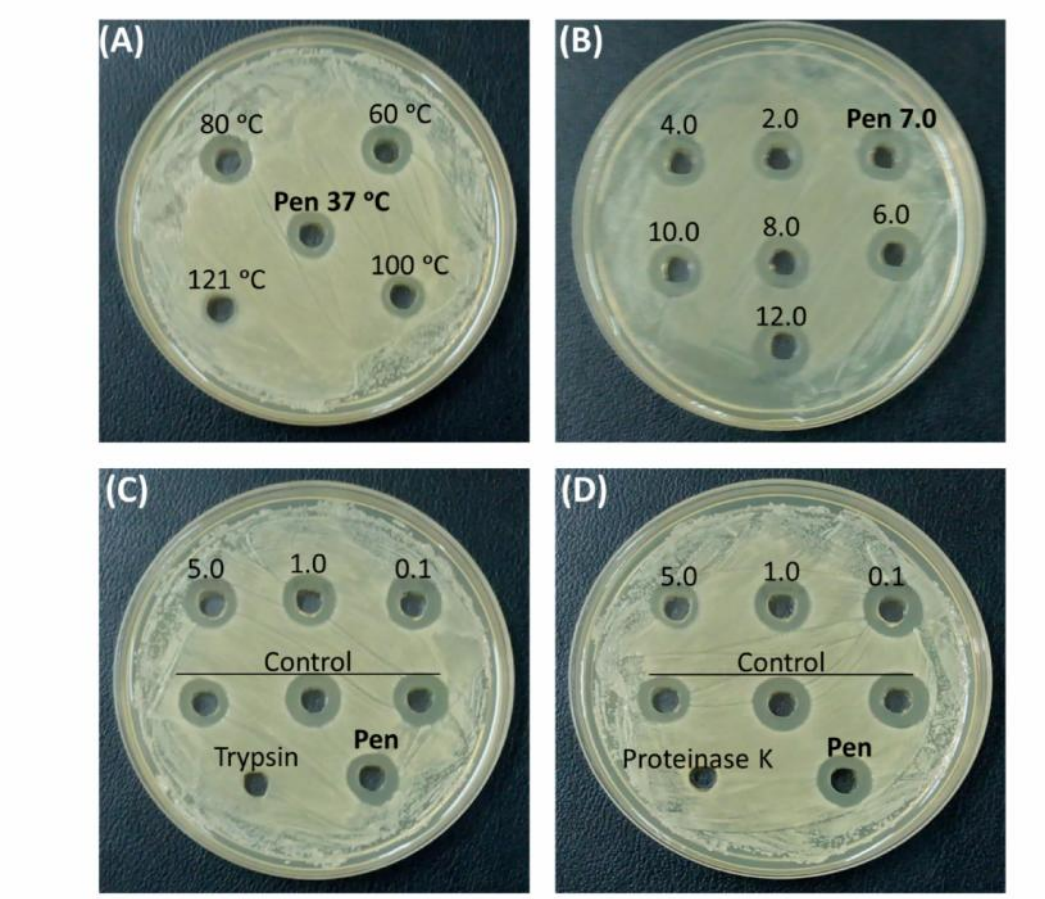
Supplementary Figure S1: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of *Paenibacillus* species showing the phylogenetic relationship between strain A3 and other members. Bootstrap values greater than 50% are given at the nodes. Bar indicates 0.01 substitutions per site.



Supplementary Figure S2: Two dimensional TLC of total polar lipid analysis of strain A3. The total polar lipids were detected by spraying with 5% phosphomolybdate and identified based on Rf values obtained for species of the genus *Paenibacillus*.



Supplementary Figure S3: Pegylation assay of purified penisin with MPEG (maleimide PEG, 5 kDa) in presence of TCEP (*tris*(2-carboxyethyl)phosphine) to access the presence of free cysteine. Same assay has been done in the presence of denaturing agent, Urea. MPEG; maleimide PEG, 5 kDa, T; *tris*(2-carboxyethyl)phosphine, U; urea.



Supplementary Figure S4: Temperature, pH and protease test of penisin. (A) Well diffusion assay of penisin after treatment at different temperatures for 30 min. Penisin at 37°C used as a control. (B) Well diffusion of penisin at different pH range (penisin at 7.0 served as control). (C) Well diffusion assay of pensin after 6 h treatment with trypsin. Untreated penisin used as a control. (D) Well diffusion assay of penisin after 6 h treatment with proteinase K. Untreated penisin served as a control.