

SUPPLEMENTARY MATERIALS

Effects of indicitin, a novel ribosome-targeting enzyme from milky mushrooms,
towards four human cell lines and gut microbiota

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Figure S1.

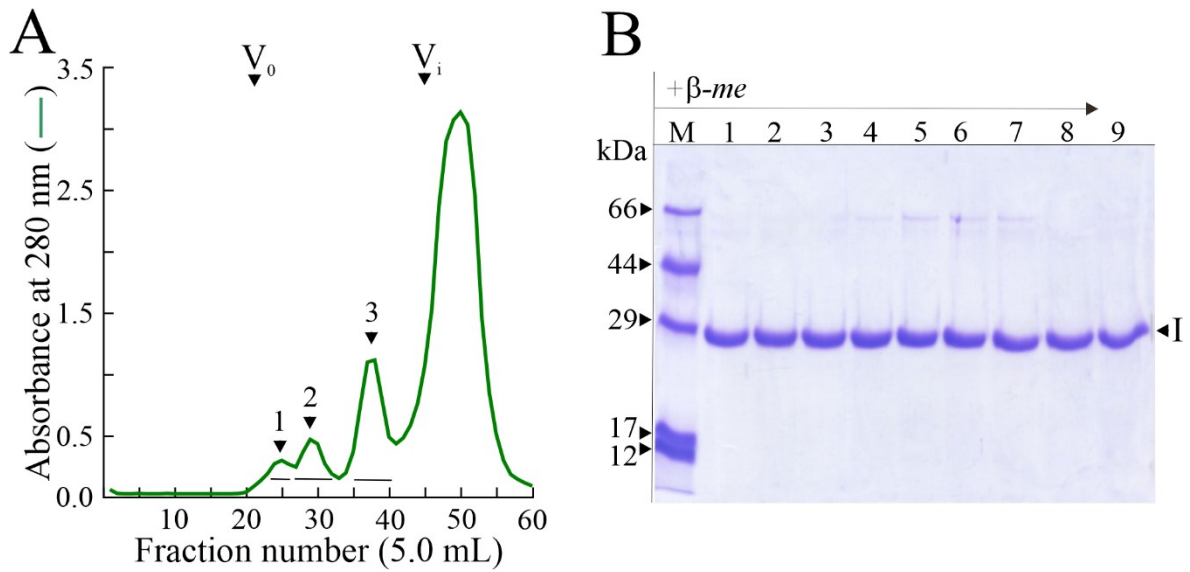


Fig. S1. (A) Elution profile from gel-filtration on HiLoad Superdex 75™ 26/60 column of basic proteins extracted from *Calocybe indica* fruiting bodies. V_0 and V_i indicate the void and inclusion volumes of the gel-filtration column, respectively., 2 and 3 indicate the protein peaks subjected to Endo's assay (see main text). (B) SDS-PAGE analysis of 240-256 fractions (3.0 μ g) from indicitin obtained after cation exchange chromatography using SP-Sephacrose. M, molecular weight markers. Lanes 2-10, fractions 240, 242, 244, 246, 248, 250, 252, 254 and 256, respectively. SDS-PAGE in the presence of β -mercaptoethanol (+ β -me) was carried out in 15% polyacrylamide separating gel and then stained with Coomassie brilliant.

Figure S2.

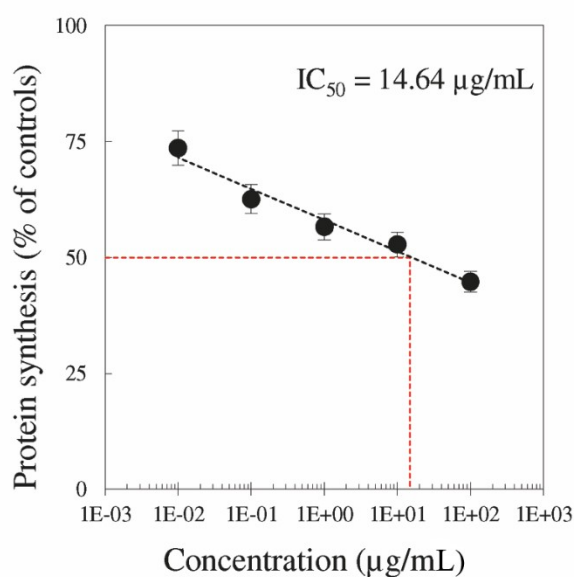


Fig. S2. Protein synthesis inhibition assay of indicitin. The inhibitory activity was evaluated using a cell-free protein synthesis system consisting of rabbit reticulocyte lysate. The concentration causing 50% inhibition of protein synthesis (IC_{50}) was calculated by linear regression analysis. Results are expressed as percentage of untreated control samples as means $\pm$ SD of two different experiments, each conducted in duplicate. SD never exceeds 10%.

Table S1. List of Gram-positive, Gram-negative bacteria, and yeast strains used to test the activity of indicitin.

Strains	Reference
<i>Salmonella enterica typhi</i> ATCC 14028	
<i>Escherichia coli</i> DH5 α	commercially available
<i>Bacillus subtilis</i> PY79	Youngmann <i>et al.</i> , 1984
<i>Bacillus cereus</i> MV19	Vittoria <i>et al.</i> , 2023
<i>Lactobacillus rhamnosus</i> GG	commercially available
<i>Candida albicans</i> ATCC 14028	
<i>Candida glabrata</i> intestinal isolate	gift of A. Tavanti (Univ. Pisa)
<i>Candida parapsilosis</i> intestinal isolate	gift of A. Tavanti (Univ. Pisa)
<i>Candida albicans</i> intestinal isolate	gift of A. Tavanti (Univ. Pisa)
<i>C. parapsilosis</i> ATCC 22019	

Table S2. NCBI Sequence Read Archive (SRA) accession numbers of data generated in this study (BioProject number PRJNA1291050).

Sample name	Accession number
Total_1	SAMN49946430
Total_6	SAMN49946432
Total_12	SAMN49946434
Untreated_2	SAMN49946436
Untreated_7	SAMN49946438
Untreated_13	SAMN49946440
Treated_Indicitin_5	SAMN49946442
Treated_Indicitin_10	SAMN49946444
Treated_Indicitin_16	SAMN49946446

Table S3. Amino acid compositions of indicitin. Residues are expressed as number of residues/mol of protein. Three letter amino acid code has been used.

Amino acid	indicitin	Note
Ala	23.16 (23)	
Arg	19.17 (19)	
Asx	29.16 (29)	Asp+Asn
Cys	15.98 (16)	
Glx	11.22 (11)	Glu+Gln
Gly	27.13 (27)	
His	5.83 (6)	
Ile	7.39 (7)	
Leu	13.38 (13)	
Lys	5.90 (6)	
Met	1.58 (2)	
Phe	13.80 (14)	
Pro	26.24 (26)	
Ser	27.65 (28)	
Thr	24.87 (25)	
Trp	n.d.	
Tyr	12.05 (12)	
Val	12.20 (12)	
(total)	~(277)	

The closest integers of the calculated number of residues are given in parentheses. n.d.= not determined.

Table S4. Average abundance of bacteria at the *Phylum* level in samples of the Control and Untreated groups.

Phylum	Control	Untreated	p-value
<i>Firmicutes</i>	97229.00	70488.33	0.0037 **
<i>Bacteroidetes</i>	30067.00	31025.33	0.9038
<i>Actinobacteria</i>	12021.33	1802.67	0.1158
<i>Proteobacteria</i>	1651.67	25001.67	0.0152 *
<i>Verrucomicrobia</i>	1372.67	0.00	0.1697
<i>Euryarchaeota</i>	93.00	0.00	0.2463
<i>Patescibacteria</i>	14.33	0.00	0.1613
<i>Lentisphaerae</i>	10.33	0.00	0.1636
<i>Nanoarchaeaeota</i>	10.00	7.67	0.7476
<i>Planctomycetes</i>	7.00	0.67	0.1511
<i>Fibrobacteres</i>	5.00	0.00	0.3739
<i>Epsilonbacteraeota</i>	3.00	20.00	0.4479
<i>Omnitrophicaeota</i>	2.00	2.33	0.9188
<i>Acetothermia</i>	0.67	0.00	0.3739
<i>Chloroflexi</i>	0.67	22.00	0.3873
<i>Fusobacteria</i>	0.67	0.00	0.3739
<i>Latescibacteria</i>	0.00	3.00	0.3739

* p-value <0.05. ** p-value <0.01

Table S5. Average abundance of bacteria at the family level of samples of the Control and Untreated groups.

Family	Control	Untreated	p-value
Haloferacaceae	91.3333	0	0.2597
U. m. of Methanofastidiosales order	1.6667	0	0.3739
U. m. of Nanohaloarchaea class	9	5	0.5265
U. m. of Woesearchaea class	1	2.6667	0.5898
U. m. of Acetothermia class	0.6667	0	0.3739
Actinomycetaceae	105.3333	0	0.0435*
Bifidobacteriaceae	9357.3333	385.6667	0.1379
Corynebacteriaceae	3.3333	0	0.3739
Nocardiaceae	0	1	0.3739
Microbacteriaceae	3	0	0.3739
Micrococcaceae	3	0	0.3739
Propionibacteriaceae	4.6667	0	0.1475
Pseudonocardiaceae	0	0.6667	0.3739
Coriobacteriaceae	2223	0	0.1363
U. m. of Coriobacteriales order	35	0	0.3739
Eggerthellaceae	286.6667	1415.3333	0.0351*
Bacteroidaceae	12150.3333	30793.6667	0.0772
Barnesiellaceae	1692.6667	0	0.1176
Marinifilaceae	339.3333	0	0.2011
Porphyromonadaceae	0.6667	0	0.3739
Prevotellaceae	5914.6667	1	0.3388
Rikenellaceae	7680.6667	20.3333	0.0093*
Tannerellaceae	2288.6667	210.3333	0.0048*
U. m. of RBG-13-54-9 order	0	22	0.3739
Chloroflexaceae	0.6667	0	0.3739
Campylobacteraceae	3	20	0.4479
U. m. of Fibrobacterales order	5	0	0.3739
Bacillaceae	72	89	0.8891
Family XI (Bacillales order)	15.6667	0	0.1754
Staphylococcaceae	2.3333	2.6667	0.9148
Carnobacteriaceae	2.6667	0	0.3739
Enterococcaceae	34	29	0.7787
Lactobacillaceae	28	0	0.2915
Leuconostocaceae	3.3333	0	0.3739
Streptococcaceae	637	2.6667	0.1438
Christensenellaceae	1389	12.6667	0.0752*
Clostridiaceae 1	1309.3333	54.6667	0.1657
Clostridiales vadinBB60 group	440	0	0.1391
Defluviitaleaceae	44.6667	0	0.1217
Eubacteriaceae	63.6667	12.6667	0.4471
Family XI (Clostridiales order)	11	60.3333	0.3037
Family XIII (Clostridiales order)	689	11.6667	0.0061*
Family XVIII (Clostridiales order)	0	7.3333	0.0480*
Lachnospiraceae	39893	44420.3333	0.7032

Peptococcaceae	58	0	0.0411
Peptostreptococcaceae	4038.6667	9.6667	0.0984
Ruminococcaceae	42091	20466	0.0982
Erysipelotrichaceae	1419.6667	1414.3333	0.9967
Acidaminococcaceae	529.3333	1	0.3719
Veillonellaceae	4457.6667	3894.3333	0.8120
Fusobacteriaceae	0.6667	0	0.3739
Latescibacteraceae	0	3	0.3739
U. m. of Victivallales order	7	0	0.3739
Cytophaga sp. PRPR22	3.3333	0	0.3739
U. m. of Omnitrophicaeota	2	2.3333	0.9188
Saccharimonadaceae	14.3333	0	0.1613
U. m. of Pla4 lineage class	0.6667	0	0.3739
Gemmataceae	5.6667	0	0.1493
U. m. of vadinHA49 class	0.66667	0.6666	1
Beijerinckiaceae	1	2	0.6779
Desulfovibrionaceae	234	9	0.12023
Burkholderiaceae	485.6667	228	0.4877
Neisseriaceae	0.6667	0	0.3739
Enterobacteriaceae	921.6667	24762.6666	0.0157*
Pasteurellaceae	8.6667	0	0.1368
Akkermansiaceae	1372.6667	0	0.1697

* p-value <0.05. ** p-value <0.01

Table S6. Average abundance of bacteria at the *Phylum* level of Treated and Untreated groups.

<i>Phylum</i>	Untreated	Treated	p-value
<i>Firmicutes</i>	70488.33	71853.33	0.9136
<i>Bacteroidetes</i>	31025.33	29657.00	0.8784
<i>Proteobacteria</i>	25001.67	28255.67	0.6024
<i>Actinobacteria</i>	1802.67	2359.33	0.5234
<i>Chloroflexi</i>	22.00	30.33	0.8349
<i>Epsilonbacteraeota</i>	20.00	32.67	0.7575
<i>Nanoarchaeaeota</i>	7.67	15.67	0.6531
<i>Latescibacteria</i>	3.00	3.33	0.9283
<i>Omnitrophicaeota</i>	2.33	1.67	0.8276
<i>Planctomycetes</i>	0.67	1.33	0.6779
<i>BRC1</i>	0.00	4.67	0.3739
<i>Patescibacteria</i>	0.00	3.33	0.2378
<i>TA06</i>	0.00	1.00	0.3739

Table S7. Average abundance of bacteria at the *genus* level in samples of the Treated and Untreated groups.

Genus	Untreated	Treated	p-value
<i>Bacteroides</i>	30793.6667	29267.6667	0.8661
<i>Escherichia-Shigella</i>	24733.3333	27955.6667	0.6181
<i>Lachnoclostridium</i>	23226.6667	25343.6667	0.6631
<i>Hungatella</i>	12005.3333	11140.6667	0.9109
<i>Flavonifractor</i>	9273.3333	8715.0000	0.9304
<i>Intestinimonas</i>	5622.3333	8074.3333	0.6931
<i>Oscillospira</i>	3828.3333	4041.3333	0.9553
<i>Dialister</i>	3605.3333	2839.0000	0.7725
<i>Roseburia</i>	3247.3333	2063.3333	0.6129
<i>Blautia</i>	2076.0000	3072.3333	0.6654
<i>Eggerthella</i>	1342.6667	1920.0000	0.6076
<i>Dorea</i>	982.3333	680.6667	0.6427
<i>Erysipelatoclostridium</i>	810.6667	478.6667	0.7421
<i>Herbinix</i>	729.6667	730.0000	0.9986
<i>Candidatus Soleaferrea</i>	542.0000	527.3333	0.9789
<i>Pseudobutyrvibrio</i>	511.0000	457.3333	0.8874
<i>Butyricicoccus</i>	462.0000	425.3333	0.9523
<i>Holdemania</i>	428.3333	388.3333	0.8858
<i>Tyzzerella 4</i>	426.3333	372.6667	0.9028
<i>Bifidobacterium</i>	385.6667	365.3333	0.9408
<i>Lachnospiraceae NK4A136 group</i>	370.6667	470.6667	0.8629
<i>Megasphaera</i>	288.3333	107.0000	0.5871
<i>Oscillibacter</i>	239.0000	167.6667	0.8106
<i>Sutterella</i>	227.3333	231.6667	0.9900
<i>Parabacteroides</i>	210.3333	346.0000	0.6513
<i>Ruminiclostridium 9</i>	191.6667	183.3333	0.9690
<i>Anaerostipes</i>	190.0000	106.3333	0.4861
<i>Clostridium innocuum group</i>	175.3333	78.3333	0.6400
<i>Sellimonas</i>	136.6667	234.6667	0.4167
<i>Faecalibacterium</i>	136.6667	115.6667	0.8061
<i>ASF356</i>	119.6667	130.0000	0.9562
<i>Eubacterium ventriosum group</i>	112.6667	90.6667	0.7167
<i>GCA-900066755</i>	106.6667	162.0000	0.7896
<i>Agathobacter</i>	96.6667	87.3333	0.9463
<i>Bacillus</i>	89.0000	0.0000	0.3739
<i>Gordonibacter</i>	72.6667	71.0000	0.9857
<i>Peptoniphilus</i>	60.3333	45.6667	0.7883
<i>Clostridium sensu stricto 1</i>	54.6667	46.3333	0.7642
<i>Eubacterium hallii group</i>	53.3333	43.3333	0.7745

<i>Ruminiclostridium</i> 5	39.0000	15.0000	0.5634
<i>Enterococcus</i>	29.0000	27.3333	0.8616
<i>Ruminiclostridium</i>	29.0000	23.6667	0.8294
<i>Klebsiella</i>	27.6667	22.0000	0.8804
<i>Ruminococcaceae</i> NK4A214 group	25.6667	23.6667	0.9571
<i>U. m. of</i> RBG-13-54-9 order	22.0000	30.3333	0.8349
<i>Alistipes</i>	20.3333	39.0000	0.4037
<i>Campylobacter</i>	20.0000	32.6667	0.7575
GCA-900066225	14.6667	19.0000	0.8655
<i>Eubacterium</i>	12.6667	115.6667	0.4261
<i>Ruminococcaceae</i> UCG-005	12.6667	10.3333	0.7744
UBA1819	12.0000	22.3333	0.3152
Family XIII AD3011 group	11.6667	6.0000	0.6880
<i>Eubacterium eligens</i> group	11.3333	11.6667	0.9846
<i>Fournierella</i>	10.6667	17.3333	0.7597
<i>Phoea</i>	10.6667	2.0000	0.4693
<i>Christensenellaceae</i> R-7 group	10.3333	17.0000	0.7279
<i>U. m. of</i> Ruminococcaceae family	9.0000	12.6667	0.7418
<i>Bilophila</i>	9.0000	43.0000	0.2481
GCA-900066575	8.3333	9.6667	0.9218
<i>Symbiobacterium</i>	7.3333	8.6667	0.6934
<i>Romboutsia</i>	6.0000	22.3333	0.1351
<i>U. m. of</i> Nanohaloarchaeia class	5.0000	10.3333	0.6424
<i>Eubacterium oxidoreducens</i> group	4.3333	0.0000	0.3739
<i>Anaerotruncus</i>	4.0000	12.0000	0.5614
<i>Lachnospiraceae</i> UCG-004	3.6667	4.3333	0.9122
<i>Paeniclostridium</i>	3.6667	0.0000	0.3739
<i>Candidatus Latescibacter</i>	3.0000	3.3333	0.9283
<i>U. m. of</i> Woesearchaeia class	2.6667	5.3333	0.6779
<i>Staphylococcus</i>	2.6667	0.0000	0.3739
<i>Streptococcus</i>	2.6667	0.0000	0.3739
<i>Caproiciproducens</i>	2.6667	0.0000	0.3739
<i>U. m. of</i> Christensenellaceae family	2.3333	2.3333	1.0000
<i>U. m. of</i> Omnitrophicaeota phylum	2.3333	1.6667	0.8276
<i>Methylobacterium</i>	2.0000	1.0000	0.6779
<i>U. m. of</i> Lachnospiraceae family	1.6667	0.0000	0.3739
<i>Salmonella</i>	1.6667	0.0000	0.3739
<i>Rhodococcus</i>	1.0000	0.0000	0.3739
<i>Prevotella</i> 7	1.0000	0.0000	0.3739
<i>Phascolarctobacterium</i>	1.0000	0.0000	0.3739
<i>Saccharomonospora</i>	0.6667	3.0000	0.4900
<i>Ruminiclostridium</i> 6	0.6667	1.0000	0.7953
<i>Megamonas</i>	0.6667	0.0000	0.3739
<i>U. m. of</i> Planctomycetes phylum	0.6667	0.0000	0.3739

<i>Parasutterella</i>	0.6667	1.0000	0.7953
<i>Barnesiella</i>	0.0000	2.6667	0.1404
<i>Odoribacter</i>	0.0000	0.6667	0.3739
<i>Prevotella 9</i>	0.0000	1.0000	0.3739
<i>U. m. of BRC1 phylum</i>	0.0000	4.6667	0.3739
<i>Finegoldia</i>	0.0000	2.0000	0.3739
<i>Fusicatenibacter</i>	0.0000	13.3333	0.3739
<i>Lachnospiraceae UCG-010</i>	0.0000	3.0000	0.3739
<i>Marvinbryantia</i>	0.0000	22.3333	0.3739
<i>Ruminococcaceae UCG-002</i>	0.0000	4.0000	0.3739
<i>Ruminococcaceae UCG-008</i>	0.0000	4.0000	0.3739
<i>Subdoligranulum</i>	0.0000	1.6667	0.3739
<i>U. m. of Parcubacteria class</i>	0.0000	2.0000	0.3739
<i>U. m. of WS6 (Dojkabacteria) class</i>	0.0000	0.6667	0.3739
<i>U. m. of WWE3 class</i>	0.0000	0.6667	0.3739
<i>U. m. of OM190 class</i>	0.0000	1.3333	0.3739
<i>Desulfovibrio</i>	0.0000	0.6667	0.3739
<i>Pseudomonas</i>	0.0000	0.6667	0.3739
<i>U. m. of TA06 phylum</i>	0.0000	1.0000	0.3739

References

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