

Supplementary Material

Figure S1. Primary sequence alignment of biotin-binding proteins in fungi. Protein names or identifiers are displayed for each sequences found in *Pleurotus cornucopiae* (Pc), *Galerina marginata* (Gm), *Hypholoma sublateritium* (Hs). Conserved residues are highlighted. Residues that interact with biotin in tamavidin 2 are indicated (*).

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      1      10      20      30      40      50      60
Pc_Tamavidin2  .MSDVQSSLTGTTWYNELNSKMELTANKDGLTGTGKYLSKVGDVYVVPYPLSGRYNLOPPAGQ.....GVA
Pc_Tamavidin1  .MKDVQSSLTGTWYNELGSTMNLTKNDGSLTGTYHSNVGVEVPPTYHLSGRYNLOPPSSGQ.....GVT
Gm2_1328802    MSKKFGIETLSGDWYNQLGSTVHFVADLNGGLVGGKYIISAVGHAEDGYRLNGRFDADPPAGE.....GVS
Gm3_78940      MSYKFDPKALSGDWHNQLGSKVHFADADGGITGKYHSAVGKVEDFYILLGRFDTNPEENE.....GVS
Gm4_253228     MTNKFDPNTLACDWHNQLGSKVHFVTDPNCGITGKYNSAVGRAEDFYVLTGRFDANPPLDE.....GVS
Hs_173356      .MSNRLSGTWYNELGSTMLTLTADATGCLSGKYKSAVGNADFFVLTGRYDTNAPSDK.....GVS
Gm6_159069     MSNKFDYSNLSGDWYNRIQLQVNLIPADGSLKGGKLYDD...KKAVIDLTGRFDTPPSGD.....GAS
Gm5_25497      .....TLAGDWYNELGSHLILTPDSSKGLTGMYDSAVGRAHYFYVLTGRFDTNPPQDDKDGPNYGV
                                     * * * *
      70      80      90      100     110     120
Pc_Tamavidin2  LGWAVSWENNS....K.IHSATTWSGOFFSESSPV...ILTQWLLSSSTARGDVVESTLVGNDSTTKTA
Pc_Tamavidin1  LGWAVSFENNT....SANVHSVSTWSGQYFSEPAEV...ILTQWLLSSSEREDLWQSTHVGHDFRSKTK
Gm2_1328802    LGWTVNWRNVLPDGTVNNAHSTTTWSGQYFDESSVGERIVTNWLLTOSTDPSDIWNSTNVGNDATFRIM
Gm3_78940      VGWTVNWRNVV.DGKENNRHSSTTWSGQYFDTTSGV.BRIVTNWLLTRSTEAKDIWSTNVGNDTFVRDK
Gm4_253228     VGWTVNWRNFI.NGKENNHSSTTWSGQYFDKSTGV.BRIVTNWLLTOSTTTNDSWSTNVGNDTFVRDK
Hs_173356      LAWTVAYNNSL....RNAHSTAGWSGQYFDDDDGE.EKILTHWLLTSSSTSESVWSTNVGNTLFTNRN
Gm6_159069     VGWVVTRENVV...KPTHEHNNSTTWSGQYFDWNGGV.ESIAASWLLTOSTIARKSNSTNVGNDLFTREK
Gm5_25497      VGWAVTRENNDT...AGNNSSTTWSGQYFAATKNVVERILTQWLLTOSTDRILINDATNVGADTFVREK
                                     * * * *
      130     140
Pc_Tamavidin2  PTEQQIAHAQLHCRAPRLK.....
Pc_Tamavidin1  PTKKQIAQAQLLRRGLKFE.....
Gm2_1328802    PSAAETSKAKALSFGSPHPQQILAKK.....
Gm3_78940      PSAAETSMDETLSVGSPPNPEQLLARR.....
Gm4_253228     PSAAETAKAKALSFGSPHPPEELLARR.....
Hs_173356      PSTADTAKARAILAESATKSEKVAABESRRSGSRLARL
Gm6_159069     PSDAETAKAKAVLFGRR.....
Gm5_25497      PTDSEIAKAMQLR.....

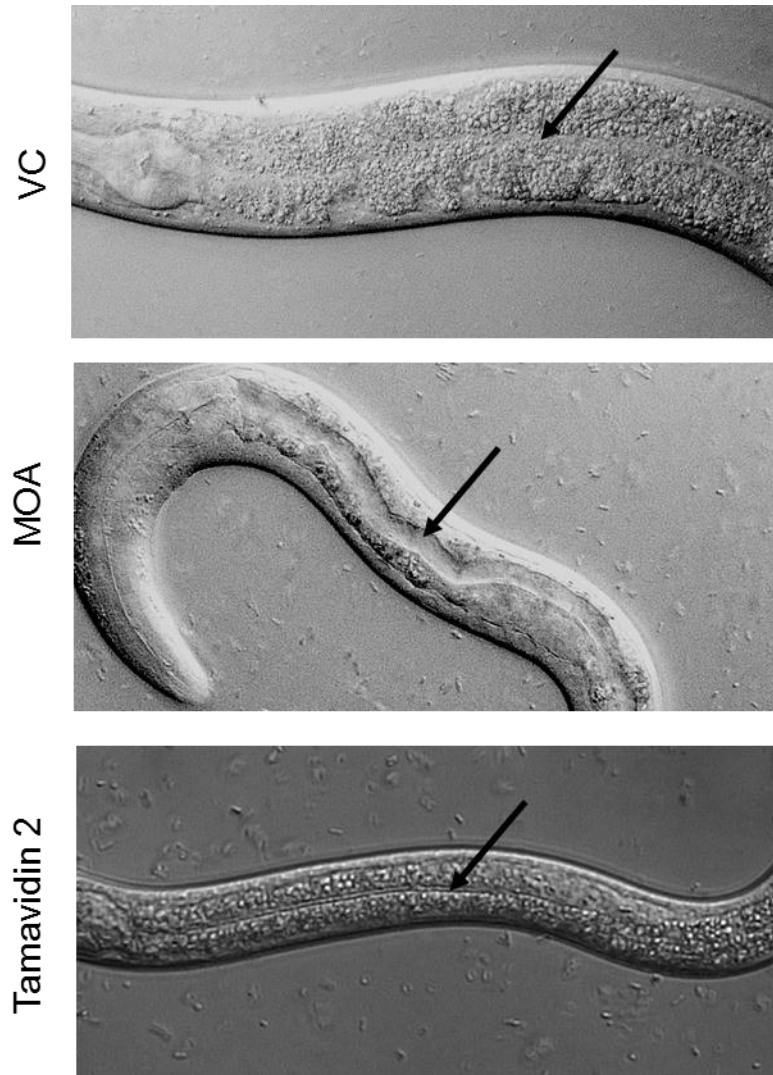
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Table S2. Overview of characterized avidin-like, biotin-binding proteins.

| Name | Organism | pI | MW (kDa) /Oligomeric | Localization | Reference |
|---------------|--------------------------------------|------|-------------------------|--------------|-----------|
| Avidin | <i>Gallus gallus</i> | 10.0 | 15.6 / tetramer | Secreted | (5) |
| Streptavidin | <i>Streptomyces svidinii</i> | 6.1 | 16.5 / tetramer | Secreted | (2) |
| Bradavidin I | <i>Bradyrhizobium japonicum</i> | 6.3 | 14.3 / tetramer | Secreted | (10) |
| Bradavidin II | <i>Bradyrhizobium japonicum</i> | 9.6 | 12.56 / tetramer | Secreted | (6) |
| Rhizavidin | <i>Rhizobium etli</i> | 4.0 | 14.0 / dimer | Secreted | (7) |
| Burkavidin | <i>Burkholderia pseudomallei</i> | 7.4 | 14.2 / tetramer | Secreted | (11) |
| Tamavidin 2 | <i>Pleurotus cornucopiae</i> | 7.4 | 15.5 / tetramer | Cytoplasmic | (13) |
| Xenavidin | <i>Xenopus tropicalis</i> | 9.2 | 18.6 / tetramer | Secreted | (8) |

Method S3. Purification of recombinant tamavidin 2. A plasmid encoding a N-terminally His-tagged version of the protein was constructed and expressed in *E.coli* (BL21). The protein was purified via metal affinity chromatography using the Ni-NTA resin (Qiagen) according to manufacturer's protocol.

Figure S4. Comparison of the gut phenotype of *C. elegans* when fed either on recombinant *E.coli* expressing the nematotoxic lectin MOA and Tamavidin 2. *E.coli* expressing a vector control (VC) was used as control. Arrows point to the intestine.



Discussion S5

Considering the ability of these proteins to sequester biotin, the question arises of how organisms producing BBPs avoid reduction or depletion of biotin required for normal metabolism. In the case of plants, where no BBPs have been identified, most biotin exists as a free pool in the cytoplasm, and the rest in protein-bound form in the soluble fractions of chloroplasts and mitochondria (1). For this reason, heterologous expression of BBPs for biological control has to be targeted to the apoplast (12) or the vacuoles (9) in order not to interfere with plant viability. In *E. coli*, most of intracellular biotin is bound to proteins making the intracellular pool of free biotin very low (3, 4). This may explain why recombinant expression of tamavidins and other BBPs in the cytoplasm of *E. coli* is not toxic for the cell. The same may be true for fungi. The tamavidins are most likely synthesized in the cytoplasm of *P. cornucopiae* as they do not have a signal sequence for secretion. Accordingly, recombinant expression of tamavidins in the cytoplasm of the ascomycete *Ashbya gossypii* is possible without deleterious effects for this fungus (unpublished data). This result suggests that also in fungi, free-biotin pools are absent or minimal in the cytoplasm.

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