

Pcitrinum -----GATCAGTCGACAAACTACCCAATCCCTTCC **F1.2** AACAAGGCGGAACCAACGCTTC  
 Psizovae -----  
 Psteckii CTGAAATGATCAATCAGCAAACTACCCAATTCCCTCC **AACAAGGCGGAACCAACG**CTGC  
 \*\*\*\*\* \*\* \*\*\*\*\* \*\* \*\*\*\*\* \*\*\*\*\*  
  
 Pcitrinum ACGATGGTCGACAGTCGCCTATTGCGGACCATGAAGGACATCTCCGTAAAGATCTACCTC  
 Psizovae -----CCGTAAAGACCTGCCTA  
 Psteckii GCGATGGCCGACAGTCACCTATTGCGGATCATGAGGGAAACCTGCGAAAGGATCTACCTG  
 \*\*:\*.\*. \*.\*.\*\*\*  
  
 Pcitrinum **F2**  
 AAGATGTAAAGGAGCACAATAAAGATATG **GACAATCGATACGATAAGC**CATACAATCATA  
 Psizovae AGGATGTAAAGGAGCACAATAAAGATATG **GACAATCGGTACGATAAGC**CATACAATCATA  
 Psteckii AGGATGTAAAGGAACACAATAAAGAGATG **GACAGTCGGTACGATAAGC**CATATAATCATA  
 \*.\*\*\*\*\*.\*\*\*\*\* \*\*\*\*\*.\*\*\*.\*\*\*\*\*  
  
 Pcitrinum TTGCGGACGAAGGGAATATCGAAGGCGGCTGGGAGAAGAAATAAATTGGGATTGTCTGCA  
 Psizovae TCGCGGACGAAGGGAATGTCTGAAGGCGGTTGGGAGAAGAAATAGATTGGGACGATCTGCA  
 Psteckii TTGCCGATGAAGGAAATGTCTGAAGGCGGCTGGGAGAAGAAATGAATTTGATTGAATGTCTG  
 \* \*\* \* \*.\*\*\*.\*\*\*.\*\*\*\*\*.\*\*\*\*\* \*\*\*\*\*.\*\*\* \*. : . : \*.  
  
 Pcitrinum GGTATCCGCTATGTGTAGCAAGATTACACTGGTGCAAGGCCTTTTATGTCAATTCAGCTTG  
 Psizovae GATGTTCTGTTATGTGTAGCGAGATTATACTGGTGCAAAG-TTTTTATGTCAATTTAAGTTG  
 Psteckii -----TTATGTCAATTTAAATTG  
 \*\*\*\*\* \*. \*\*  
  
 Pcitrinum GAGAATACT-----ACT---TCTGCAATCTCCAGTTTCTCTCATAT-CACACTATTA  
 Psizovae GATAATTTCT-----ACTGGTTCTGCAATCTCCCTGTCTGTCTCAAAT-TATACTGTAC  
 Psteckii GAAGATACATCATCTGACTGCTTCTGTGATCTCCAAGCTCCTCTCATACGGATGCTATTT  
 \*\* .\*:\*: \*\* \* \* \*.\*\*\*\*\*.:\* \*\*\*\*\*:\* \* .\*\*.\*:  
  
 Pcitrinum TGTCTTACTGTCTCATCAATTTAGGCAAGAG----AGGAAATAAATTAGAAAATAAATAATG  
 Psizovae TAGTACGTCTTTCTCCATTATGACATGGGGAATAGGGGAAGGAAAGATCAATAAATAATG  
 Psteckii AGTACGTCTTTCTGTCAATTGTATCGCAGGG---GGGAATAAAGTAAATAAATAACCAATG  
 :. \* .\*\*\* :. \* .\*. \* .\*..\*: ..\*: \* .\*\*.:\*. \*\*\*  
  
 Pcitrinum TTCAGCGAGTTTATTTCATTATTTACAGCTAATATCGATGTTTTTGTAACTACCTATCTGG  
 Psizovae TTCTGCGAAGTTACTCTCTATTTACTGCTAGTATCAATGTTTTTGTAACTACCTACGTGG  
 Psteckii TTCTGCGAAATTCTTCTCTATTTATGGCTAG-----CCATATGG  
 \*\*\*:\*\*\*. \*\*. \*\*: \*\*\*\*\* \*\*\*, \* \* \*\*  
  
 Pcitrinum GTATGTAGGAGTCAAATCAAAGATCGGCCCATATGTTATTTGTTCTTCTTGCAACCTTCT  
 Psizovae GTATGTAGGGGTCAAGTCAAAGAGTGGCCTATATGTCATCGG---TTTTTGCAATCTTCT  
 Psteckii -----ATCAAATTAAGATCGATCTAAGTGTCATTAT-----TCCCAATAGTCT  
 .\*\*\*\*.\* \*\*\*\*\* \*. \* \*.:\*\*\* \*\* \* \*\*\* . \*\*  
  
 Pcitrinum CTCTCAGAAGTTAAGACTAGGCATTTGTGTTTAGAGCAAATGTCGACGCAATCTCAGTGA  
 Psizovae CTGCCAAACACTAG-TCTAGGCATTTGTATTAAGAGAAAAGGTTGAAGCAATCTCAGTGA  
 Psteckii CTTCTAAATTTTAG-ACTAGGCATTTGTATTTAAAGCAAATGTTGAAGCAATTTCAGTGA  
 \*\* \*. \* \*\*. :\*\*\*\*\*.\*\*\*:\*.\*\*\* \*\* \*\*.\*\*\*\*\*\*  
  
 Pcitrinum **FASP2**  
 TGTGTTTGAAGTTCTGCGCAGCAATAGTCCCAGTAATATACGAGATTTT**TGAGGGTTCAAGT**  
 Psizovae TGTGCTCGACTTCGCTAGCAATAGTCCCAGTAATATACGGGATTTT**TGAGGGTTCAAGT**  
 Psteckii TGTGCTTGAAGTTTCTGCTAGCAATAGTCCAAGTAATATACGGGATTTCTGAG**GGTTCAAGT**  
 \*\*\*\*\* \* \*\*\*\*\* \*\* \*\*\*\*\*.\*\*\*\*\*.\*\*\*\*\* \*\*\*\*\*  
  
 Pcitrinum **RASP2.1**  
**ATCCAC**TGGCAATATGAGTGGCAGTGCTGCTGTCGACGTCTGACAATGGCACCTCACCAT  
 Psizovae **ATCCAC**TGGCAATATGAGTGGCACTGGTGCTTTTCGACGTCTGACAATGGCACTTCACCAT  
 Psteckii **ATCCACTAGCA**ATATGAGTGGCACTGTTGCTTTTCGACGTCTGACAAGGGAACCTCACCAT  
 \*\*\*\*\*.\*\*\*\*\* \*\*\*\*\* \*\* \*\*\*\*\* \*\*\*\*\* \*\*.\* \*\*\*\*\*  
  
 Pcitrinum TGACCGTGCGCATACTTTGAATAATTGGAATTCGAAGGCGATTGATAGCGTCTTCAATAG  
 Psizovae TGACCGTAGCATACTTTGAATTATTGGGATTCGAGGCGATTGATAGCGTCTTCAATAG  
 Psteckii TCACAGTAGCATACTTTGGATTATCGGGATTCGAGGCGGTTGATAGCGTCTCCTCCATGG  
 \* \*\*.\*.\*\*\*\*\*.\*\*\*:\*\*\* \*\*.\*\*\*\*\*\*.\*\*\*\*\*.\*\*\*\*\*.\*\*\* \*\*.\*.\*  
  
 Pcitrinum CATAGTTGAATGATGTTGTGACGCCTCCTGCTCCAGCTCCTGCAATCTACGACTAGTCA

|                                   |   |
|-----------------------------------|---|
| Psizovae<br>Psteckii              | CATAGTTGAATGACGTTGTGACTCCTCCGGCCCCAGCCCCTGCAATCTACGACCAGTCAG<br>CATAATTGAATGATGTTGTACACCTCCGGCCCCGGCCCCTGCAATCTACGAACAGTCAG<br>****.***** ** ** ** ** ** ** ** ** ** ** ** ** ** ** **  |
| Pcitrinum<br>Psizovae<br>Psteckii | TTATTGAACAAAATTC-ACAGAAAATGTGCTTATGTCAATTGTGAAAGTGTAACCTACCA<br>CTGTTAAACAACATTT-ACGGGAAATATTTTCTGTCAATTCTGAAAGGATGACCTACCA<br>CTATTGAACAATTTTGTACAGAAATGTACTTCTGCCTAATTTGAATGGATGACCTACCA<br>*.*.***** :* **.*.*.*.* **.* *.:* ****.* .*.***** |
| Pcitrinum<br>Psizovae<br>Psteckii | CGATGCCCTTTGCGCCACTATCGATGGCATTGTACAAGGTGTCATTGTGCATATCCTCGT<br>CGATGCCCTTTGCGCCACTTTTCGATGGCATTGTACAAGGTGTCATTATGCATATCCTCAT<br>CAATGCCCTTTGCACCACTTTCAACCGCGTTGTATAAGGTGTCGTTATGCATATCCTCAT<br>*.*****.*****.*.* **.* **** **.*.*****.*       |
| Pcitrinum<br>Psizovae<br>Psteckii | AAGAGAAGAGGATATCAACTCTCGGAATTTCCGTGACGTTGGCAATATCGAAGTTCTTCT<br>AAGAGAAGAGAATATCAACTCTCGGAATTTCCGTGACGTTGGCAATGTTGAAGTCCTTCT<br>AGGAGAAGAGAATATCGACTCTCGGATTTCTTGACGTTGGAATATCAAGATCCTTCT<br>*.*****.*****.*****.***** *****.***.* .**** *      |
| Pcitrinum<br>Psizovae<br>Psteckii | TTCCCGTCGGCTGTACGGGAGGGTAGAAAAAGAATGGAGTATTAGAGATCATCTCTCCTA<br>TTCCCGTCGGCTGTACGGGGGGGTAGAAAAAGAATGGGGTATTAGAGATCATTTCTCCTA<br>TTCCCGTTGGCTGTACAGGAGGGTAGAAAAAGAATGGTGTATTAGAGATCATCTCTCCTA<br>***** *****.*.*.***** ***** ***** *****         |
|                                   | <b>FASP3</b>  |
| Pcitrinum<br>Psizovae<br>Psteckii | GGAATCCCATTTCATTGCTTTTAAACGTATCAATGGTATTGGCGTTGGTCTTGGTCGTGT<br>AGTATCCCATTTCATTGCTTTTGAACGTATCCATGGTGTGGCGTTGGTCTTGGTCGTGT<br>GGAATCCCATTTCATTGCTTTTGAATGTATCCATGGTGTAGCGTTGGTCTTAGTTGTGT<br>.*.*****.*.* *****.*****.*.*.*****.*.* ****       |
| Pcitrinum<br>Psizovae<br>Psteckii | AGTAGGCGGATGCGATGCGATCATTCATGACGATCATGGCACCGCGGTTCTTGGCCTTCG<br>AGTAAGCGGATGCGATGCGATCATTCATGACAATCATGGCACCGCGGTTCTTAGCCTTCG<br>AGTAAGCGGATGCGATGCGATCATTCATAACAATCATGGCACCGCGATTCTTGGCCTTCG<br>****.*****.*.*.*****.*****.*****                |
| Pcitrinum<br>Psizovae<br>Psteckii | GGGATGCTGCGACTGTAACAGACTCAAGGAGATTGAAGGGCCCATCGGCTGAGATGGCGG<br>GAGATGCTGCGACGGTAACGGATTCAAGGAGATTGAAGGGTCCATCAGCTGAGATGGCGG<br>TAGATGCCGCGACAGTAACGTATTGAGAAAGATTGAAGGGTCCGTGGCTGAGATGGCGG<br>.*.***** ***** ** **.*.*.***** **.*.*.*****      |
| Pcitrinum<br>Psizovae<br>Psteckii | TCGAAGGGCGCATGGCGCCAACGATGATGACGGGTTTACCACAATTGATTGTAGCGTCGA<br>TTGAAGGACGCATGGCGCCAACAATGATGACGGGTTTACCACAATTGATCGTAGCGTCAA<br>TCGAAGGGCGCATAGCGCCAACAATGATAACCGGTTTACCACAATTGACCGTAGCATCCA<br>* *****.*****.*****.*****.* ***** ***** ** *    |
| Pcitrinum<br>Psizovae<br>Psteckii | GGAAAAAGGCAGTTTCTCCTCAAAGTGTGAGTGCCATGAGTGACAACTGCTCCAGCCATGG<br>GGAAAAAGGCAGTTTCTCCTCAAAGTATCGGTTCCATGAGTAACAACAGCCCCAGCCATGG<br>AGAAAAAGGCAGTTTCTCCTCAAAGTATCGGTGCCATGAGTGACAACAGCTCCAGCCATGG<br>.*.*****.*****.*.* ** *****.*****.* *****    |
| Pcitrinum<br>Psizovae<br>Psteckii | TTGGATCGTCGCAGACAAGCTTATGATCTGCTTTGACAGTGAAATTAGAATGTCGGAGG<br>TTGGATCGTCGCAGACAACTTGTGATCTGCTTTGATAGTGAAATTAAGATGTCAGATG<br>TTGAATCATCGCAGACAAGCTTGTGATCTGTTTTGATAGTGAAATGAGGATGTCGGATG<br>***.*.*.*****.*.*.***** ***** ***** *.*.*****.* *   |
| Pcitrinum<br>Psizovae<br>Psteckii | TGATATCTTCACTTCCAACATTTGCTGTCTGAACACCCGCTACATTTGCAACGTCGAGCA<br>TGATATCTTCACTTCCAACATTTGCTGTCTGGACACCCGCGACATTTGCAATATCGAGCA<br>TGATATCTTCACTTCCACATTCGCTGTCTGGACACCCGCTACATTTGCAATATCGAGCA<br>*****.***** *****.***** ***** *****              |
| Pcitrinum<br>Psizovae<br>Psteckii | TAGATGGAACAGCATCGATTAGAGCACGAACGCCAACCGCTCCAGAAGGTGTAGCCTGTTG<br>TGATGGAACGGCATCGATTAGAGCACGAACGCCAACCGCTCCAGAAGGTGTAGCCTGTCTG<br>TGATGGGACGGCATCAATTAGAGCACGAACCTCAACCGCTCCAGAGGTGTAGCCTGTCTG<br>*.*.*****.*.*.***** ***** *****.***** *       |
| Pcitrinum<br>Psizovae<br>Psteckii | TTGCCGTTGAGCTTGAATCTGAGCCTGCAATTGTCCCTCCTAAGAGGTGTTAGGAAAATA<br>TTGCCGTTGAGCTTGAATCTGAGCCTGCAATTGTCCCTCCTAGGGAGTTTGTAGATAAAAA<br>TGGCCGTCGAGCTTGAATCTGAGCCTGCTATAGTTCTCCTATAAGGTGTGAGTAAAAATA<br>* ***** *****.:*:* ***** ..* * * *:***:*       |

|           |   |              |
|-----------|---|--------------|
|           |   | <b>RASP6</b> |
| Pcitrinum | TTAGCATTTGAACGCAGAAATTGGATATAGGGGGCCAAACGAACCTGTAGCAAAGATGGTT |              |
| Psizovae  | TTAGGATAGAAACGTG-----GGATAAAGGAGTCGGACTGACCTGTAGCAAAAATGGTT   |              |
| Psteckii  | CTAGCATAATTACATG-----GGAGAACC GGTTGGACTAACCTGTAGCAAAAATGGTG   |              |
|           | *** **: :*. . *: :* *. * ..** .*****.*****                    |              |
| Pcitrinum | ACATTAGGAAGGGTATGATTCATCTGAGTGAAATCAACCCATTTCGCATTGGTGAAAACA  |              |
| Psizovae  | ATATTAGGAAGGGTATGATTCATCTGGGTGAAATTCAGACCATTTCGCATTGGTGAAAACA |              |
| Psteckii  | ATATTAGGAAGGGTATGATTCATCTGGGTGAAATTCACCCATTTCGCATTGGTGAAAACG  |              |
|           | * *****.*****.*****.*****.                                    |              |
| Pcitrinum | AACCCTGTGCCAATGGCTCCACGGCCATATAGCAAGGGCGATGCCGAACCTCTGACAAGCA |              |
| Psizovae  | AACCCTGTGCCATTGGTTCCACGGCCATACAGCAGGGGCGAAGCCGAACCTCTGACAAGCA |              |
| Psteckii  | AACCCTGTACCATTGGCGCCACGACTATACAGCAGGGGCGAAGCCGAGCTCTGCCAAGCA  |              |
|           | *****.***:*** *****. * *** *****.*****:*****.*****.*****      |              |
| Pcitrinum | AGAGTGGCCAACGTTATCAGGAAGCTCTTTATTGACACCATTTGTGGAAATGCTTGGTCAG |              |
| Psizovae  | AAAGTAGCCAACGCTACGAGGAAGCTCTTAATTGACACCATCGTGAAAAGGCCTGATCAG  |              |
| Psteckii  | AGAGTAGCCAACGCTACGAGGAAGCTTTTAATTGGCACCATTTGTGGAAAGGCTTGCTTGG |              |
|           | *.***.***** ** ***** **:*****.***** ***. ** * * * *           |              |
| Pcitrinum | ACTTGGGGTTCAACCTTAGAGACAATTTAATCCATGTCAACGATTTATGCCATTCTTAAG  |              |
| Psizovae  | ACTGGGG-----TTCAGCCAATTCAATCCATGTCAACGATT-----                |              |
| Psteckii  | ACTTGGTACTCAACCTTAGAATCAATTCATCAATGGCAACGATTTATGCCATTCTTAAG   |              |
|           | *** ** *: *. ***** *****.*** *****                            |              |
| Pcitrinum | GAA-TTTGTGATCTTATCGTACTACTTCATACATCCGCGATATACCACTAATTATCGGTT  |              |
| Psizovae  | -----   |              |
| Psteckii  | GAAATCCGTGATCTTATCGTACTGCTTCATACTTCCGTGGAGTAC-AGTAATTATCGGTA  |              |
|           | *** * ***** ***** ***** * *** * *****                         |              |
|           |   | <b>R2</b>    |
| Pcitrinum | TGCCTATTACGTGGAATTGTGAATCTACTAG                               |              |
| Psizovae  | -----   |              |
| Psteckii  | TGTCTATTACGTGGAATTGTGAATCTTCTAG                               |              |
|           | ** ***** *  |              |

**Figure S1.** Nucleotide alignment of *P. citrinum* and *P. steckii* sequences to construct the L-asparaginase gene sequence of *P. sizovae* with the degenerate primers used in identification (reverse complement). Forward primers F1.2, F2, FASP2 and FASP3 (blue); reverse primers RASP2.1, RASP6 and R2 (pink); initiation codon (green); stop codon (red).

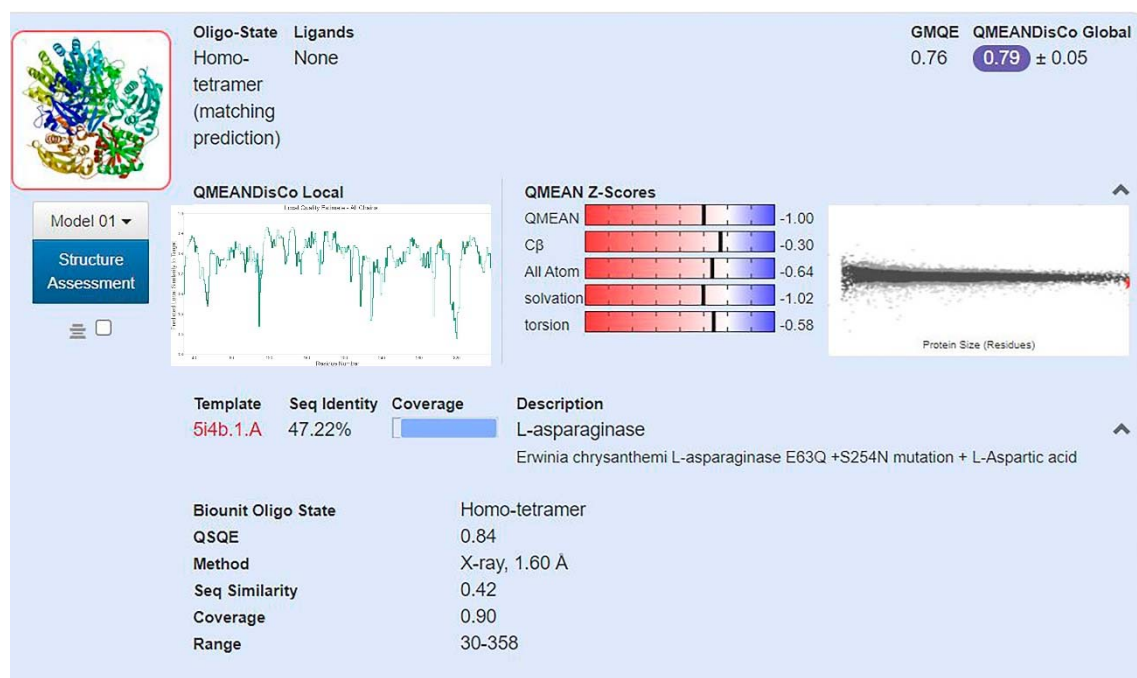
5' AATCGTTGACATGGATTGAATTGGCTGAACCCAGTCTGATCAGGCCTTTTCACGATGGTGT  
CAATTAAGAGCTTCCTCGTAGCGTTGGCTACTTTTGCTTGTCAGAGTTCGGCTTCGCCCTGCT  
GTATGGCCGTGGAACCAATGGCACAGGGTTTGTTCACCAATGCGAATGGTCTGAATTTACCC  
CAGATGAATCATACCCTTCCTAATATAACCATTTTGTCTACAGGTCAGTCCGACTCCTTTATCC  
CACGTTTCTATCCTAATTTTATCTAAACTCCCTAGGAGGAACAATTGCAGGCTCAGATTCAA  
GCTCAACGGCAACGACAGGCTACACTTCTGGAGCGGTTGGCGTTTCGTGCTCTAATCGATGCCGT  
TCCATCCATGCTCGATATTGCAAATGTCGCGGGTGTCCAGACAGCAAATGTTGGAAGTGAAGAT  
ATCACATCTGACATCTTAATTTCACTATCAAAGCAGATCAACAAGTTTGTCTGCGACGATCCAA  
CCATGGCTGGGGCTGTTGTTACTCATGGAACCGATACTTTGGAGGAACTGCTTTTTCTTGA  
CGCTACGATCAATTGTGGTAAACCGTCATCATTGTTGGCGCCATGCGTCCTTCAACCGCCATC  
TCAGCTGATGGACCCTTCAATCTCCTTGAATCCGTTACCGTCGCAGCATCTCCGAAGGCTAAGA  
ACCGCGGTGCCATGATTGTCATGAATGATCGCATCGCATCCGCTTACTACAGACCAAGACCAA  
CGCCAACACCATGGATACGTTCAAAGCAATGGAAATGGGATACTTAGGAGAAATGATCTCTAAT  
ACCCATTCTTTTTCTACCCCCCGTACAGCCGACGGGAAAGAAGGACTTCAACATTGCCAACG  
TCACGGAAATCCGAGAGTTGATATTCTTCTTCTTATGAGGATATGCATAATGACACCTTGTA  
CAATGCCATCGAAAGTGGCGCAAAGGGCATCGTGGTAGGTCATCCTTTCAGAATTGACAGAAAA  
ATATTTCCCGTAAATGTTGTTTAACAGCTGACTGGTCGTAGATTGCAGGGGCTGGGGCCGGAGG

AGTCACAACGTCATTCAACTATGCCATTGAAGACGCTATCAATCGCCTCGGAATCCCAATAATT  
CAAAGTATGCGTACGGTCAATGGTGAAGTGCCATTGTCAGACGTCGAAAGCACCAGTGCCACTC  
ATATTGCCAGTGGATACTTGAACCCTCAAAAATCCCGTATATTACTGGGACTATTGCTAGCGAA  
GTCGAGCAACATCACTGAGATTGCTTCAACCTTTTCTCTTAATACAAATGCCTAGACTAGTGTT  
TGGCAGAGAAGATTGCAAAAACCGATGACATATAGGCCACTCTTTGACTTGACCCCTACATACC  
CACGTAGGTAGTTACAAAAACATTGATACTAGCAGTAAATAGAGAGTAACTTCGCAGAACATTA  
TTTATTGATCTTTCCTTCCCCTATTCCCCTATGTCATAATGGAGAAAGACGTACTAGTACAGTAT  
AATTTGAGACAGACAGGGAGATTGCAGAACAGTAGAATTATCCAACCTAAATGACATAAAAAAC  
TTTGCACCAGTATAATCTCGCTACACATAACGAACATCTGCAGATCGTCCCAATCTATTTCTTC  
TCCCAACCGCCTTCGACATTCCCTTCGTCCGCGATATGATTGTATGGCTTATCGTACCGATTGT  
CCATATCTTTATTGTGCTCCTTTACATCCTTAGGCAGGTCCTTACGG3'

**Figure S2.** *Penicillium sizovae* gene sequence. L-asparaginase (bold) and introns (underlined).

MVSIKSFLVALATFACQSSASPLLYGRGTNGTG FVFTNANGLNFTQMNHTLP  
NITIFATGGTIAGSDSSSTATTGYTSGAVGVRLIDAVPSMLDIANVAGVQT  
ANVGSEDTSDILISLSKQINKFVCDDPTMAGAVVTHGTDLEETAFFLDATI  
NCGKPVHVGAMRPSTAISADGPFNLLESVTVAASPKAKNRGAMIVMNDRI  
ASAYYTTKTNANTMDTFKAMEMGYLGEMISNTPFFFYPPVQPTGKKDFNI  
ANVTEIPRVDILFSYEDMHNDTLNAIESGAKGIVIAGAGAGGVTTTSFNYAI  
EDAINRLGIPIHQSMRTVNGEVPLSDVESTSATHIASGYLNPQKSRILLGLLL  
AKSSNITEIASTFSLNTNA-

**Figure S3.** Predicted amino acid sequence of the native and partial (bold) L-asparaginase gene from *Penicillium sizovae*.



**Figure S4.** L-asparaginase crystal structure using Swiss model. Homology model prediction quality evaluation. QMQE: Global Model Quality Estimate (range 0-1); QMEANDisCo Global [60]: Model Quality Estimation with Distance Constraints.

Feature 3

|          | #  | HR          | ASFL  |                | #####           | #               |              |               |              |       |     |
|----------|----|-------------|-------|----------------|-----------------|-----------------|--------------|---------------|--------------|-------|-----|
| query    | 32 | PNITIFATGGT | AGSD  | ssstattgysgav  | GVRALIDAVPSMLdi | -anVAGVQTANVGS  | EDITS        | SDI           | ILSLKQINKFV  | cd    | 110 |
| 1HFJ_A   | 5  | PNIVILATGGT | AGSA  | atgtttgkagav   | GVDTLINAVPEVKk1 | -anVKGQFSNMASE  | NMT          | GDVVLKLSQRV   | NELLar       | 83    |     |
| 1DJP_A   | 3  | ANVILATGGT  | AGAG  | saasatyqaav    | GVDKLIAGVPELAd1 | -anVRGEQVMQIAS  | ESIT         | NDDLLKLGKRV   | AEAdS        | 81    |     |
| ACJ75594 | 2  | KKVILTTGGT  | IAHVK | heag----       | vvpyDKGSALISEI  | PSLKnlgvKIEV    | REFSNIPSPHMT | PKH           | WELSRIDEIQkd | 76    |     |
| EEX68565 | 3  | KHIYILATGGT | AGKA  | seaattgyeagav  | GIADLLAAVPELRqy | -adVEGEQIASIDS  | KDHT         | SAIHLRLAARCK  | ELLar        | 81    |     |
| EEG08105 | 34 | PNVILATGGT  | AGT   | sttttgytaav    | GVDKMIESVPELKa  | -anVRGEQVQVQIAS | ESHT         | NDVHLKLAQRV   | NELLaq       | 112   |     |
| EEU48991 | 44 | PNITIFATGGT | AGSA  | gsadqttgyqagav | GKVLIDAVPELcni  | -sNVRGQVIANVDS  | SDIT         | STI           | TNLTHQIQ     | AQLds | 122 |
| ACZ12201 | 20 | PNVVILATGGT | AGSG  | ysstqygyqaav   | GVDKLLQAVPELKn1 | -anLSGEQVAVASQD | IS           | TDIHLKLAQRV   | NLSLkq       | 98    |     |
| EAY64500 | 41 | PRIAVLATGGT | AGAA  | pdaastagyqagav | GVNFLVDVAPALAsv | -aRIDAEQVASIDS  | KDLALPL      | NTLAARIDAL    | Mad          | 119   |     |
| ACB74433 | 13 | PRIRLATGGT  | AGAC  | cdavar-gyhaaaf | SIDALVAAPVQLat1 | -aRLDVEQVAAIGS  | QDM          | DEGVHLQLAARTE | EALLaq       | 90    |     |

Feature 3

|          | #   | *            | #     | *            | #         | ###         | ##      |         | ###        | #           |             |     |
|----------|-----|--------------|-------|--------------|-----------|-------------|---------|---------|------------|-------------|-------------|-----|
| query    | 111 | dptMAGAVVTHG | TDTLE | ETAFFLDATINC | GKPVII    | VGAMRPSTAI  | SADGPF  | NLLES   | VTVAAS     | SpaknRGAMIV | NDRIAS      | 190 |
| 1HFJ_A   | 84  | -ddVDGVVITHG | TDTVE | ESAYFLHLTVK  | SDKPVV    | FVAAMRPATA  | ISADGP  | PNLLE   | AVRVAGD    | ksrgRGVMV   | NDRIAS      | 162 |
| 1DJP_A   | 82  | -ndVDGIVITHG | TDTLE | ETAYFLNVLQ   | TKDPIV    | VVGSHRPGTA  | MSADGML | NLYNAV  | AVASNkds   | rgKGVLT     | NDRIAS      | 160 |
| ACJ75594 | 77  | -ddVIGVVITHG | TDTLE | ETSYLLDLTLK  | SEKPVVCTA | AMRNIGEL    | GTGDP   | PNVYSSV | LTVLSpqanq | MGVMVCL     | NDRIAS      | 155 |
| EEX68565 | 82  | -edVDGVVITHG | TDTME | ETAYFLHLTVH  | SAKPIVLT  | GAMRPATAL   | SADGPM  | NLLQAV  | RVAA       | deavqQGV    | LIVLDTIES   | 160 |
| EEG08105 | 113 | -kdVDGVVITHG | TDTLE | ETAYFLDLTVK  | SKPVVIV   | SGMRPSTAI   | SADGPF  | NLNLNA  | VLLAGS     | keavqKGV    | LVTLNDQINA  | 191 |
| EEU48991 | 123 | -pyTQGVVTHG  | TDTLE | ESSFFLDLT    | VQSDKPVV  | VVGSHRPGTA  | ISADGP  | PNLLE   | SAVLAAS    | dsakkRGAL   | ITLNDRIAS   | 201 |
| ACZ12201 | 99  | -dnVDGVVITHG | TDTME | ETAYFLNVLV   | SKKPVV    | MGAMRPATA   | ISADGPM | NLLYDA  | VLTAGS     | keavqKGV    | MIVLNDRIAS  | 177 |
| EAY64500 | 120 | -paIDGVVITHG | TDTLE | ETAYALHLV    | VRGDKPV   | LTAAAMRPATA | LSSDGP  | NLLNLA  | VTVA       | hpaargQGV   | LVAFNNDRIAS | 198 |
| ACB74433 | 91  | -pdTAGVIVTHG | TDTME | ETAFFLNLV    | RSAPVVL   | VVGAMRPATA  | ISADGPM | NLNLNA  | VAAH       | hpdthgRGV   | LVVANDRIAS  | 169 |

Feature 3

|          | #   | ####      | *          | ###    | #       | *        | ####    | *      | ###    | #        | *       | #       |
|----------|-----|-----------|------------|--------|---------|----------|---------|--------|--------|----------|---------|---------|
| query    | 191 | AYYITKTAN | MDTFKAMem  | GYLGE  | IsntP   | FFFYPPV  | QPTGK   | KDfnia | ---    | nvtEIP   | PRVDIL  | FSyedM  |
| 1HFJ_A   | 163 | ARYITKTAN | SLDTFRAnee | GYLGI  | IgnrI   | YYQNRIDK | LHTR    | Svfdvr | --     | gltsLP   | KVDILY  | GyqdpE  |
| 1DJP_A   | 161 | GRDVS     | KSIINLT    | EAFKSA | -wG     | PLGMV    | VegkSYH | FRLP   | AKRHT  | VNSF     | edik--  | qissLP  |
| ACJ75594 | 156 | AREVT     | KTYTSH     | VATFDS | SpgyG   | PLGIV    | DednV   | IFRKS  | LTREK  | ILVdk    | -----   | iEERVAL |
| EEX68565 | 161 | ARDAV     | KIHTAL     | DTFQSP | evGAL   | SGSHDge  | PVFR    | GRPLR  | RHTAQ  | Stfsve   | --      | gvieLPR |
| EEG08105 | 192 | GRDVT     | KTNTS      | ADTFKT | pelG    | FLGY     | QdnkP   | HFYRL  | PKRHTA | Etefdvs  | --      | kldkLP  |
| EEU48991 | 202 | ARYIT     | KTANAL     | DTFKA  | eeqGYL  | GAFEniq  | PVFHY   | PPVPL  | GHYfn  | issisase | PLKVD   | LYGQq   |
| ACZ12201 | 178 | ARDV      | QKTD       | SIITDT | FKapif  | GYLGI    | QIVdg   | KVFFK  | NPLNK  | HTYES    | efdis-- | titkLP  |
| EAY64500 | 199 | ARDV      | VKTST      | YAVDA  | FQSP    | elGAL    | GWQdgr  | VEFAR  | RVTR   | TDQTL    | Laia    | ----    |
| ACB74433 | 170 | AREVA     | KTNIT      | LTGFR  | atrhrGL | AGV      | NagrL   | HL     | YAPP   | VRHT     | CTSe    | fsva--  |

Feature 3

|          | #   | *      | ####    | *     | ###  | #       | *     | ####   | *    | ###   | #    |
|----------|-----|--------|---------|-------|------|---------|-------|--------|------|-------|------|
| query    | 268 | GAKGIV | IAGAGAG | GVtts | FNYA | IEDAIN  | Rlgi  | PIIQSH | RTv  | -nGE  | VP   |
| 1HFJ_A   | 241 | GvKGI  | VYAGHG  | AGSVS | rVr  | GIAGMR  | KALEK | -VVM   | RS   | TRTg  | -nG  |
| 1DJP_A   | 238 | GAKAL  | IHAGT   | NGSVS | rVr  | VPALQ   | QLRK  | Ng     | -TQ  | IRSSH | Vnqg |
| ACJ75594 | 228 | GvKGI  | ILEG    | GRGIV | Pp   | VAEAVE  | EVKE  | qIP    | VIIT | SRFC  | -kGR |
| EEX68565 | 239 | GvKGI  | VYAGHG  | NGSIP | Par  | AEALARA | AAEg  | -VAV   | VR   | STRSA | -aGR |
| EEG08105 | 270 | GAKGIV | QAGV    | GDG   | MAa  | qHLPA   | FRARQ | Kg     | -VIV | VR    | SRVg |
| EEU48991 | 282 | GAKGIV | VAGV    | GAGG  | Wptk | AKALEE  | EVVK  | tkV    | PPV  | VS    | RRTA |
| ACZ12201 | 256 | GAKGIV | HAGAG   | TAS   | Msef | VLP     | SEKAT | Kg     | -VA  | IV    | TRP  |
| EAY64500 | 273 | GvRGL  | VVAGT   | NGS   | I    | atLQ    | TALAD | AVN    | Ag   | -VAV  | VR   |
| ACB74433 | 248 | GvRGI  | VYAGV   | GDG   | N    | arALRA  | AA    | AAQ    | Rg   | -VIV  | VR   |

Feature 3

|          | #   | *      | ####    | *     | ### | #                | *            | ####         | * | ### | # |
|----------|-----|--------|---------|-------|-----|------------------|--------------|--------------|---|-----|---|
| query    | 341 | LLLAK  | SSN-ITE | IA    | 353 |                  |              |              |   |     |   |
| 1HFJ_A   | 309 | LALTR  | TS      | PKVIQ | 321 | Pectobacterium   | chrysanthemi |              |   |     |   |
| 1DJP_A   | 312 | VAMTK  | TQD-SKE | L     | 324 | Pseudomonas      | sp.          |              |   |     |   |
| ACJ75594 | 307 | VVLGK  | TSN-LEE | IR    | 319 | Thermosiphon     | africanus    | TCF528       |   |     |   |
| EEX68565 | 311 | LTLLQ  | TDD-TAA | IR    | 323 | Mitsuokella      | multacida    | DSM 20544    |   |     |   |
| EEG08105 | 343 | LAMTK  | TND-TKK | IK    | 355 | Chromobacterium  | sp. 2002     |              |   |     |   |
| EEU48991 | 349 | LALAK  | KLP-AKE | IK    | 361 | Nectria          | haematococca | mpVI 77-13-4 |   |     |   |
| ACZ12201 | 329 | LGLTK  | SMN-PKY | LQ    | 341 | Sulfurospirillum | deleyianum   | DSM 6946     |   |     |   |
| EAY64500 | 346 | LALANG | HDR     | ALQ   | 359 | Burkholderia     | cenocopia    | PC184        |   |     |   |
| ACB74433 | 321 | LALTK  | TPD-PR  | AVQ   | 333 | Opitut           | terrae       | PB90-1       |   |     |   |

**Figure S5.** Alignment of L-asparaginases Conserved Protein Domain Family of type II L-asparaginases with the enzyme from *P. sizovae* (query). In yellow are the conserved residues of homotetrameric interfaces. HR: Hinge Region, ASFL: Active Site Flexible Loop. \* Residues that form the active site.