

Supplementary materials

Recent Progress in the Production of Cyanide-Converting Nitrilases— Comparison with Nitrile-Hydrolyzing Enzymes

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Table S1. Percentage of amino acid sequence identities between typical representatives of cyanide dihydratase (CynD), cyanide hydratase (CynH) and nitrile-converting nitrilase (Nit).

	CynD_{pum}	CynD_{stut}	CynD_{ind}	CynH_{sorg}	Nit4	Nit6803	NitPf	NitTv1
CynD_{pum}	100	79.75	32.71	35.62	29.55	34.67	37.77	27.60
CynD_{stut}		100	33.86	35.18	31.49	34.70	41.10	29.65
CynD_{ind}			100	32.29	31.80	33.55	36.79	29.48
CynH_{sorg}				100	26.43	30.10	37.82	26.75
Nit4					100	34.89	32.94	52.20
Nit6803						100	36.78	32.10
NitPf							100	30.61
NitTv1								100

CynD_{pum}: CynD from *Bacillus pumilus* (AAN77004.1)

CynD_{stut}: CynD from *Stutzerimonas stutzeri* (BAA11653.1)

CynD_{ind}: CynD from *Flavobacterium indicum* (CCG52320.1)

CynH_{sorg}: CynH from *Microdochium sorghi* (AAA33353.1)

Nit4: Nitrilase from *Arabidopsis thaliana* (pdb 6i00)

Nit6803: Nitrilase from *Synechocystis* sp. PCC6803 (pdb 3wuy; Q55949)

NitPf: Nitrilase from *Pseudomonas fluorescens* EBC191 (pdb 6zby; AAW79573)

NitTv1: Nitrilase from *Trametes versicolor* (XP_008032838.1)

Phylogenetic tree showing relationships between various *Bacillus* species and other bacteria. The tree is rooted at the bottom left with a bootstrap value of 0.49. Major clades are highlighted with grey triangles and labels:

- Paenibacillus clade**: Includes WP 227893389.1 *Burkholderia vietnamiensis*, WP 023485281.1 *Paenibacillus larvae*, QOT01313.1 *Brevibacterium* sp. JNUCC-42, and WP 078990636.1 *Priestia flexa*.
- Bacillus clade 1**: Includes AIG27261.1 *Brevibacillus laterosporus* LMG15441, WP 068791844.1 *Brevibacillus laterosporus*, UQM69643.1 *Brevibacillus laterosporus*, WP 022746654.1 *Clostridium saccharobutylicum*, WP 077867457.1 *Clostridium saccharobutylicum*, WP 039630894.1 *Clostridium argentinense*, RDC48628.1 *Acinetobacter* sp. RIT592, WP 039657448.1 *Clostridium tyrobutyricum*, WP 063554590.1 *Clostridium ljungdahlii*, WP 127719682.1 *Clostridium* sp. AWRP, WP 046131591.1 *Bacillus*, WP 077320578.1 *Virgibacillus proomii*, and WP 077320578.1 *Virgibacillus proomii*.
- Bacillus clade 2 (*CynD_{pum}*)**: Includes WP 077320578.1 *Virgibacillus proomii*.
- Flavobacterium homologs clade**: Includes WP 077320578.1 *Virgibacillus proomii*.

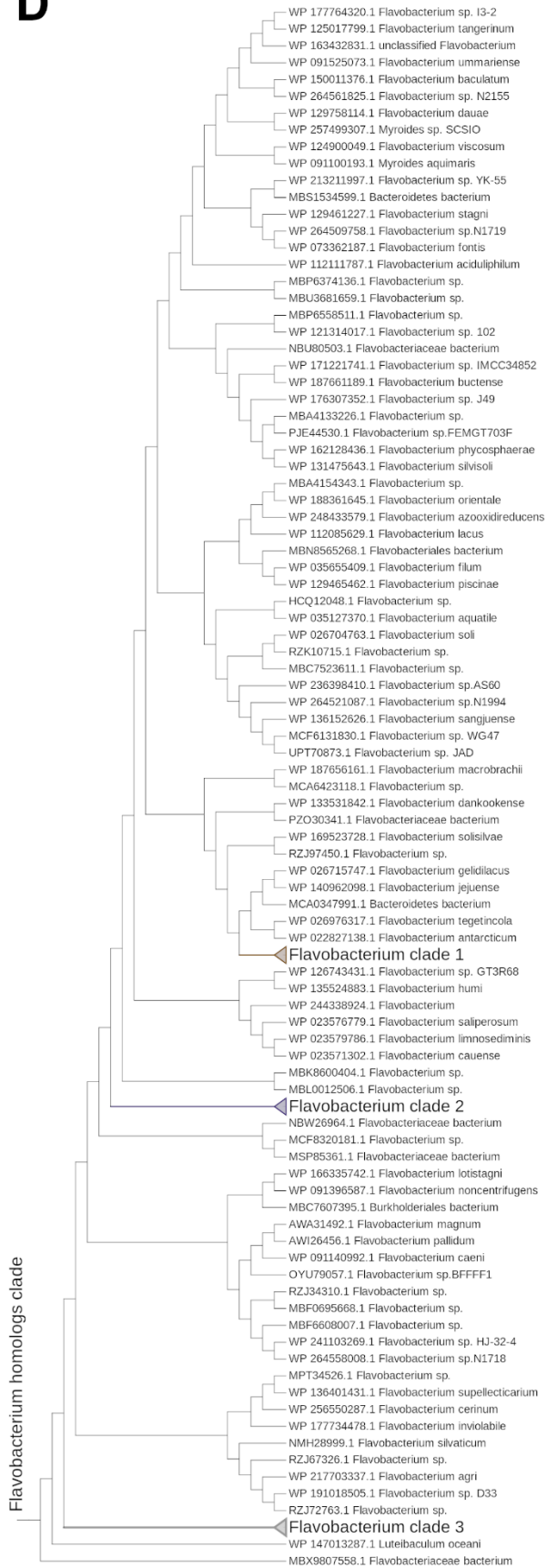
Other sequences shown include XP 001383097.1 *Scheffersomyces stipitis* CBS 6054, WP 207641342.1 *Lacrimispora aerotolerans*, and BAA11653.1 *CynD Stutzerimonas stutzeri*.

WP 00551642.1 *Paenibacillus alvei*
 NOJ74130.1 *Paenibacillus alvei*
 WP 187698875.1 *Paenibacillus alvei*
 WP 216634001.1 *Paenibacillus alvei*
 WP 010348885.1 *Paenibacillus peoriae*
 WP 175400617.1 *Paenibacillus* sp. KS1
 KJB88517.1 *Paenibacillus* sp. E194
 EPY14880.1 *Paenibacillus alvei* A6-6i-x
 WP 138187048.1 *Paenibacillus alvei*
 WP 142544163.1 *Paenibacillus* sp.
 EPY08098.1 *Paenibacillus alvei* TS-15

WP 001089880.1 *Bacillus thuringiensis*
 EEM92870.1 *Bacillus thuringiensis*
 WP 153594547.1 *Bacillus thuringiensis*
 WP 179884080.1 *Bacillus thuringiensis*
 WP 242260565.1 *Bacillus cereus* group
 WP 176504922.1 *Bacillus cereus* group
 QGV10588.1 *Bacillus cereus*
 WP 173927869.1 *Bacillus thuringiensis*
 WP 172583219.1 *Bacillus cereus*
 WP 182812604.1 *Bacillus* sp. ME78
 WP 183048393.1 *Bacillus toyonensis*

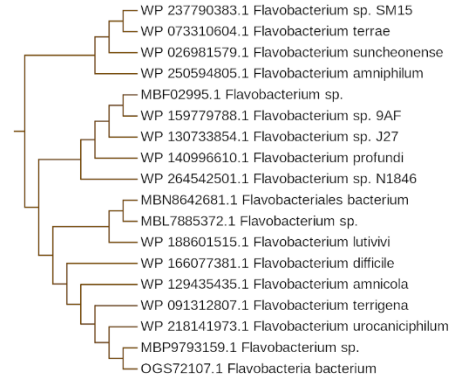
WP 105925761.1 *Bacillus* sp.
WP 099726715.1 *Bacillus pumilus*
UJM26251.1 *Bacillus aerophilus*
MBC3641955.1 *Bacillus pumilus*
WP 251184298.1 *Bacillus pumilus*
MBQ4817818.1 *Bacillus pumilus*
WP 254109567.1 *Bacillus pumilus*
MBU8726676.1 *Bacillus pumilus*
WP 144521087.1 *Bacillus pumilus*
QLI77745.1 *Bacillus pumilus*
WP 257474624.1 *Bacillus pumilus*
WP 106067145.1 *Bacillus pumilus*
MBU8607118.1 *Bacillus pumilus*
EDW20979.1 *Bacillus pumilus*
WP 106033142.1 *Bacillus pumilus*
MBR8688472.1 *Bacillus australimaris*
WP 060697273.1 *Bacillus australimaris*
MBU5207213.1 *Bacillus safensis*
WP 073979723.1 *Bacillus safensis*
WP 039183195.1 *Bacillus* sp.WP8
WP 251219491.1 *Bacillus safensis*
AAN77004.1 CynD *Bacillus pumilus*
WP 197198237.1 *Bacillus safensis*
KML13663.1 *Bacillus safensis*
WP 207382894.1 *Bacillus safensis*
WP 252056255.1 *Bacillus safensis*
WP 065214768.1 *Bacillus safensis*
AYW35867.1 *Bacillus* sp.
WP 098677504.1 *Bacillus safensis*
WP 101676965.1 *Bacillus safensis*
MBT2262508.1 *Bacillus safensis*
WP 120208335.1 *Bacillus safensis*
WP 251180457.1 *Bacillus safensis*
WP 212051676.1 *Bacillus safensis*
ACD88988.1 *Bacillus pumilus*
WP 254241452.1 *Bacillus safensis*
NRF05629.1 *Bacillus safensis*
WP 234107210.1 *Bacillus safensis*
WP 200956595.1 *Bacillus* sp. Root920
KRE15580.1 *Bacillus* sp. Root920

D

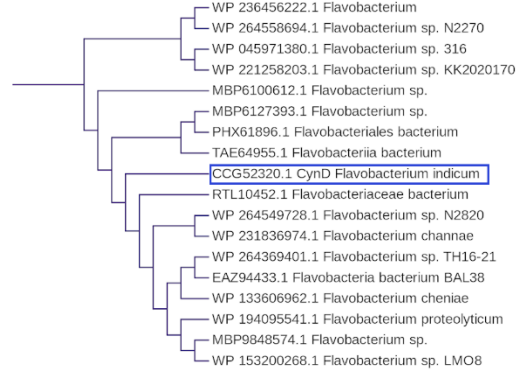


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Flavobacterium clade 1



Flavobacterium clade 2



Flavobacterium clade 3

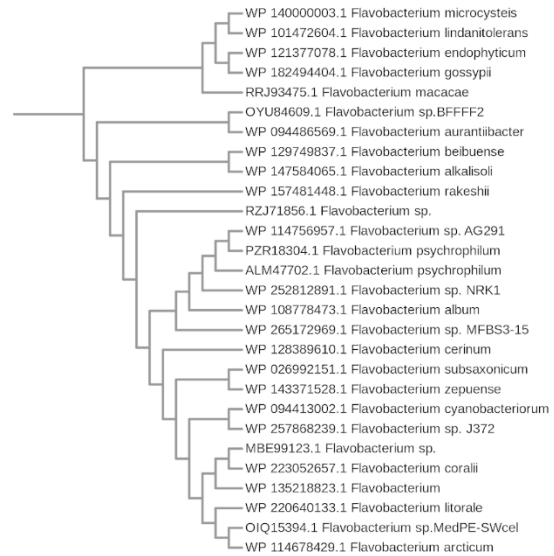
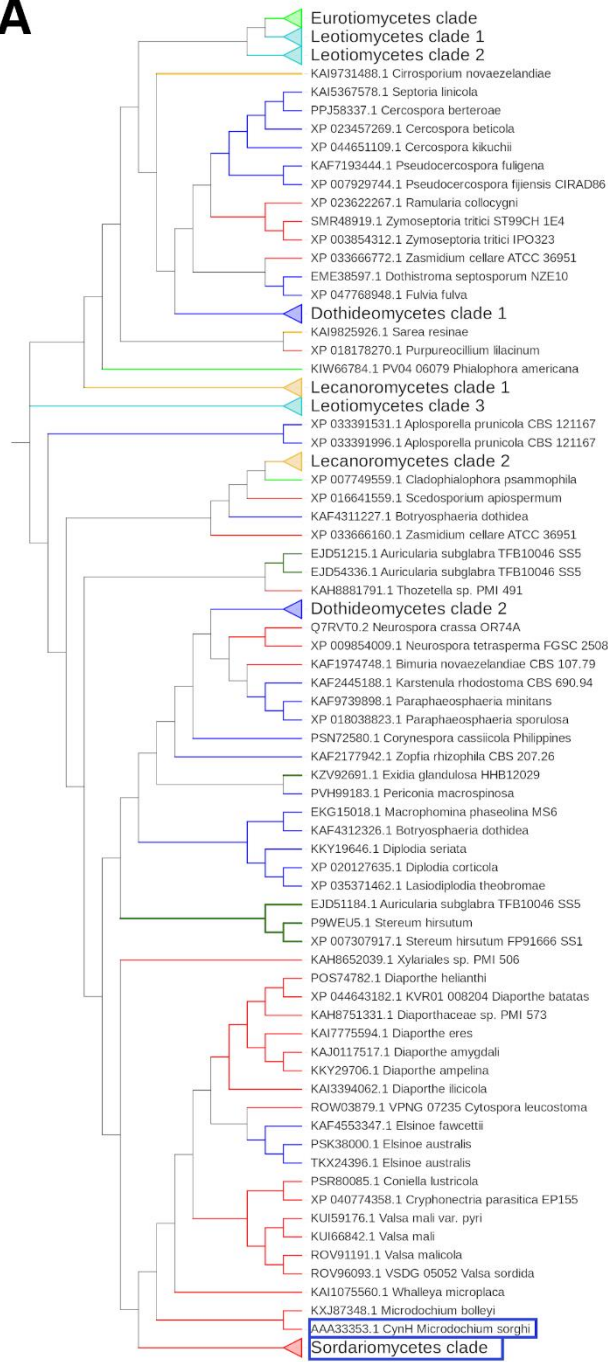
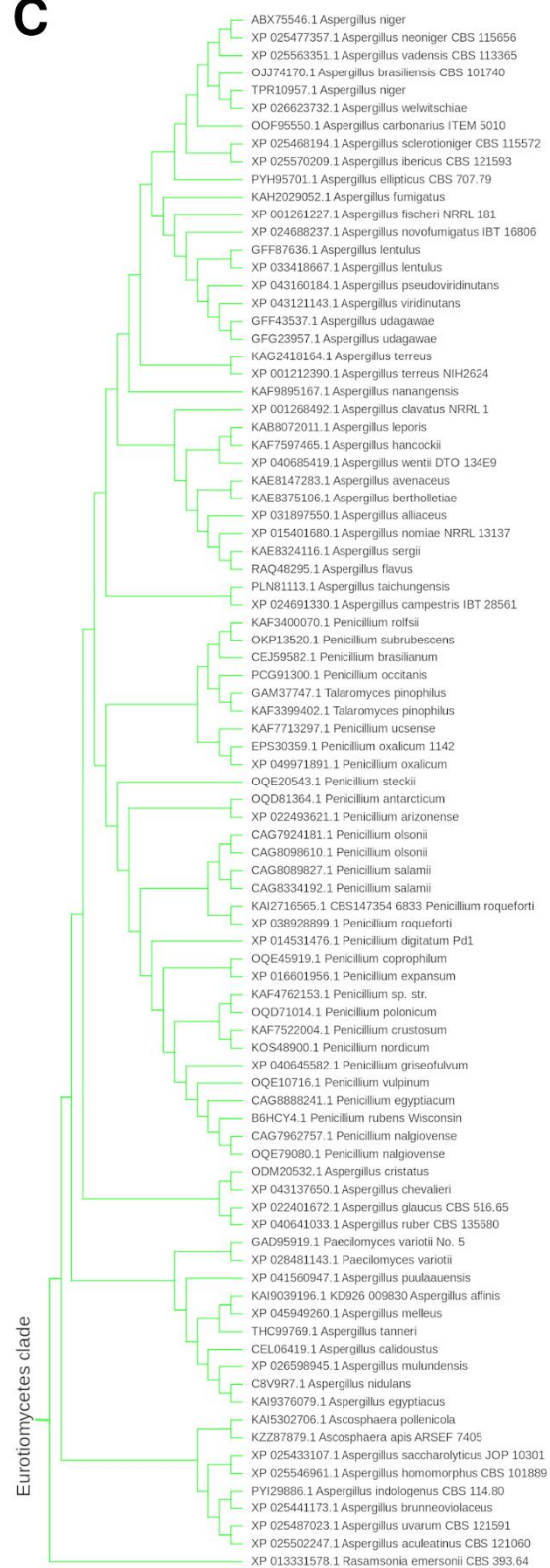
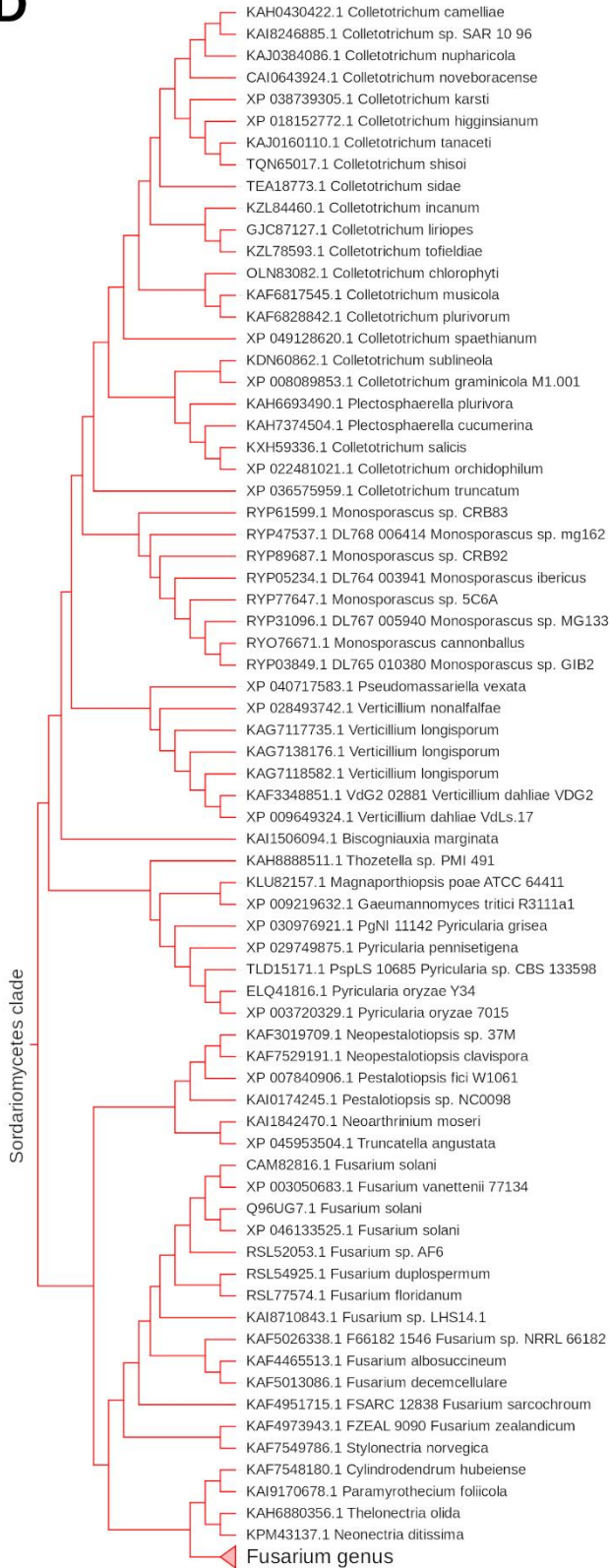


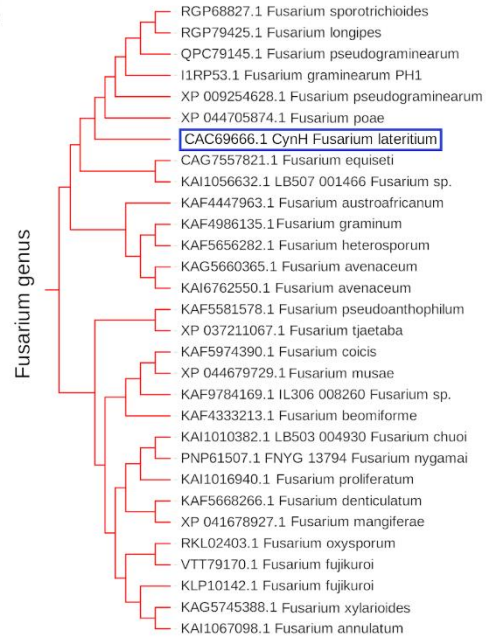
Figure S1. Complete phylogenetic tree of cyanide dihydratase homologs. (A) Full tree with some clades collapsed; (B) *Paenibacillus* clade and *Bacillus* clade 1 expanded; (C) *Bacillus* clade 2 expanded; (D) *Flavobacterium* homologs clade expanded; (E) *Flavobacterium* clades 1-3 expanded. Phylogenetic tree of cyanide dihydratase homologs. Pairwise distances were estimated using JTT model [70]. Initial tree for the heuristic search was obtained automatically using Neighbor-Join and BioNJ algorithms [71]. Then the tree with the highest log likelihood was selected using Maximum Likelihood method [72]. All positions with less than 90% site coverage were eliminated. Evolutionary analyses were conducted using MEGA X [73]. The tree was visualized with iTOL tool [74]. Branch lengths are indicated above the lines (data are rounded to two decimal places). The position of the template sequences is indicated by the blue boxes. The tree is rooted on midpoint. The complete tree is available at <https://itol.embl.de/tree/147231254143208591671005621>.

A**B****Lecanoromycetes clade 1****Lecanoromycetes clade 2****C**

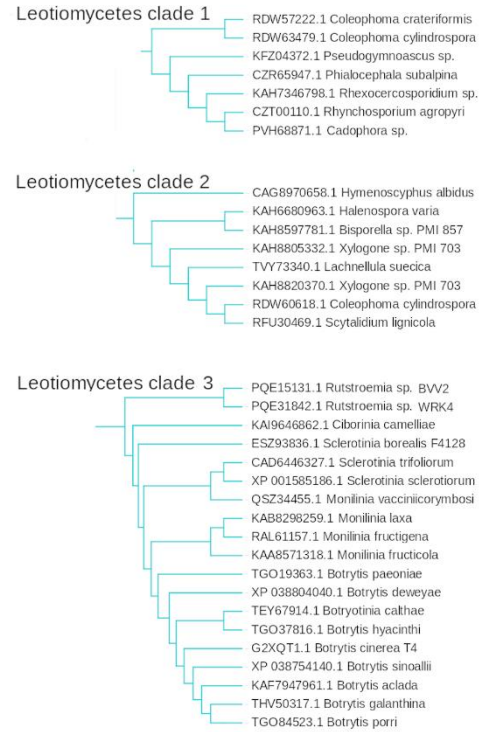
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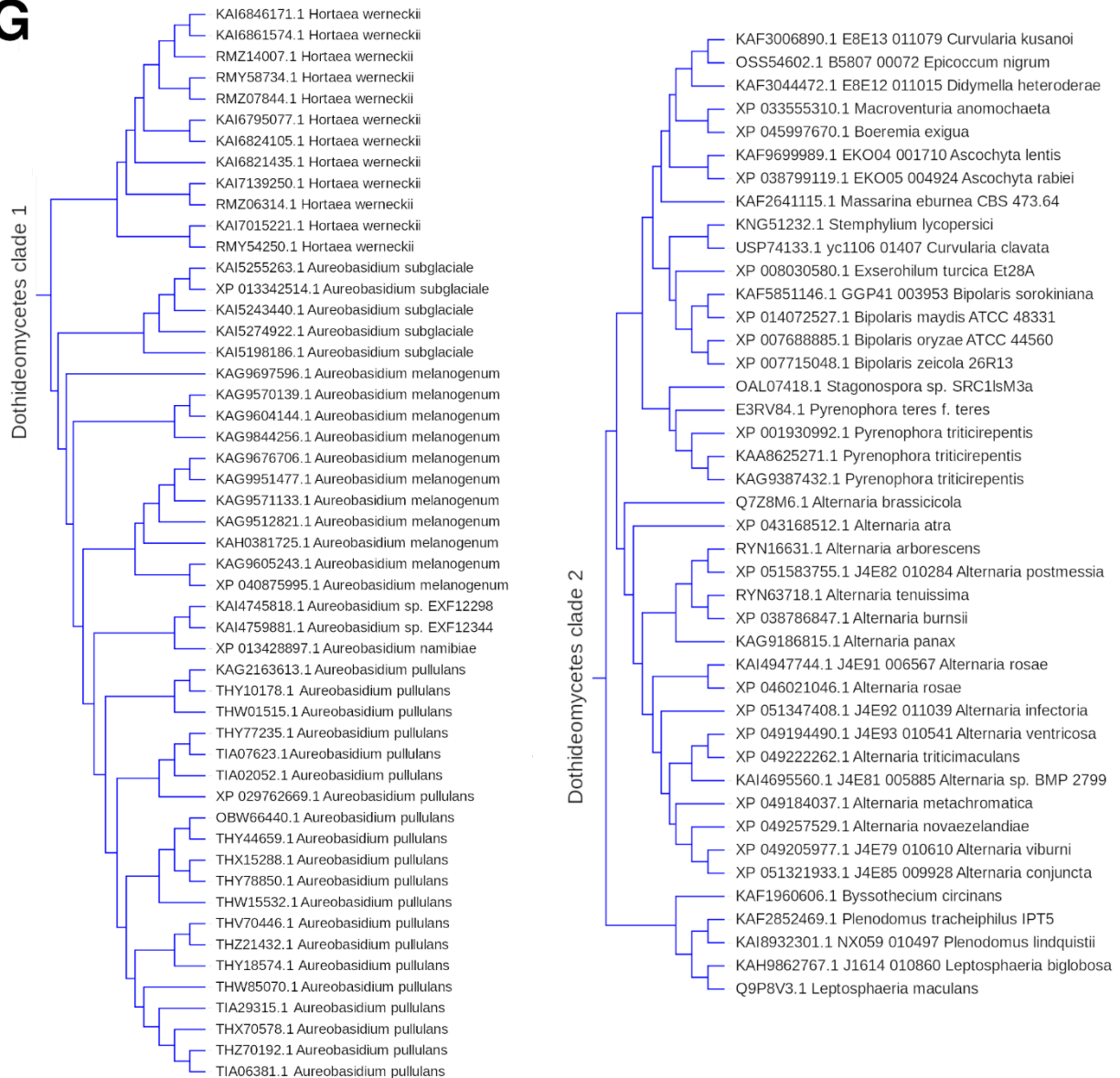
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Figure S2. Complete phylogenetic tree of cyanide hydratase homologs. (A) Full tree with some clades collapsed; (B) Lecanoromycetes clade 1 and 2 expanded; (C) Eurotiomycetes clade expanded; (D); (E) *Fusarium* clade expanded; (F) Leotiomycetes clade 1-3 expanded; (G) Dothideomycetes clade 1-2 expanded. See Fig. S1 legend for methods used for the construction of the phylogenetic tree. The previously reported phylogenetic tree of the CynHs [15]) was extended. The complete tree is available at <https://itol.embl.de/tree/17825516828117701671187763>.