

Crystal Structure Analysis and Characterization of NADP-Dependent Glutamate Dehydrogenase

with Alcohols Activity from *Geotrichum candidum*

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Fig. S1 Gene (a) and amino acid (b) sequence alignment of the GDH with *Yarrowia lipolytica*. Identical residues are highlighted in dark blue and denoted by lowercase. Highly conserved residues are denoted by colored cyan. White color depicts less conserved denoted by blank. KJ442577.1: *Geotrichum candidum* glutamate dehydrogenase (GDH) mRNA, complete cds; XM_505553.1: *Yarrowia lipolytica* YALI0F17820p (YALI0F17820g) mRNA, complete cds; AHX58293.1: GDH-S12 from *G. candidum*; XP_505553.1: YALI0F17820p from a *Yarrowia lipolytica* CLIB122.

Fig. S2 Mass spectrum obtained for tryptic peptides eluted from 1-D gel band (Fig. 2a). After a baseline correction, a background subtraction, and peak deisotoping, 17 ions were submitted to Mascot. Twenty one of the submitted ions were matched to theoretical tryptic peptides from glutamate dehydrogenase; the sequences of these peptides are shown next to the mass of the monoisotopic, singly charged ions. The full protein sequence and the sequenced peptides are in red color.

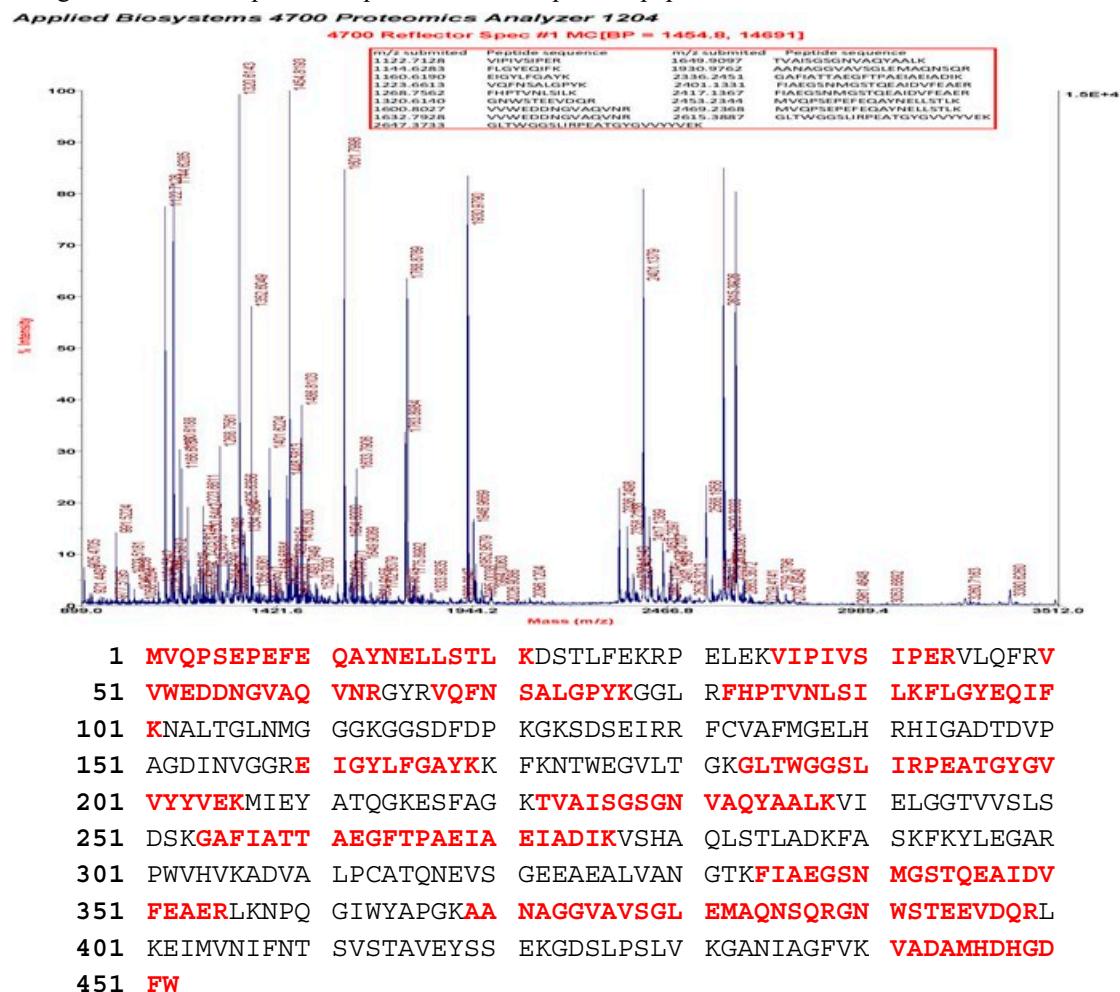


Fig. S3 It showed that the differences of GDHs were more notable when proteins were from different microorganism. All the sequence data were extracted from NCBI database.

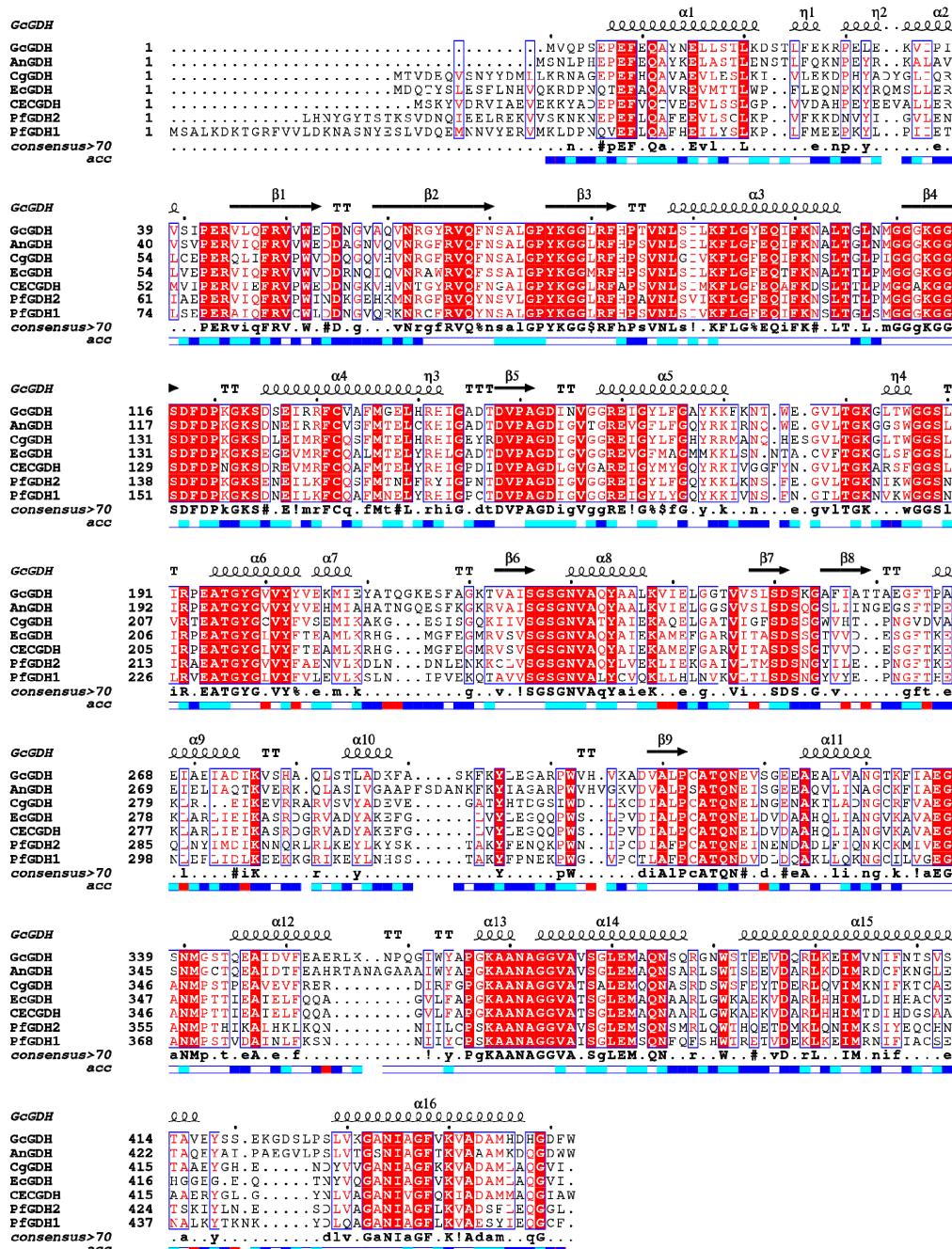
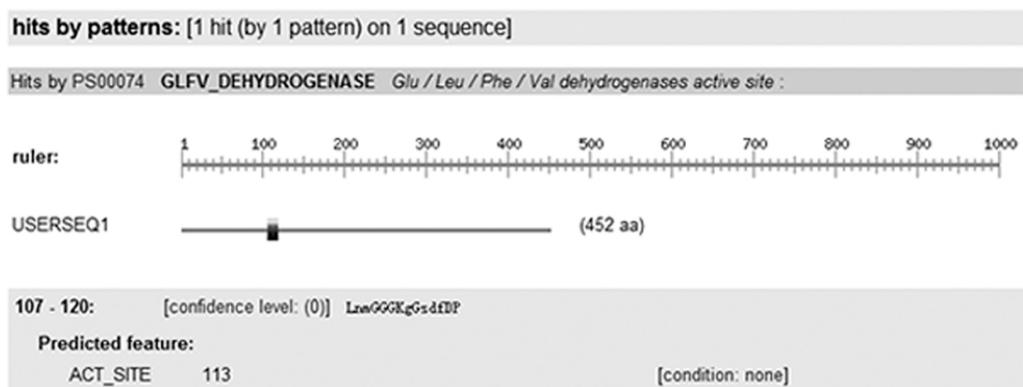


Fig. S4 The results of motif identification determined from the online tool Scanprosite (a) and SDS-PAGE of purified K113Ala (I) and K113Gly (II)(b). M: Protein molecular weight markers; 1, Crude GDH; 2, Flowed crude GDH from Ni column; 3-7, Purification processes (elution buffer containing 10-120 mM imidazole) 8, Purified pET-28as-GDH (elution buffer containing 200 mM imidazole); 5, Arrow mark indicates the target protein.

a



b

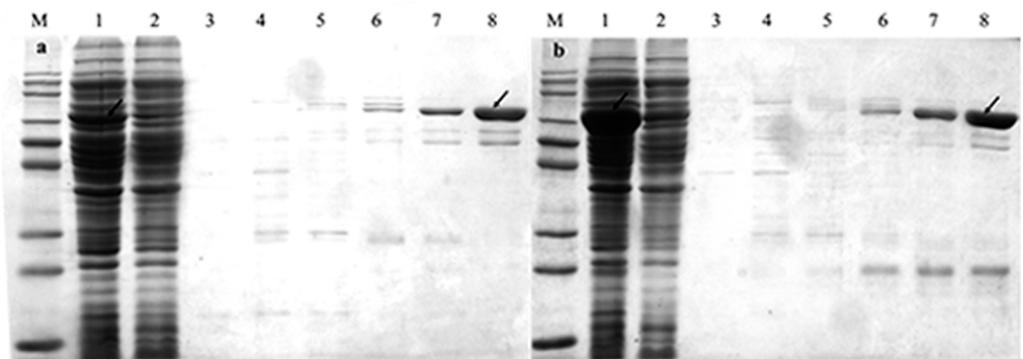


Fig. S5 GC-MS analysis. Total ion chromatograms of the products after GDH treatment using hexanol and isoamyl alcohol.

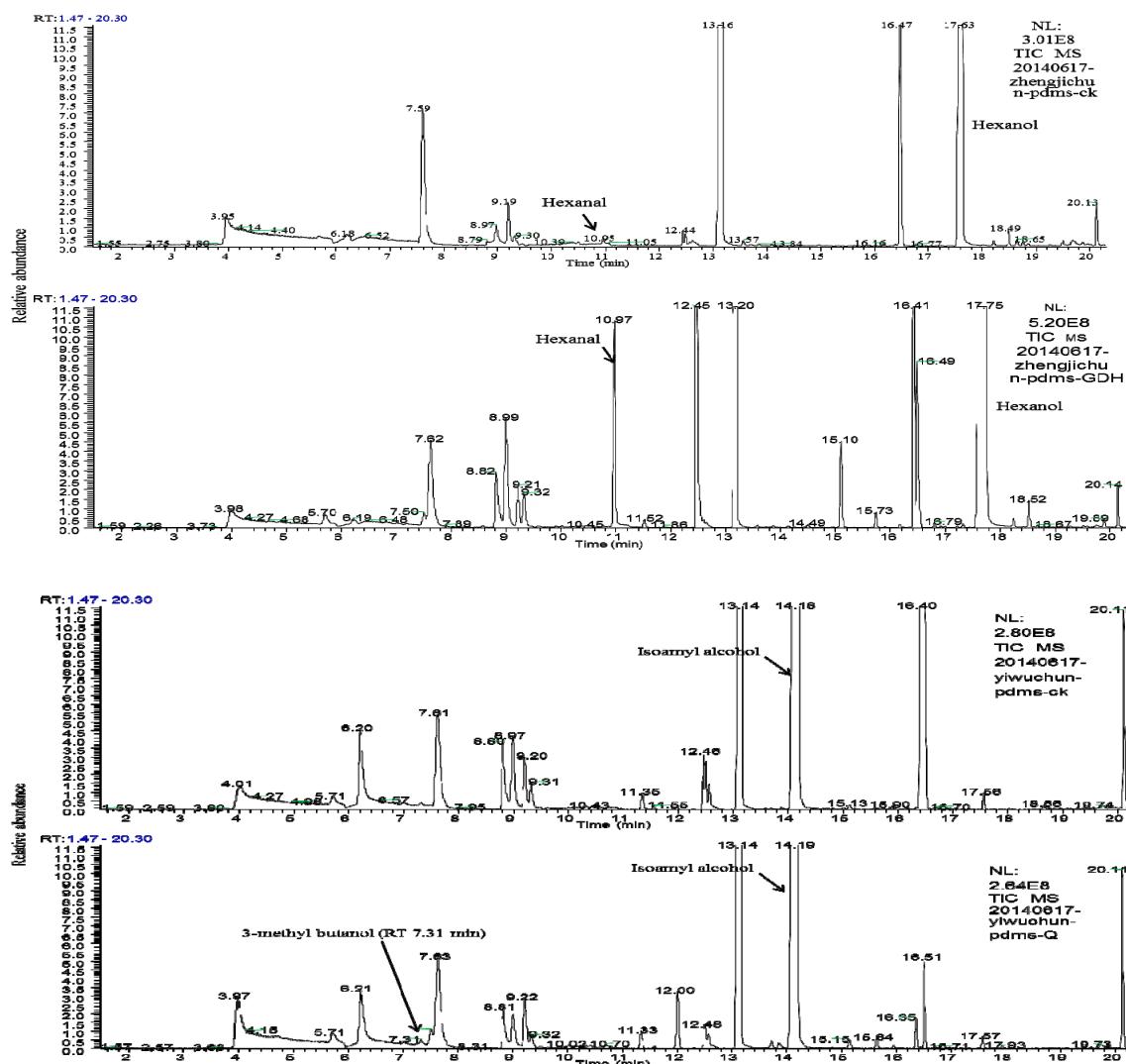


Table S1 List of primers used in the 5' Race. I : verify primers II :linker-adapter, III: 5'Race trans-specific primers.

| Name | Sequence (5'-3') |
|----------------|--|
| RC75-F (I) | GTTGTCTCTCTTCCGACTCCA |
| RC75-R (I) | ACGAAACCAGCAATGTTAGCA |
| 5'adaptor (II) | GCTGTCAACGATACTGCTACGTAACGGCATGACAGTGGGG |
| SG-outer (II) | GCTGTCAACGATACTGCTACGTAAC |
| SG-inner (II) | GCTACGTAACGGCATGACAGTG |
| RC75-RT1 (III) | CAGAGACAGCGACACCACCGCGTT |
| RC75-R1 (III) | GGAACCCATGTTAGAACCCCTCGGCAAT |
| RC75-R2 (III) | AGCAACATCAGCCTGACGTGGACCC |
| RC75-R3 (III) | CGACGGTCTGCCAGCGAAGGACTC |
| RC75-R4 (III) | GGAACATCGGTATCAGCACCAATGTGTCT |
| RC75-Fi | TCAAAACATCAAAATGGTCCAG |