Fungal Biology and Biotechnology

REVIEW

Open Access

Glutamate dehydrogenases in fungi: From biology to biotechnology

Ejaj K. Pathan[1*], Himal Sapkota[1], Subrata Dasgupta[2] and Narayan S. Punekar[3]

Abstract

Glutamate dehydrogenases (GDH; EC 1.4.1.2 and EC 1.4.1.4) play a pivotal role in fungal nitrogen metabolism by catalyzing the reversible conversion of 2-ketoglutarate to L-glutamate. In fungi, NAD- as well as NADP-dependent GDHs function at the interface of ammonia assimilation and glutamate catabolism, contributing to growth, differentiation, and morphogenesis. The evolution of fungi to adapt and occupy various ecological niches is closely aligned to the diversity of regulations of the functions of GDHs, their localisation and biochemical characteristics. This review explores the biochemical, molecular, and structural studies on fungal GDHs, emphasizing their catalytic diversity, coenzyme specificity, and regulatory mechanisms, including phosphorylation, thiol modulation, and allosteric control. Structural elucidations of NADP-GDHs from *Aspergillus niger*, *Aspergillus terreus*, and *Candida albicans* provide new insights into cofactor binding, substrate recognition, and inhibitor interactions. Molecular analyses reveal distinct evolutionary trajectories for NAD- and NADP-GDHs across fungal taxa, with GDH-mediated transitions linked to morphogenetic processes such as the yeast-to-hypha (Y-H) switch, highlighting GDHs as promising antifungal drug targets. The comprehensive survey of fungal GDHs presented here emphasises their biochemical versatility, evolutionary significance, and translational potential in agriculture, biosensor development and in industry. The review also highlights gaps in our understanding of fungal GDHs and potential areas for further research.

Keywords Allostery, Ammonia assimilation, Antifungal, Dimorphism, Evolution, Glutamate dehydrogenase, Fungal physiology, NADPH, NADH

Introduction

Glutamate dehydrogenases (GDHs) are found in archaea, protozoans and eukaryotic organisms. These enzymes play a role in balancing and harmonising carbon and nitrogen metabolism in living systems. Based on coenzyme specificity, GDHs fall into three categories: (1) NADP-GDHs, which use NADPH as cofactor and are involved in reductive amination, converting 2-ketoglutarate to L-glutamate, (2) NAD-GDHs, which use NAD as cofactor and catabolize oxidative deamination, converting glutamate to 2-ketoglutarate, and (3) GDHs with dual specificities, which can use both NADH and NADPH as cofactors.

*Correspondence:
Ejaj K. Pathan
ek.pathan@ssbs.edu.in
[1]Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Lavale, Pune, Maharashtra 412115, India
[2]RIKEN Center for Biosystems Dynamics Research, Yokohama, Kanagawa 230-0045, Japan
[3]Department of Biosciences and Bioengineering, Indian Institute of Technology Dharwad, Dharwad, Karnataka 580011, India

The third category of GDHs is present in archaea and vertebrates [1]. In animals, the expression of ammonia assimilating enzymes is tissue specific [2]. Ammonia assimilation is different in higher plants and bacteria. In 1974, glutamate synthase was discovered in higher plants, and soon NAD-GDH was considered the major route for ammonia assimilation in higher plants and in yeasts. Subsequently, it was suggested that a glutamine synthetase/glutamate synthase (GS/GOGAT) system is the main, if not the sole, pathway of ammonia assimilation in higher plants. The GS/GOGAT pathway plays a significant role in bacteria as well [3].

Fungal carbon and nitrogen metabolism is connected via ammonia assimilation. The participating enzymes are NAD-dependent (EC 1.4.1.2) and NADP-dependent (EC 1.4.1.4) glutamate dehydrogenase (GDH), glutamate synthase (GOGAT; EC 1.4.1.13), and glutamine synthetase (GS; EC 6.3.1.2). An interconnection between these nitrogen metabolizing enzymes that regulate ammonia assimilation in fungi is depicted in Supplementary Figure S1.

The significant contribution of the NAD- and NADP-GDH pathway to ammonia assimilation has been highlighted in fungi such as *Saccharomyces cerevisiae* [4], *Mucor racemosus* [5], *Benjaminiella poitrasii* [6, 7], *Candida boidinii* [8], *Candida utilis* [9] and *Aspergillus niger* and *Aspergillus nidulans* [10, 11].

In this review, we focus on NAD- and NADP-dependent GDHs in fungi. These enzymes catalyse the reversible reaction depicted in the lower portion of Fig. 1 and also as Supplementary Fig. S1. Since this reaction is central to carbon and nitrogen metabolism in fungi, we argue that controlling these enzymes can change the cellular functioning of fungi and help us not only in medical applications but also to develop fungal cell factories for agriculture and for industrial use. To substantiate this argument, we first explore the diversity of physiological roles that GDHs play besides nitrogen assimilation. We provide evidence of their roles in redox homeostasis, secondary metabolism, and morphogenesis. We then proceed to examine the evolution of fungal GDHs and available information on the localisation of GDHs in fungal cells. We then argue that the localisation of specific GDHs and their biochemical properties are inter-related. We summarise available literature on the kinetics of GDHs, molecular and structural studies on GDHs as well

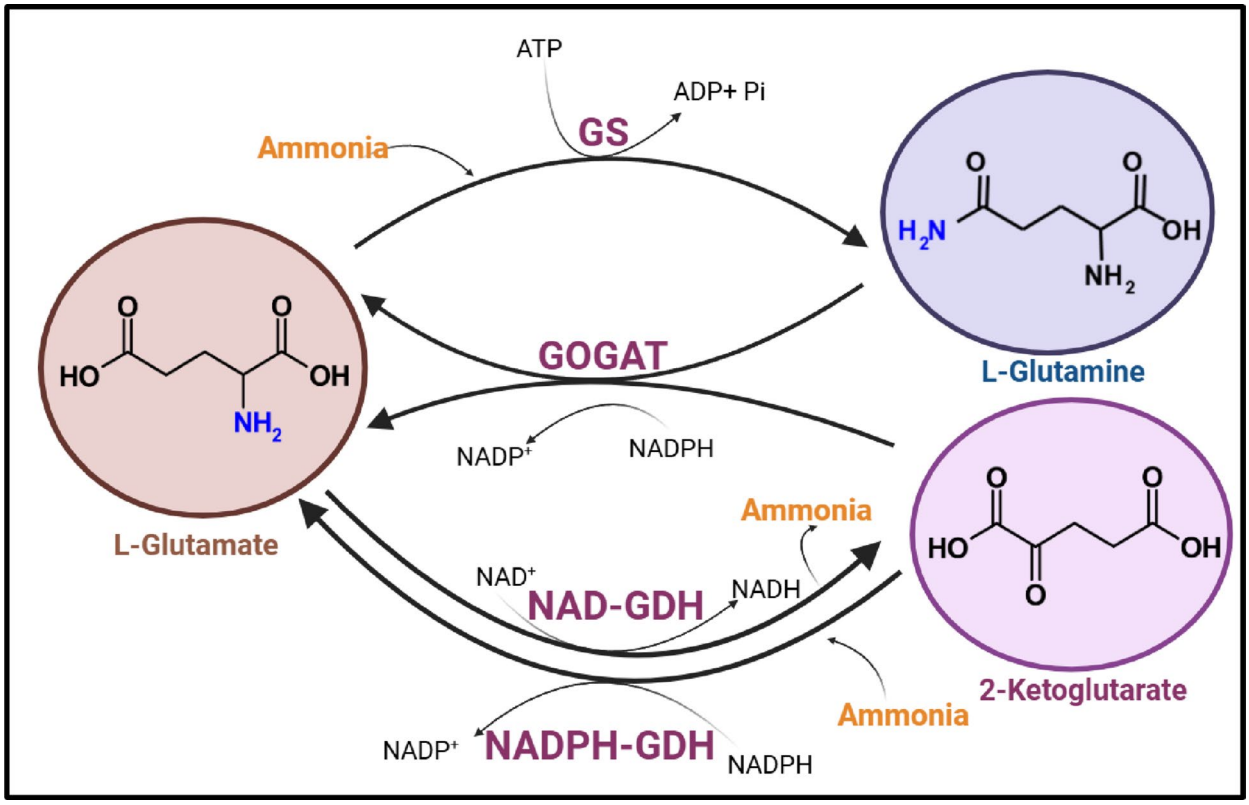

Fig. 1 Scheme representing the interconnection of ammonia-assimilating pathways in fungi. Glutamine synthetase (GS) converts L-glutamate to L-glutamine using ammonium. Glutamate synthase (GOGAT) regenerates L-glutamate from L-glutamine and 2-ketoglutarate. NADP-dependent glutamate dehydrogenase (NADP-GDH) catalyzes the reductive amination of 2-ketoglutarate to form L-glutamate. NAD-dependent glutamate dehydrogenase (NAD-GDH) mediates the oxidative deamination of L-glutamate to 2-ketoglutarate. In fungi, these interconnected reactions act together to regulate ammonia assimilation and maintain the metabolic flux between 2-ketoglutarate, L-glutamate, and L-glutamine.

as the inhibition and regulation of GDHs. We stress the role of GDHs in the reversible dimorphic switch between yeast (Y) and hyphal (H) forms in fungi. Since the Y-H switch is associated with fungal pathogenesis in animals and plants, we proceed to explore GDHs as antifungal drug targets. We discuss possible uses of fungal GDHs in agriculture and in industries. This comprehensive account of what is known about the biochemical, structural and molecular properties of fungal glutamate dehydrogenases reveals gaps in our knowledge about fungal GDHs. Therefore, where appropriate, we point out areas that need further research.

Physiological significance of glutamate dehydrogenases in fungi

Fungal glutamate dehydrogenases catalyze the reversible conversion of 2-ketoglutarate and glutamate, linking nitrogen assimilation, carbon flux, and energy balance. By maintaining equilibrium between amino acid pools and tricarboxylic acid intermediates, GDHs confer adaptive flexibility under variable nutritional and environmental conditions.

Cofactor specificity distinguishes two major GDH types. $NADP^+$-dependent GDH (NADP-GDH) functions primarily in the anabolic direction, assimilating ammonia into glutamate to support biosynthetic pathways, whereas NAD^+-dependent GDH (NAD-GDH) catalyzes oxidative deamination, releasing ammonia and replenishing 2-ketoglutarate during nutrient limitation. In nitrogen-rich conditions, NADP-GDH activity predominates, promoting ammonia assimilation and anabolic metabolism [12]. But, in nitrogen-limited or carbon-deficient states, NAD-GDH becomes dominant, enabling amino-nitrogen recycling and energy generation [11]. This reciprocal regulation allows fungi to switch between growth and maintenance phases. In *S. cerevisiae* and *Candida utilis*, for example, GDH isoenzymes complement each other in the glutamine synthetase–glutamate synthase pathway depending on nitrogen source availability [13].

Mara et al. (2018) extensively reviewed the roles of the NADP-GDH isoforms, GDH1 and GDH3, beyond their anabolic function in glutamate biosynthesis in *S. cerevisiae*. These GDH isoforms modulate multiple cellular processes including gene regulation, stress responses, metabolic adaptation, and cytoskeletal organization, highlighting the crucial role of GDH-mediated glutamate homeostasis in yeast physiology [14].

In *C. albicans*, *GDH2* (NAD-dependent) and *GDH3* (NADP-dependent) mutants show distinct metabolic and morphological defects, including impaired amino-acid use and disrupted redox homeostasis [15, 16]. In *A. terreus*, NADP-GDH exhibits unique kinetic regulation, including noncompetitive ammonia inhibition, suggesting fine-tuning at the carbon–nitrogen interface [11]. In the rice blast fungus, *Magnaporthe oryzae*, *MoGDH2* participates in nitrogen metabolism, conidiation, and virulence [17]. Similarly, the disruption of *gdh1* in *Penicillium chrysogenum* significantly affected morphology and reduced penicillin biosynthesis, linking NADP-GDH activity with morphology and with central carbon flow and secondary metabolite production [18, 19]. In *B. poitrasii*, *gdh1* and *gdh3* (coding for NADP-GDH isoforms) and *gdh2* (coding for NAD-GDH) are differentially expressed during the yeast–hypha transition, establishing a cause-effect relationship of GDH genes in dimorphism [20, 21]. Elevated *gdh1* drives yeast development, while *gdh2* and *gdh3* support the hyphal phase, corroborating earlier enzymatic observations by Amin et al. (2004) [22]. In the insect-pathogenic fungus, *Metarhizium robertsii*, NAD-GDH (*MrGDH2*) induces environmental alkalization during growth on the host cuticle and hemolymph, and its loss leads to impaired mycelial growth, reduced conidiation, defective hemolymph alkalization, and markedly attenuated virulence [23]. Notably, *MrGDH2* has no role in appressorium formation but is essential for colonization in the insect hemocoel, partly through the modulation of the pH responsive transcription factor, *PacC*, suggesting a GDH-dependent pH-regulatory mechanism distinct from plant and human fungal pathogens.

Collectively, these results suggest that fungal GDHs function not merely as metabolic enzymes but as integrative regulators as well. They coordinate nitrogen assimilation, redox homeostasis, secondary metabolism, and morphogenesis, aligning metabolic flux with developmental and environmental cues.

Evolution of fungal glutamate dehydrogenases

GDHs from fungi of different classes have been studied and characterized extensively [10–12, 24–28]. More than forty species of early-diverging fungi, including Mucoromycetes, Chytridiomycetes, Blastocladiomycetes, were found to possess only a NAD-linked glutamate dehydrogenase while higher fungi, belonging to the Deuteromycetes, Ascomycetes and Basidiomycetes classes, showed two distinct enzymes, a NAD-linked and a NADP-linked glutamate dehydrogenase. The Hypochytridiomycetes class exhibits an atypical NAD-GDH, allosterically regulated by NADPH, representing an evolutionary intermediate between the two enzyme classes [29].

Le John (1971) reported the significant contribution of NAD-GDH to the understanding of the evolution of fungi, especially from the phycomycetes genera [29]. Comparative studies of enzyme evolution indicate that GDHs serve as valuable molecular markers for tracing fungal diversification. The observed similarity between plant and archaeal GDHs [30] and the regulatory diversity among phycomycete GDHs [29], collectively

demonstrate the conserved, yet evolutionarily adaptable nature of this enzyme family, reflecting both fungal phylogeny and metabolic specialization. Lateral gene transfer has further contributed to GDH diversification across kingdoms [31]. Gene duplication events likely gave rise to yeast *gdh1* and *gdh3*, supporting their use as ancient paralogs for rooting the universal tree of life [32].

For the purpose of this review, we analysed the NADP-GDH gene sequences (*gdh1*) to understand evolutionary relationships among fungi. The minimum-evolution tree based on *gdh1* sequences showed grouping of fungal species broadly consistent with their taxonomy. Ascomycota species, including *S. cerevisiae*, *C. albicans*, *P. guilliermondii*, *D. hansenii*, *P. chrysogenum*, *A. niger* and others, with moderate sequence variation observed within the group, formed a well-defined cluster, while Basidiomycetous fungi such as *C. cinerea*, *H. cylindrosporum* and *L. bicolor* formed a separate but related group. This separation reflects inter-class *gdh1* sequence divergence among the studied fungi. Notably, *C. neoformans*, a Basidiomycetous fungus, occupied an intermediate position between the two clusters, while *B. poitrasii* branched distinctly from both Ascomycetes and Basidomycetes fungi, consistent with its early-diverging lineage. Overall, the tree reflects lineage-based clustering and evolutionary relationships among fungal *gdh1* sequences (Fig. 2). A similar pattern was observed in the phylogenetic tree constructed based on amino acid sequences derived from fungal *gdh2* nucleotides. The minimum-evolutionary tree constructed in *MEGA11* showed class-specific clustering of fungi belonging to Ascomycetes, Basidiomycetes, and Zygomycetes, classes (Supplementary Fig. S2).

To further examine the evolutionary relationships in fungal GDHs, a phylogenetic analysis was performed using representative *gdh1* (NADP-GDH) and *gdh2* (NAD-GDH) sequences from different fungal classes, with two representatives from each group wherever available (Supplementary Fig. S3). Due to limited sequence availability, only a single *gdh1* sequence from zygomycetes was included. The tree was rooted using the *gdh1* sequence from *E. coli* as an outgroup. The resulting topology showed a primary separation based on cofactor specificity, with fungi forming two distinct clusters based on *gdh2* and *gdh1* sequences (Supplementary Fig. S3). Within each of these groups, fungi further clustered according to their respective taxonomic classes, including Ascomycetes, Basidiomycetes, and Zygomycetes (Supplementary Fig. S3). This pattern suggests that

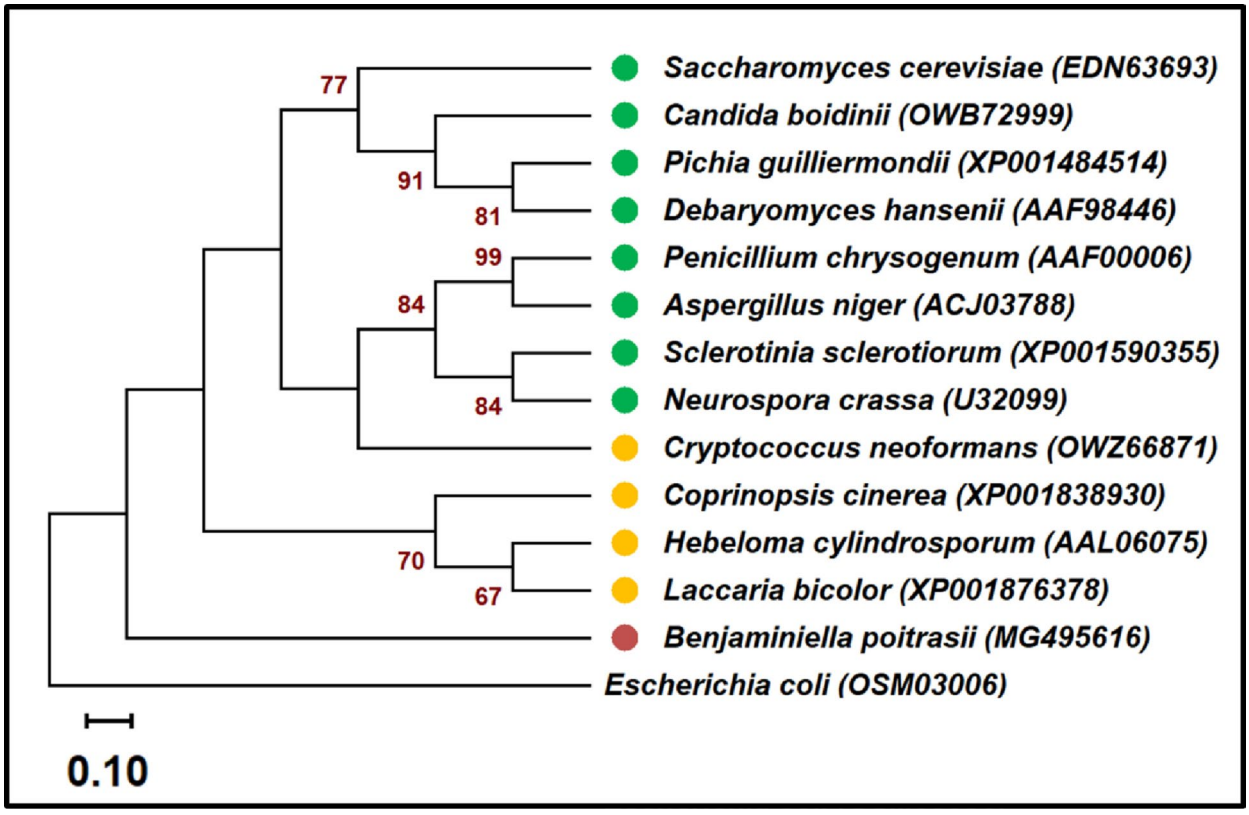

Fig. 2 The evolutionary history of different classes of fungi was inferred based on NADP-GDH1 (*gdh1*) nucleotides, translated into amino acid sequences using the minimum-evolution method in MEGA11. The fungi from different classes, Ascomycetes (filled green circle), Basidiomycetes (filled yellow circle) and Zygomycetes (filled maroon circle), branched separately from each other on a phylogenetic tree. The tree was rooted with an *E. coli* NADP-GDH sequence. The numbers (in maroon) on the branches indicate bootstrap values and the text in parentheses represents the accession numbers.

divergence based on cofactor specificity is a major evolutionary feature of fungal GDHs, followed by lineage-specific diversification within fungal taxa. Overall, the analysis highlights both functional and taxonomic diversification of GDHs evolution in fungi.

Localization of fungal glutamate dehydrogenases

The evolutionary diversity of glutamate dehydrogenases is reflected in the localization of the enzyme within fungal cells. Glutamate dehydrogenases in fungi are reported to be in the cytosol, nucleus or in mitochondria.

Le John (1971) [29] reported that, in *Chytridiomycetes*, *Blastocladiella*, and *Achlya* as well as *Saprolegnia*, most GDH activities are in mitochondria. Cytosolic GDHs have been reported in ascomycetes, such as *Neurospora* [33] and *Candida* [34]. In the zygomycetes fungus, *B. poitrasii*, major activities of NAD- and NADP-GDHs were observed in the cytosol [22]. The model yeast, *S. cerevisiae*, contains three GDHs: one using NADH as cofactor (GDH2) and two isoenzymes using NADPH as cofactor (GDH1 and GDH3). The GDH1 of *S. cerevisiae* is localized in the nucleus and cytosol, whereas GDH3 is localized in mitochondria and in the nucleus [35, 36].

Since the localization of GDHs affects their biochemical activity and physiological significance, further research is needed to understand the relationship between the evolution of the diversity and distribution of GDHs within fungal cells. However, the biochemical properties of fungal dehydrogenases provide clues about their localisation, as the next section reveals.

Biochemical properties of fungal glutamate dehydrogenases

In most fungi, NAD-GDHs have been reported to catalyze the oxidative deamination of glutamate, optimally at alkaline pH (8–10), while the optimum pH for reductive amination was 7.0–7.5 [26, 27].

Purified NAD-GDH of the zygomycete fungus, *B. poitrasii*, showed an optimum pH of 8.5 for oxidative deamination and 8.0 for the reductive amination of 2-ketoglutarate [27]. On the other hand, NADP-GDH isoenzymes of *S. cerevisiae* were reported to have an optimum pH of 6.8 but were active in the pH range of 4.5–9.5 [25]. However, NADP-GDHs from *A. niger*, *A. terreus*, *A. bisporus* and *Schizosaccharomyces pombe* catalyzed the reductive amination of 2-ketoglutarate optimally at pH 7.5–8.5, whereas the oxidative deamination of L-glutamate was catalyzed optimally at slightly alkaline pH (9.0–9.75) [10, 11, 37, 38]. The preliminary biochemical characterization of NADP-GDH isozymes from a crude extract of *B. poitrasii* showed optimum activity of both isoenzymes at pH 7.0 [22]. However, purified BpNADPGDH I and II catalyzed the reductive amination of 2-ketoglutarate optimally at pH 7.0 and 8.0, respectively, while the optima for oxidative deamination were pH 7.5 and 8.5, respectively. While BpNADPGDH I remained stable in the pH range of 6.0–9.0, purified BpNADPGDH II remained stable in the pH range of 7.0–9.5 [28].

In *Saccharomyces cerevisiae*, GDH1 and GDH3 catalyze the reductive amination of 2-ketoglutarate with optimal activity near pH 6.8–7.0, while GDH2 catalyzes oxidative deamination most effectively at alkaline pH (8.5–9.0) [25, 39]. The distinct pH optima of these isoenzymes reflect their metabolic roles, with cytosolic NADP-GDHs functioning under neutral conditions during biosynthetic growth, and mitochondrial NAD-GDH operating preferentially under alkaline or nitrogen-limited conditions to support catabolic flux. Thus, the variations in the optimal pH for different GDHs in fungi likely reflect the diversity of the intracellular microenvironments in which they function. The slightly alkaline mitochondrial matrix and near-neutral cytosol may differentially favor deamination and amination reactions, linking GDH activity to subcellular compartmentalization and metabolic state. This interpretation is consistent with *in vivo* measurements in *Saccharomyces cerevisiae*, which demonstrate distinct cytosolic (~ 7.2) and mitochondrial (~ 7.5) pH values and their modulation in response to nutrient availability and growth conditions [40]. However, direct evidence for the dynamic relocalization of GDHs or the differential expression of mitochondria-targeted isoforms remains limited. To strengthen this observation, further studies on GDH localization and role of pH in their regulation are required.

Data on the temperature optima of various fungal GDHs show similar variations. Most fungal NAD- and NADP-GDHs show maximum activity in the temperature range of 25 °C-35 °C [10, 11, 37]. However, NADP-GDH from *S. pombe* showed reductive amination of 2-ketoglutarate optimally at 56 °C [38]. The optimum temperature for the reductive amination of 2-ketoglutarate by *B. poitrasii*-purified NAD-GDH was 30 °C and, for oxidative deamination, it was 25 °C [27]. In contrast, the temperature optima of NADP-GDHs showed differences: 32 °C for the yeast form and 28 °C for the hyphal isoform [22, 28].

To interpret this data on the variations in temperature optima of the enzymes, we may need to consider differences in their functions. The reductive amination of 2-ketoglutarate to glutamate is an exothermic process in the direction of oxidation but can be an endothermic process in the direction of synthesis. The reaction catalyzed by glutamate dehydrogenase is reversible. It can proceed in either direction depending on the needs of the organism. Thus, temperature optima may reflect nanoscale local temperature conditions under which the enzymes work within the fungal cells and/or microscale

local temperature provided by the environmental conditions in which the fungi grow.

Kinetic studies of fungal GDHs

Although glutamate dehydrogenases from many sources have been studied, the kinetics of only a few have been characterized in detail [1, 41, 42].

In *Blastocladiella emersonii*, the K_m of NAD-GDH towards L - glutamate and NAD^+ was significantly lower than the K_m of 2-ketoglutarate and NADH [43]. K_m values for NAD-GDH from fungi, such as *N. crassa* and *A. bisporus*, towards 2-ketoglutarate were 4.6 and 3.5 mM, respectively, and, for glutamate, they were 5.5 and 37.1 mM, respectively. On the other hand, NADP-GDHs reported from a variety of sources (yeast and filamentous fungi) showed greater affinity towards 2-ketoglutarate (K_m, 2–6 mM) and NADPH (K_m, 0.011–0.074 mM) than towards L-glutamate (K_m, 6.36–34.6 mM) and $NADP^+$ (K_m, 0.01–0.11 mM) [10, 11, 37, 38, 44–46]. For most substrates, Walvekar et al. (2014) observed a slight increase in the K_m and V_{max} values for *A. niger* NADP-GDH, heterologously expressed and purified from *E. coli* [46].

These variations in the kinetics of GDHs can be understood when we consider that GDHs function in both directions, *i.e.*, they can use both 2-ketoglutarate and L-glutamate. NADP-specific GDH is a biosynthetic enzyme, while NAD-GDH serves a catabolic function. According to Smith et al. (1975), the K_ms for 2-ketoglutarate, $NADP^+$ and NADPH tend to be similar for enzymes from a variety of sources [1], whereas the K_ms for L-glutamate, ammonia and NAD(P)H may differ widely [26]. From a thermodynamic point of view, it would, therefore, be interesting and necessary to explore if there are any correlations of the kinetic data with variations in the temperature optima of the GDHs.

Having examined the physiological roles, biochemical properties, and kinetics of the GDHs, let us explore how the enzymes are coded, structured and regulated to play their crucial roles in fungi.

Molecular studies of fungal GDHs

Fungal GDH genes follow the characteristic fungal exon-intron organization, with introns occurring mainly in filamentous fungi belonging to the Ascomycetes and Basidiomycetes classes, while yeasts often harbour intron-less genes. Based on comparative genomic and protein sequence analyses, we observed that fungal NAD-GDH genes are usually large (2.5–3.5 kb) and typically contain 2–6 introns, whereas fungal NADP-GDH genes are relatively short (1.4–1.6 kb) with 0–2 introns [20]. NAD-GDHs possess an N-terminal Rossman-fold with a glycine-rich motif (VIGLGPGXG) and a conserved Lys crucial for NAD^+ binding [47, 48]. On the other hand, NADP-GDHs contain three distinct conserved motifs *viz.*, PSVNL, KFLGFEQ, and RPEATGY/F, and a characteristic NADP-binding region (GSGNVAQYAALKVIELG). The Lys residue between positions 110 and 120 serves as a catalytic residue in fungal NADP-GDHs [20, 49].

Gene sequence translation yields putative enzymes of ~1000 amino acids for NAD-GDH and ~450 amino acids for NADP-GDH. Both enzymes maintain the conserved structural, catalytic, and oligomerization elements required for the efficient interconversion of L-glutamate and 2-ketoglutarate. A graphical representation of fungal NAD- and NADP-GDH gene sequences, along with their transcribed mRNAs and translated polypeptides, are summarized in Fig. 3.

Molecular investigations into nitrogen metabolism in fungi reveal the multifaceted roles of glutamate dehydrogenases (GDHs) in ammonium assimilation and glutamate biosynthesis. In *Saccharomyces cerevisiae*, three distinct pathways contribute to glutamate formation: the NADP-GDH encoded by *GDH1*, the glutamate synthase (*GLT1*)-dependent GS-GOGAT route, and a third pathway involving *GDH3*, which encodes an isozyme functionally related to *GDH1* [4, 20, 25, 50, 51].

In *Kluyveromyces lactis*, NADP-GDH and GS-GOGAT activities have been confirmed through null mutations [52, 53]. In *Neurospora crassa*, molecular analyses delineated distinct anabolic functions for NADP-GDH and catabolic functions for NAD-GDH, with the *am* gene encoding NADP-GDH extensively characterized at biochemical and genetic levels [54, 55]. Further, the NAD- and NADP-GDH genes in *N. crassa* were concurrently regulated by a repression-derepression mechanism. In the presence of glutamate, NADP-GDH was repressed while NAD-GDH was simultaneously de-repressed [54, 55].

In *Aspergillus nidulans*, NADP-GDH (encoded by *gdhA*) plays a pivotal role in nitrogen metabolism and regulation [56]. The enzyme is strongly expressed in the presence of glucose and inorganic nitrogen, reflecting its importance in ammonium assimilation [57]. Mutants deficient in *gdhA* exhibit slow, leaky growth, while *gltA*–*gdhA* double mutants fail to use ammonium, confirming the indispensable role of GDH in nitrogen assimilation [58]. In *Aspergillus awamori*, the transcriptional regulation of *gdhA* is strongly influenced by nitrogen source availability, consistent with its involvement in extracellular protein secretion [59]. In *Penicillium chrysogenum*, the disruption of the NADP-GDH gene adversely affected penicillin biosynthesis and cell morphology, linking GDH function to secondary metabolism [18].

In *B. poitrasii*, NAD- and NADP-dependent GDHs are strongly correlated with the yeast–hypha transition [22]. Molecular characterization revealed one NAD-GDH

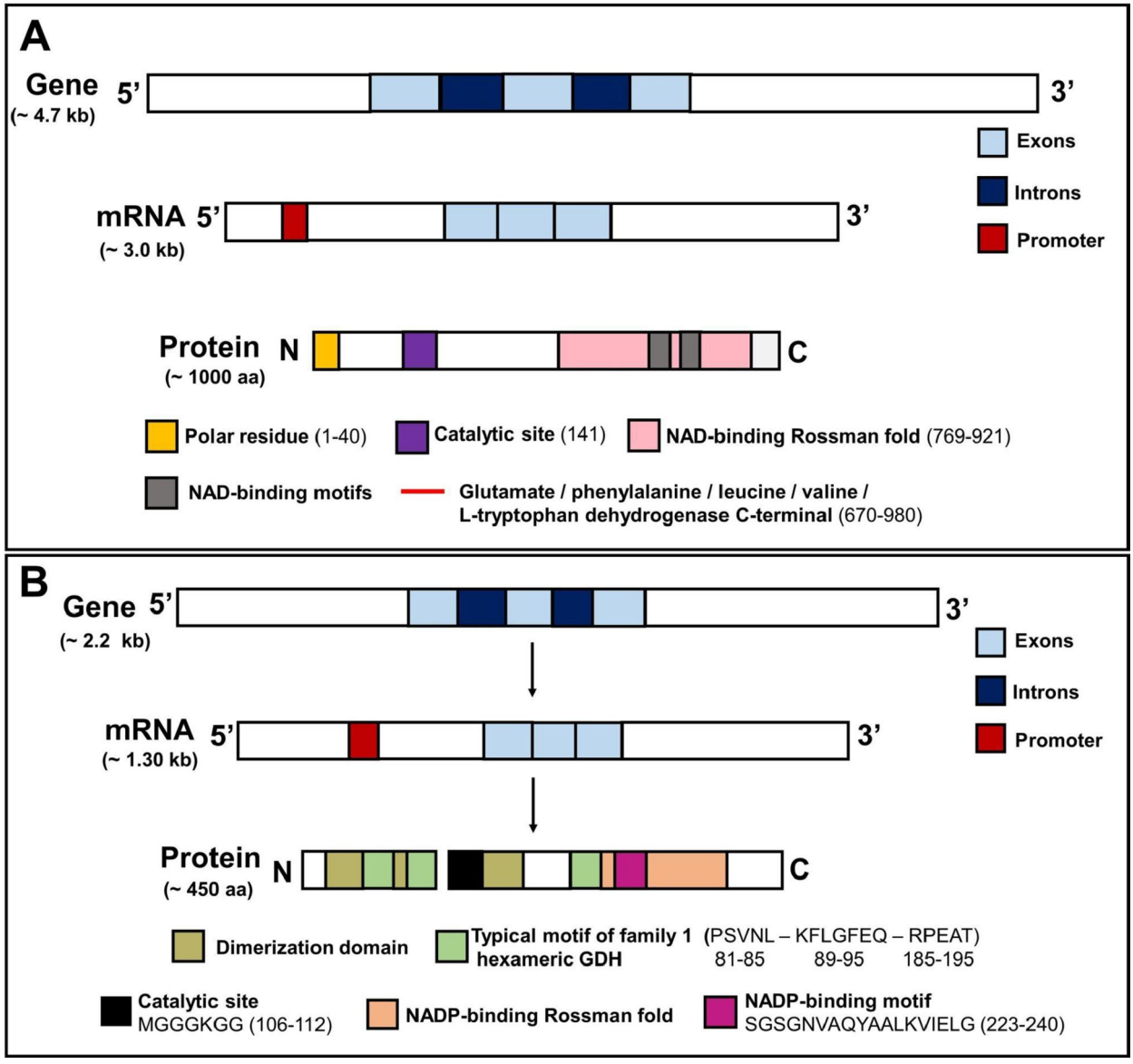

Fig. 3 The generalized molecular structure of fungal NAD-dependent (**A**) and NADP-dependent (**B**) glutamate dehydrogenase genes, mRNAs, and proteins. Gene architecture, cofactor binding sites, conserved domains and catalytically important amino acids are highlighted. The numbers in parentheses indicate the tentative amino acid positions.

(*BpNADGDH*) and two NADP-GDH genes (*BpNADPGDH I* and *II*) [20]. *BpNADPGDH I* was yeast-form specific, while *BpNADPGDH II* was hyphal-form specific. In *B. poitrasii*, the expression of *BpNAD-* and *BpNADP-GDH* genes was affected by dimorphism triggering factors, such as glucose, temperature, glycerol and serum [20, 60]. The expression of *BpNADPGDH I* was induced in the presence of glucose and glycerol at 37 °C, whereas, under similar conditions, *BpNADPGDH II* was repressed. On the other hand, serum induces the expression of *BpNADGDH* and *BpNADPGDH II*, and represses the *BpNADPGDH I* levels [60].

Collectively, these findings establish GDHs as central regulators of nitrogen metabolism, metabolic adaptation and morphogenesis in fungi.

Structural studies of fungal GDHs

Fungal glutamate dehydrogenases are multimeric proteins. NAD-GDHs are generally reported to be homotetramers while NADP-GDHs are homohexamers. NAD-GDHs from *Agaricus bisporus*, *B. poitrasii*, *C. utilis*, *N. crassa*, *S. cerevisiae* have a native *MW* of around 350–500 kDa with a subunit *MW* of 90–120 kDa [25, 27, 34, 61–63]. However, it has been reported that the NAD-GDH from *Phycomyces* had an exceptionally low subunit

MW of 54 kDa and was dimeric with a native *MW* of 98 kDa. This deviation from the typical tetrameric organization highlights gaps in our understanding of the structural diversity of GDHs.

Homo-hexameric NADP-GDHs, reported in *(A) niger*, *(B) poitrasii*, *P. chrysogenum*, *S. cerevisiae* and *Tuber borchii*, have a native *MW* of 270–350 kDa with a subunit *MW* 46–58 kDa [10, 12, 24, 25, 28]. However, it remains unclear why such a large subunit molecular weight difference exists between the NAD- and NADP-GDHs – despite the fact that the two enzymes catalyze essentially the same reversible chemistries. This unexplained disparity suggests fundamental structural or evolutionary divergences that remain insufficiently addressed.

Each subunit of the tetrameric NAD-GDHs and hexameric NADP-GDHs enzymes has one cofactor and one substrate binding site as per the structural models derived from a limited number of species. The first high-resolution crystal structure of a fungal NADP-dependent glutamate dehydrogenase (NADP-GDH) was reported from *A. niger* [49]. The apo form (PDB ID: 5XVI; 2.8 Å) and the ternary complex with 2-ketoglutarate and NADPH (PDB ID: 5XVX; 1.8 Å) revealed a hexameric 'dimer of trimers' assembly, with each subunit composed of two domains: domain I for substrate recognition and domain II for coenzyme binding. The catalytic residues, Lys78, Gln99, Lys102, Lys114, Asp154, Arg193, and Asn346, interact directly with 2-ketoglutarate, whereas Ser253, Lys277, and Gln282 stabilize the 2′-phosphate of NADPH. The 2.8 Å proximity between the carbonyl carbon of 2-ketoglutarate and the hydride-donating carbon of NADPH supports an efficient hydride transfer chemistry. These observations largely reinforce established catalytic mechanisms rather than provide novel mechanistic insights. The asymmetric arrangement of three open and three partially closed subunits suggest an allosterically coordinated catalytic mechanism consistent with a Monod–Wyman–Changeux (MWC) model. Direct experimental validation of this proposed allosteric mechanism in fungal GDHs, however, remains limited.

High-resolution structures of *A. terreus* NADP-GDH (AtGDH; PDB IDs 7ECR, 7ECS, 7ECT) complexed with NADPH and dicarboxylic acid inhibitors, such as succinate, malonate, and tartrate, revealed nearly identical conformations [64]. Each subunit contains 16 α-helices and 13 β-strands organized into two major domains connected by a hinge-like helix, facilitating interdomain motion during catalysis. The AtGDH structure closely resembles that of *A. niger* NADP-GDH (RMSD 0.34 Å), reinforcing structural conservation among filamentous fungal GDHs. Such high structural conservation suggests functional redundancy, but this has not been critically evaluated.

Building on these crystallographic insights, recent Cryo-EM-based structural analyses of *A. niger* and *A. terreus* NADP-GDHs revealed distinct conformational states associated with enzyme cooperativity [65]. The cooperative *A. niger* NADP-GDH exhibited a wider opening (~ 21 Å) at the active site for substrate binding than observed in the non-cooperative *A. terreus* GDH (~ 17 Å) in an open conformation, demonstrating how structural flexibility governs catalytic behavior. A R246S substitution in domain II was shown to modulate this flexibility, converting the *A. terreus* NADP-GDH into a cooperative enzyme similar to the *A. niger* NADP-GDH. While these findings shed light on the role of conformational flexibility in enzyme cooperativity, the molecular basis of this modulation remains unclear calling for further investigation into long-range structural dynamics. Collectively, however, these findings provide insight into the allosteric regulation and structural determinants of functional diversity in fungal NADP-GDHs.

Similarly, the *Candida albicans* NADP-GDH isoenzyme, CaGdh3 [66], exhibits a comparable hexameric architecture, with domain II rotated by ~ 10° relative to AnGDH, indicating conformational flexibility and possible allosteric regulation. The binding of 2-ketoglutarate and NADPH within the interdomain cleft induces the closure of the catalytic site, suggesting substrate-driven conformational transitions. This conformational flexibility is central to the enzyme's catalytic behavior. Yet the specific triggers for these transitions *in vivo*, beyond simple ligand binding, remain underexplored.

To investigate the structural fold and ligand binding site of fungal NADP-GDHs, the available crystal structures were obtained from the RCSB Protein Data Bank (PDB ID: 5XVX [49], 7ECR [64], 7F79 [66]) and were superimposed (Fig. 4). The volume of the substrate and cofactor binding site is observed to be ~ 504.67 $Å^3$. The cofactor NADP(H) is lined by several polar residues that stabilize the cofactor through hydrogen bond interaction (Fig. 4). The phosphate group attached to the adenine moiety of NADP(H) is stabilized by $Lys274_{NZ}$ and $Lys121_{NZ}$ through hydrogen bond interaction where the distances are ~ 2.96 to 2.56 Å, respectively. Further, the substrate (2-ketoglutarate) is stabilized through Lys114 and Lys102 on one carboxyl terminal and Lys78 and Arg193 on the other terminal, forming a salt-bridge interaction (Fig. 4).

The structural data provide valuable insights into the NADP-GDH reaction mechanism in closely related *Aspergillus* species and in the yeast, *C. albicans*. These findings offer a foundation for understanding the structural basis of fungal NADP-GDH regulation. However, available structural information remains limited, particularly regarding the enzyme's allosteric communication and long-range conformational dynamics - a research gap that needs to be closed sooner than later.

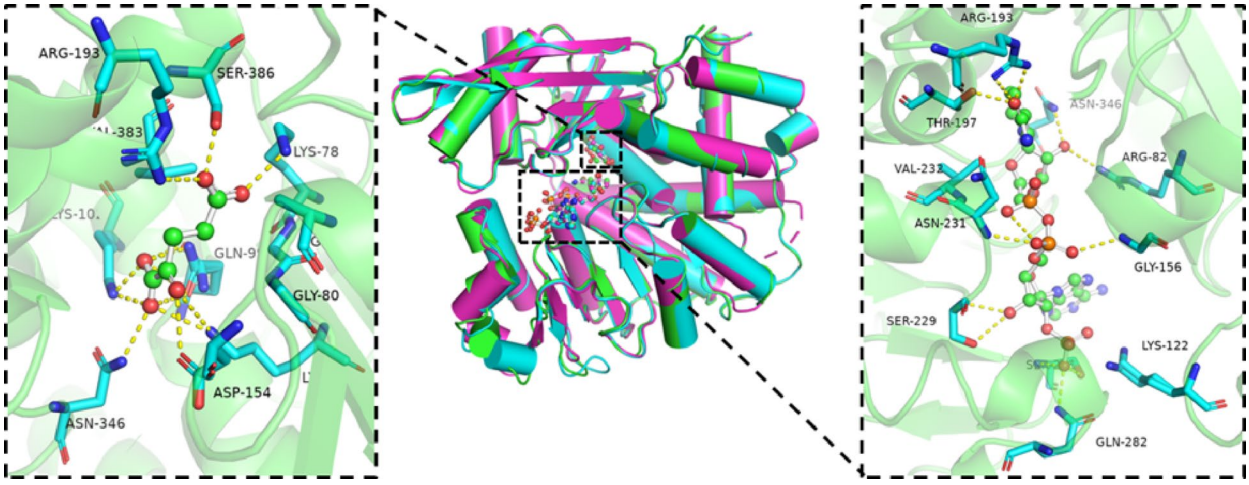

Fig. 4 The structural arrangement of fungal NADP-glutamate dehydrogenases (NADP-GDHs). The superimposed crystal structure of NADP-GDHs with PDB ID: 5XVX (green), 7ECR (cyan), 7F79 (magenta) from *A. niger*, *A. terreus* and *C. albicans*, respectively. The active site and cofactor binding residues are shown in cyan. The substrate (2-ketoglutarate) and cofactor (NADP) are shown in green. The yellow dotted lines indicate hydrogen bond interactions.

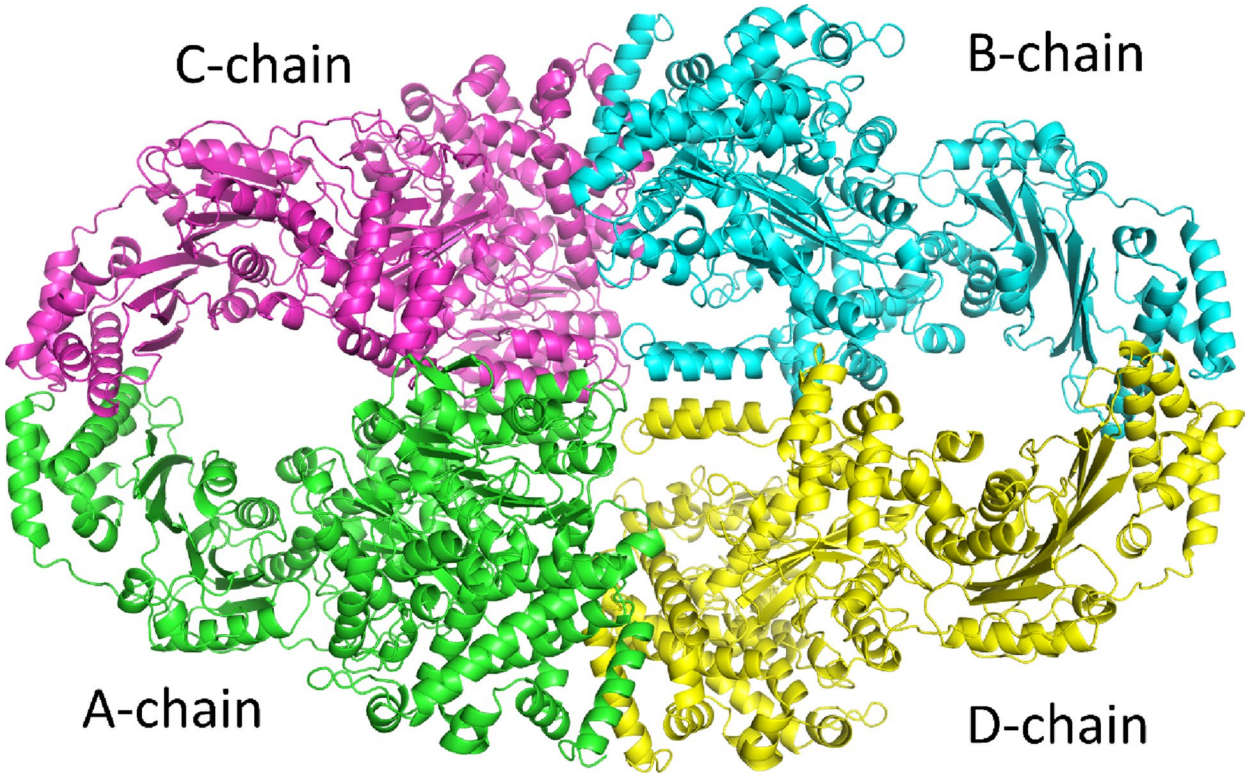

Fig. 5 Modeled tetrameric structure of apo-NAD-glutamate dehydrogenase (GDH2) from *Candida albicans*. Each of the four chains is shown in a different color.

In the absence of structural data on fungal NAD-GDH, we modeled the tetrameric structure of NAD-GDH from *Candida albicans* (Uniprot ID: XP_716032.2) using AlphaFold 3 (Fig. 5). The four different colors indicate four different chains of the tetrameric protein. Each subunit contains two main domains, and the active site (where the substrate and coenzyme bind) is located in the cleft between these domains. Domain I is primarily involved in binding the glutamate substrate and facilitating the assembly of the subunits into the tetrameric structure. Domain II is structurally analogous to the conserved dinucleotide-binding fold, specifically designed to bind the coenzyme (NADH). The binding of the coenzyme within the cleft positions the nicotinamide moiety

deep within the active site, facilitating the stereospecific hydride transfer during the catalytic cycle.

Though AlphaFold 3 has shown remarkable accuracy, experimental corroboration of the structure of NAD-GDH is advisable.

Typically, fungal tetrameric NAD-GDHs are larger than the corresponding NADP-GDHs. Therefore, to further explore the basis of cofactor specificity, we examined the structural relationships between NADP- and NAD-GDHs from *C. albicans*, as well as between mammalian GDH (where the same enzyme uses NADP as well as NAD as cofactor) and fungal NADP-GDHs. For this purpose, the respective structures were superimposed, allowing direct comparison of conserved and divergent features. As shown in Fig. 6A, an extra arm domain is observed in NAD-GDH, connecting the palm and finger

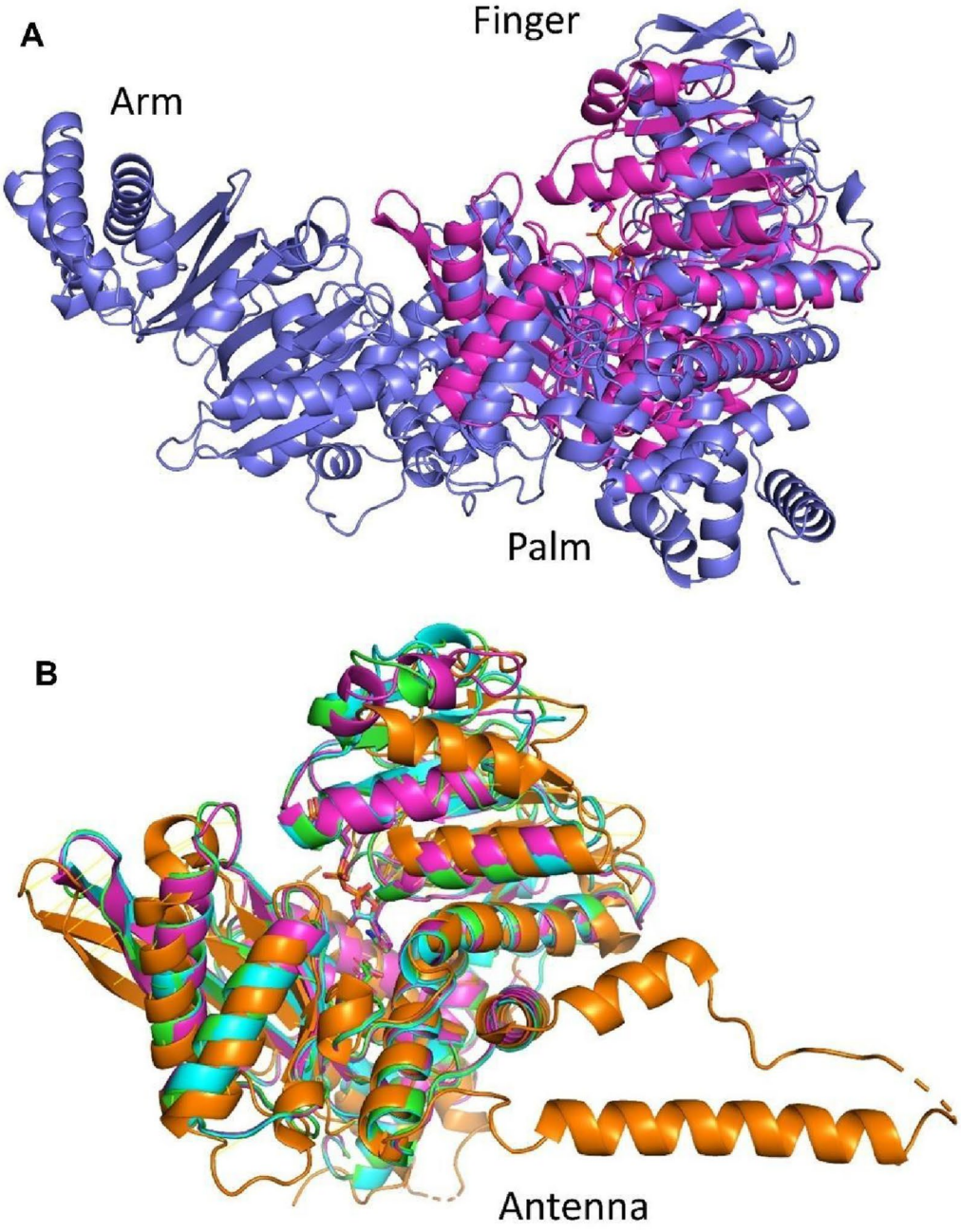

Fig. 6 **A** Superimposition of the modeled monomer of NAD-GDH (purple) from *C. albicans* (Uniprot ID: XP_716032.2) over its monomeric NADP-GDH (magenta) crystal structure (PDB ID: 7F79). **B** Superposition of fungal NADP-GDHs (PDB ID: 5XVX (green), 7ECR (cyan), 7F79 (magenta)) from *A. niger*, *A. terrius* and *C. albicans*, respectively, with human GDH1 (PDB ID: 8KGY (orange)) showing the conservation of the core fold and the presence of a distinct antenna domain in mammalian GDH, which is absent in fungal enzymes.

domains. The palm and finger domains, forming a deep cleft for substrate and coenzyme binding, characterized by the presence of a Rossman fold, were found to be conserved in all fungal GDH.

Together, these observations suggest that, while the overall catalytic architecture is conserved, the additional arm domain in NAD-GDH may contribute to functional specificity, cofactor preference and regulatory interactions. This suggests that the evolution of the arm domain may be linked to cofactor specificity, but experimental studies on the role of this domain *in vivo* are crucial to confirm this hypothesis. As mentioned earlier, the availability of structural data on fungal NAD-GDHs will be crucial to fully elucidate the evolutionary and functional implications of this domain variation between the GDH isoenzymes.

In human GDH a unique "antenna" domain (a helix-random coil structure) in the coenzyme-binding region is observed (Fig. 6B). This antenna extends from each subunit and is essential for subunit-subunit communication and allosteric regulation, allowing the hexamer to coordinate its response to inhibitors like GTP. However, as mentioned earlier, fungal GDHs lack the complex antenna-mediated allosteric machinery found in mammalian GDH, as their regulation is primarily transcriptional and allosteric regulation is operative through domain interactions [49].

We will examine the regulation of fungal GDHs after describing their catalytic sites and summarising available information on their activators and inhibitors.

Catalytically important amino acid residues of fungal GDHs

Lysine was implicated as the most conserved catalytically important residue in GDHs from a large number of organisms. Some examples include - K126 in the beef liver enzyme [67], K113 in NADP-specific GDH from *N. crassa* [68], brain GDH iso-proteins [69] and K128 in NAD-GDH from *Clostridium symbiosum* [70]. Besides lysine, histidine was reported to be important in the phosphorylation-mediated regulation of *Escherichia coli* GDH [71]. In *B. poitrasii*, chemical modification data about histidine and lysine implicated their involvement in the activity of purified NAD-GDH [27]. Cysteine residue, present at position 141 in *A. niger* NADP-GDH, was important for its covalent modification, converting an anabolic enzyme into a catabolic enzyme [49]. Though it was not found to be catalytically essential, it may be important for the regulation of the enzyme, as suggested later, in Sect. "Thiol-mediated regulation", in this review.

Activators and inhibitors of fungal GDHs

GDH activity is induced by specific environmental and nutritional molecules across different fungal species. In *N. crassa*, ammonium and nitrate strongly activate NADP-GDH synthesis [72]. *A. nidulans* NAD-GDH is induced by glutamate and glucose deprivation [73]. In *T. borchii*, glutamate potently induces NADP-GDH activity and *gdh* expression, while urea, nitrate, and alanine promote 10-12-fold higher activity than ammonium [12].

In *M. oryzae*, NAD-GDH is activated by glutamate to release ammonia during infection [17]. Osmotic stress (high NaCl) induces NADP-GDH in *D. hansenii* [44], while glucose and glycerol activate yeast-specific NADP-GDH and hyphal form specific NADP-GDH was induced by serum in *B. poitrasii* [60].

In *Coprinus cinereus*, NAD-GDH is induced under nitrogen-limiting conditions by specific carbon sources (e.g., pyruvate, glucose, acetate), with acetyl-CoA acting as a key intracellular metabolic signal. Evidence from acetyl-CoA synthetase mutants demonstrates that acetyl-CoA synthesis is required for GDH induction, linking carbon metabolism to nitrogen assimilation [74].

Inhibiting fungal GDHs is a useful strategy to understand their role in various physiological processes involved in fungal growth as evidenced by some studies [27, 28, 75–77]. Most inhibitors used in these studies were the competitive inhibitors or analogues of the substrates, 2-ketoglutarate and L-glutamate. Isophthalate is the most reported inhibitor of both NAD-GDH [62, 77, 78] and NADP-GDH [79, 80] in fungi.

Rogers (1971) reported the inhibition of bovine GDH by structural analogues of L-glutamate, such as glutaric acid, thiodiglycolic acid, oxydiglycolic acid and iminodiacetic acid [81]. Analogues of 2-ketoglutarate, such as 2-methyleneglutarate, 2,4-pyridinedicarboxylate, and 3,5-pyrazoledicarboxylate, were screened for the inhibition of NADP-GDH from *A. niger* [10]. The purified NADP-GDH from *A. niger* was strongly inhibited by isophthalate, 2-methyleneglutarate, and 2,4-pyridinecarboxylate [10]. The presence of two carboxylate groups and the planarity of the molecules were common properties reported for effective GDH inhibitors. Haberland et al. (1980) reported the complete inhibition of the NAD-GDH of *N. crassa* at 10^{-3} M concentrations of the nucleotides, guanosine triphosphate (GTP), guanosine monophosphate (GMP) and inosine monophosphate (IMP) [82]. Furthermore, in *Fusarium* sp., ethylenediaminetetraacetic acid (EDTA) was found to inhibit NADP-GDH, with no effect on NAD-GDH [83].

Recently, Godsora et al. (2022) showed that dicarboxylic acids (fumarate, maleate, malonate, succinate, and tartrate) independently inhibit *A. terreus* NADP-GDH (AtGDH) to various extents [64]. Structural studies suggest that these dicarboxylic acids partially occupy the

AtGDH substrate-binding site and involve various polar interactions [64]. As shown in Fig. 7, malonate occupies the active sites of hexameric *A. terreus* NADP-GDH and forms conserved hydrogen-bonding interactions with key coordinating residues, demonstrating the competitive mode of inhibition. Similarly, *p*-chloro-mercuribenzoate (PCMB) also inhibited NADP-GDH, while NAD-GDH was not inhibited. This observation reflects the role of -SH groups in the enzyme activity of GDH. Clues to structural aspects of this inhibitory activity emerge in the next section where thiol-mediated regulation is discussed.

Regulation of fungal glutamate dehydrogenases

GDH gene expression and enzyme activity are tightly regulated to maintain an efficient carbon-nitrogen balance in fungal cells. This regulation operates at both the transcriptional level, which controls GDH gene expression in response to metabolic cues, and the post-translational level, which modulates enzyme activity.

Transcriptional regulation

Carbon and nitrogen catabolite repression (CCR and NCR) coordinately regulate glutamate dehydrogenase (GDH), ensuring effective integration of carbon utilization and ammonia assimilation in fungi [84, 85]. This

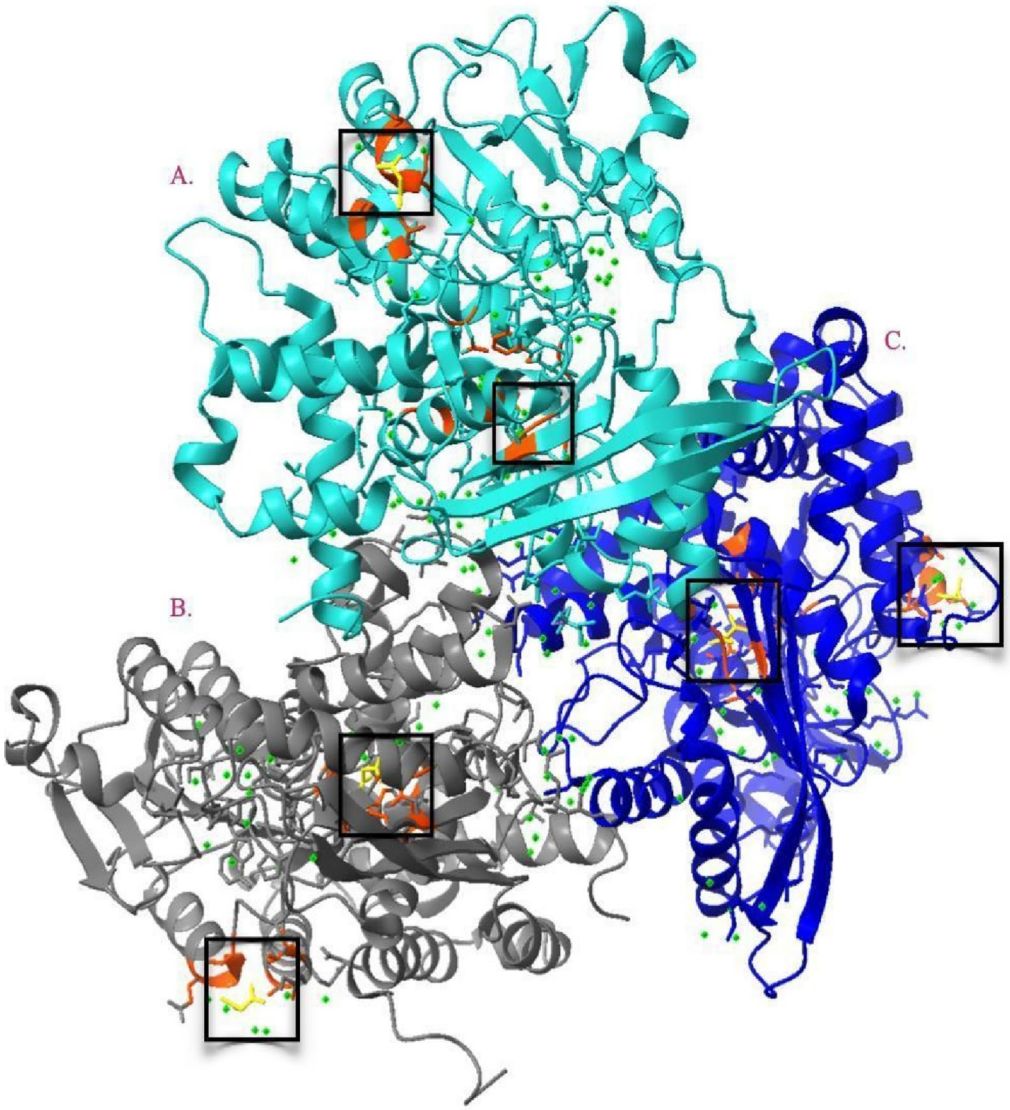

Fig. 7 *Aspergillus terreus* NADP-GDH (PDB ID: 7ECS; resolution 1.74 Å), complexed with malonate, a competitive inhibitor. The trimeric face of the hexameric enzyme is shown with chains **A** (cyan), **B** (gray), and **C** (blue). Malonate molecules (yellow) occupy active sites, where key coordinating residues are highlighted in orange-red. Green dotted lines indicate hydrogen bonds between malonate and surrounding residues. Insets **A**–**C** (black boxes) zoom in on each monomer's binding site, illustrating conserved ligand-residue interactions across the trimer.

regulation reflects a tightly interconnected CCR-NCR network, in which carbon availability influences nitrogen metabolism both directly and indirectly.

In filamentous fungi such as *A. nidulans*, *N. crassa*, and *Trichoderma reesei*, CCR is primarily mediated by *CreA/Cre1*, which represses genes required for the utilization of alternative carbon sources under glucose-rich conditions [86, 87]. This repression depends on *CreA* nuclear localization and is regulated through post-translational mechanisms involving *CreD-HulA* and *CreB-CreC* complexes, as well as signaling pathways such as *SnfA* and cAMP-PKA [88, 89]. Through interactions with nitrogen regulatory systems, particularly the GATA factor *AreA*, CCR indirectly suppresses ammonium assimilation and amino acid metabolism, repressing GDH activity; this repression is relieved under carbon limitation [88].

In *N. crassa*, CCR also directly regulates GDH isoforms. Glucose strongly represses *gdh2*, with rapid de-repression upon depletion of the carbon source, promoting glutamate catabolism. In contrast, *gdh1* is induced under carbon-rich conditions, supporting ammonia assimilation and maintaining metabolic balance [90].

In *S. cerevisiae*, GDH regulation integrates both NCR and CCR pathways. Early studies demonstrated that GDH1 must be in its catalytically active conformation to mediate NCR, functioning as a nitrogen sufficiency sensor that represses alternative nitrogen catabolic pathways independently of glutamate levels [91]. Subsequent work showed that GDH1 modulates NCR signaling *via* GATA transcription factors such as *Gln3* and *Gat1*, influencing their nuclear localization and downstream gene expression [14]. Additionally, *gdh2* (NAD-dependent) is primarily controlled by CCR, mediated by the *Cyc8-Ssn6/Tup1* co-repressor complex, and exhibits strong glucose repression, up to ~ 450-fold compared to the case with non-fermentable carbon sources [92]. Regulation is further integrated through UASNTR elements, where GATA factors activate *gdh* gene expression under nitrogen limitation and are sequestered by *Ure2* under nitrogen-rich conditions [93]. Moreover, *gdh* transcription is influenced by additional nutrient-responsive regulators such as *Gcn4* and *Hap4*, linking *gdh* gene expression to broader metabolic states [94].

In the dimorphic fungus *B. poitrasii*, the morphology-associated expression of genes encoding NADP-GDH isoenzymes was reported. The yeast-form-specific *BpNADPGDH I* is induced in the presence of glucose, whereas the hyphal-form-specific *BpNADPGDH II* is repressed under the same conditions [20]. Other dimorphism-triggering factors, including temperature, glycerol, and serum, also modulate *BpNAD* and *BpNADPGDH* gene expression, highlighting the integration of metabolic and morphological regulatory networks in *B. poitrasii* [20, 60]. Importantly, these differential expression patterns are consistent with enzyme activity data, wherein BpNADPGDH I exhibits higher affinity toward 2-ketoglutarate, while BpNADPGDH II shows more affinity toward L-glutamate. This suggests functional specialization of the isoenzymes, with BpNADPGDH I favoring reductive amination and ammonia assimilation in the Y-form, whereas BpNADPGDH II inclined toward oxidative deamination and nitrogen mobilization during H-form growth.

In *P. pastoris*, the transcription factors, *Rtg1* and *RtgX*, regulate the expression of *gdh2* at the post-transcriptional level by stabilizing the GDH2 protein. Experiments deleting either *Rtg1* or *RtgX* suggest that these factors function as a single regulatory unit, highlighting an additional layer of GDH regulation beyond transcription [95].

Beyond transcriptional control, GDH function is closely integrated with nutrient-responsive signaling pathways and autophagy. In *Magnaporthe oryzae*, the serine/threonine kinase, *Rim15*, regulates autophagy-glutaminolysis coupling by modulating NAD-GDH, with glutamate to α-ketoglutarate conversion contributing to the reactivation of TOR signaling and the suppression of autophagy upon nutrient recovery [96]. Similarly, in *P. pastoris*, *gdh2* expression is associated with carbon starvation-induced autophagy, mediated *via* a post-transcriptional mechanism that also activates phosphoenolpyruvate carboxykinase (PEPCK). Moreover, *gdh2* expression was inhibited in *Δatg1* mutants, indicating dependence on autophagy, and its deletion reduces survival under starvation conditions [97].

Collectively, these findings establish GDH as both a metabolic enzyme and a key regulatory node integrating carbon and nitrogen metabolism with cellular signaling and adaptive responses.

Post-translational regulation

The activity of fungal GDHs is known to be influenced by at least three major mechanisms: phosphorylation-dephosphorylation, thiol-mediated regulation, and allosteric regulation, collectively ensuring rapid and coordinated metabolic adaptation in fungi.

Phosphorylation-dephosphorylation

Protein phosphorylation and dephosphorylation were recognized as critical mechanisms for controlling the activity of regulatory enzymes in eukaryotic cells [98]. The NAD-GDH of the yeasts, *S. cerevisiae* and *C. utilis*, were regulated by a phosphorylation-dephosphorylation system catalyzed by protein kinases (E.C 2.7.1.37) and alkaline phosphatases (E.C 3.1.3.1). In *S. cerevisiae*, the conversion of active NAD-GDH to the inactive form was regulated by the phosphorylation of the enzyme by cAMP-dependent as well as cAMP-independent protein kinases [7, 61, 99]. Joshi et al. (2013) described the

involvement of cAMP-dependent protein kinases in the regulation of NAD- and NADP-GDH activities in the crude extract of *B. poitrasii*. The purified NAD-GDH of *B. poitrasii* was regulated by cAMP-dependent reversible phosphorylation-dephosphorylation of His residues [27]. Recently, we observed that purified isoenzymes of NADP-GDH in *B. poitrasii* were also regulated by phosphorylation-dephosphorylation, but through different kinases. BpNADPGDH I was regulated through the cAMP-dependent kinase and BpNADPGDH II through the Ca-CaM dependent protein kinase [28].

Thiol-mediated regulation

Thiol-mediated reversible regulation of NADP-GDH activity was reported in *A. niger* [46]. However, there is no evidence that such a mechanism could operate *in vivo*. Interestingly, this disulfide-modified NADP-GDH only slowed the forward activity (2-ketoglutarate to L-glutamate) without affecting the reverse activity. The key residue involved in mixed disulfide formation resulting in the inhibition of forward NADP-GDH activity in *A. niger* was identified as Cys141 [46]. This may perhaps explain the mechanism behind the inhibition of AnGDH using *p*-chloro-mercuribenzoate, discussed earlier in the section on activators and inhibitors of fungal GDHs.

Allosteric regulation

Allostery is a rapid and efficient way for enzymes to respond to changes in the concentration of ligands in the cell. In the case of *Yarrowia lipolytica*, two distinct genes code for NADP-dependent (*GDH1*) and NAD-dependent (*GDH2*) enzymes [100]. NADP-GDH helps with ammonium assimilation, while NAD-GDH is essential for glutamate catabolism. The Gdh1p and Gdh3p of *S. cerevisiae*, with 87% amino acid sequence identity, differ in their allosteric behaviour [25]. Both enzymes show a slight degree of substrate inhibition (~ 5%) when saturated with 2-ketoglutarate, with Gdh1p being more sensitive. At pH 7.2, the K_m of the two enzymes for 2-ketoglutarate show remarkable differences: 0.29 for Gdh1p and 1.27 mM for Gdh3p. They show different sigmoidal responses to 2-ketoglutarate, with the Hill coefficient (n_H) being 1.3 for Gdh1p and 1.5 for Gdh3p.

NADP-GDH from *Neurospora crassa* has been extensively studied and is under allosteric regulation by various effectors [101]. NADP-GDHs from *A. niger* (AnGDH) and *A. terreus* (AtGDH), despite sharing 88% sequence identity, show distinct kinetic properties [10, 11]. AnGDH exhibits a sigmoidal response to 2-ketoglutarate, while AtGDH displays a typical Michaelis-Menten saturation. Both enzymes experience competitive inhibition by isophthalate, but to a significantly different extent. The K_i value for isophthalate is 6.9 µM for AnGDH and 250 µM for AtGDH [76]. AnGDH selectively loses its reductive amination (forward) activity while retaining full oxidative deamination (reverse) activity on treatment with 2-hydroxyethyl disulfide (2-HED) [46]. This property is not exhibited by AtGDH, though the concerned residue (C141) is conserved in both enzymes. Subsequently, Agarwal et al. (2019) showed that 2-ketoglutarate cooperativity and ammonium biphasicity in *A. niger* NADP-GDH are structurally coupled [102].

GDHs characterized in other *Aspergillus* species (*A. nidulans*, *A. oryzae*, *A. awamori*) also showed sigmoid kinetics with 2-ketoglutarate [10]. It would be interesting to see whether this 2-ketoglutarate cooperativity is also coupled with ammonium biphasicity in these species.

Role of glutamate dehydrogenases in fungal morphogenesis

Glutamate dehydrogenases (GDHs) play a key role in fungal dimorphism, particularly in the yeast–hypha (Y–H) transition.

In *M. racemosus*, NAD- and NADP-dependent GDHs were identified, and the CO_2/N_2-dependent hypha–yeast transition correlated with decreased NAD-GDH activity [5, 103]. In *B. poitrasii*, the NADP-/NAD-GDH ratio determined morphogenesis [6], and three isoenzymes, one NAD-GDH and two NADP-GDHs, were characterized [22]. At the molecular level, *BpNADGDH* and two NADP-GDH genes (*BpNADPGDH I* and *BpNADPGDH II*) were identified [20]. *BpNADPGDH I* was yeast-form specific, whereas *BpNADPGDH II* was hyphal-form specific. The disruption of *BpNADPGDH II* abolished hyphal development, whereas its heterologous expression in the Y5 mutant of *B. poitrasii* or in *C. glabrata* induced germ tube formation, demonstrating that *BpNADPGDH II* is a critical determinant of morphogenetic transition [20, 60].

In *S. cerevisiae*, *GDH1* and *GDH3* encode NADP-GDHs, and *GDH2* encodes NAD-GDH, with expression modulated by carbon source [25, 104]. In *C. albicans*, *CaGdh3* expression increases during Y-H transition, and *ΔCaGdh3* or *ΔCaGdh2* mutants fail to use proline or arginine as nitrogen sources [15]. These findings firmly establish the role of GDHs in the regulation of fungal morphogenesis (Fig. 8).

Glutamate dehydrogenases as antifungal drug targets

Pathogenic fungi change morphology to a suitable vegetative form (unicellular yeast or filamentous hyphae), for their survival and proliferation as well as to defeat the cellular and physiological defences of the host. The microenvironment of the pathogen triggers different molecular and biochemical processes, finally leading to differentiation. These molecular and biochemical correlates of morphological change from saprophytic/less virulent to

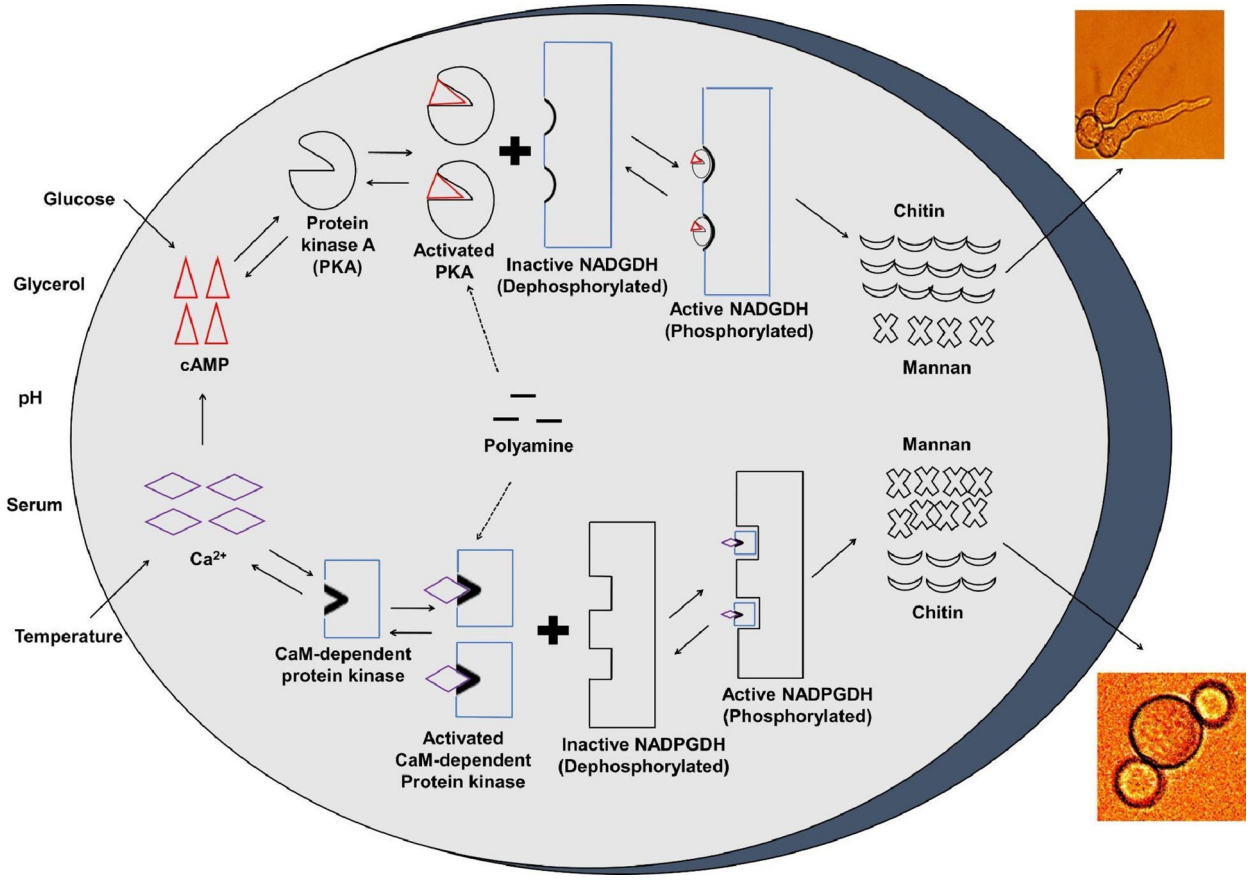

Fig. 8 Proposed signaling pathway involving the regulation of NAD- and NADP-GDH enzymes that determine the morphological outcome in fungi. Host environmental cues, such as glucose, pH, serum, glycerol and temperature, lead to a series of events ending in the activation/deactivation of GDH enzymes *via* a reversible phosphorylation/dephosphorylation mechanism. This mechanism directly regulates the flux towards chitin and mannan (cell wall polysaccharides), which in turn affects the morphological outcome in fungi [20, 28, 60, 105, 106]

pathogenic form are potential targets to develop novel antifungal agents. Since NAD- and NADP-GDHs show biochemical and molecular correlation with the yeast-hypha transition [15, 20, 27], they can be explored as antifungal drug targets.

Joshi et al. (2013) identified 1,2,3 triazole linked β-lactam-bile acid conjugates (B18, B20) as potent inhibitors of purified NAD-GDH. These inhibitors also affected Y-H transition in *B. poitrasii* as well as in *C. albicans* strains [27]. Choudhury and Punekar (2007) reported that isophthalate and 2-methyleneglutarate (structural analogue of 2-ketoglutarate), are effective inhibitors of *A. niger* NADP-GDH [76]. Choudhury et al. (2008) reported the *in vivo* inhibition of *A. niger* growth in a disc diffusion assay, by the dimethyl ester of isophthalate (DMIP) [75].

In line with these findings, twenty different derivatives of DMIP with side-chain modifications were synthesized and screened for NADP-GDH inhibition [28]. DMIP compounds containing amide side chains were the most potent NADP-GDH inhibitors. These compounds also inhibited the Y-H transition in *B. poitrasii*, *C. albicans* strains, and the dimorphic ascomycete, *Yarrowia liopolytica* [28]. The lead DMIP compounds showed antifungal potential against most tested human pathogenic yeasts and filamentous fungi at much lower concentrations than those of currently used antifungal drugs, such as fluconazole [28]. Thus, there is potential to develop more effective and safer antifungals by exploring the various derivatives of the inhibitors that target specific fungal GDHs.

In considering GDHs as antifungal targets, both specificity and metabolic flexibility must be taken into account. Fungi use multiple ammonium assimilation pathways, including GDH and the GS-GOGAT system, with the latter often predominating during active growth, while GDH becomes more relevant during differentiation. This allows GDH-targeted strategies to focus on processes linked to pathogenicity, such as the yeast-to-hypha transition in fungi like *Candida albicans*, rather than broadly inhibiting growth. In plant-associated fungi, including many mycorrhizal species, the GS-GOGAT pathway is predominant, suggesting the limited impact of GDH inhibition on beneficial interactions. GDHs in bacteria and mammals differ from fungal enzymes in

distribution, cofactor specificity, and regulatory features; notably, mammalian GDHs are dual cofactor-specific and structurally distinct. These differences provide a basis for selective targeting, as supported by studies showing that fungal NADP-GDH inhibitors are effective at low concentrations against fungi but exhibit minimal effects on human cells [28]. Although alternative pathways may compensate for GDH inhibition, targeting GDH during differentiation may still impair pathogenic development. The inhibition of such morphogenetic transitions has been linked to apoptosis-like responses in fungi, offering a potential strategy to control infections while minimizing selective pressure for resistance [60].

Utility of fungal glutamate dehydrogenases

Besides being targets for developing antifungals, fungal GDHs have important applications in agriculture, medicine, downstream processing, and for the industrial production of metabolites.

Microbial NADP-dependent glutamate dehydrogenases (GDHs), particularly those from fungi, exhibit high affinity for ammonium and can enhance nitrogen availability to plants [107–109]. The introduction of fungal *gdhA* into rice improved grain yield, photosynthetic rate, and salt tolerance through increased aminating and deaminating activities [110, 111]. GDHs from *Eurotium chevalieri* and *Trichurus* spp. increased nitrogen assimilation and yield in rice under low-nitrogen conditions [112, 113]. The expression of the *gdhA* of *Aspergillus candidus* enhanced ammonia assimilation and reduced photorespiration [114–116]. Enhanced GDH expression in maize, soybean, and tobacco also promoted biomass accumulation [115, 116]. The overexpression of the *gdhA* of *Magnaporthe grisea* conferred drought tolerance and improved survival under water deficit [108]. Collectively, these results demonstrate the potential use of fungal GDHs in agriculture.

Due to high substrate specificity and coenzyme-dependent redox activity, fungal GDHs can act as biocatalysts in biosensor technology. Biosensors employ a biological recognition element that interacts with the target molecule and a transducer that converts this interaction into a measurable signal [117]. GDH-based biosensors have been developed for the detection of L-glutamate, ammonium, and related metabolites in food and environmental samples. Co-immobilization of GDH with L-glutamate oxidase enables accurate determination of monosodium glutamate [118, 119], while immobilization within sol–gel matrices or on carbon paste electrodes provides high sensitivity and reproducibility [120, 121]. The incorporation of GDHs with multiwalled carbon nanotubes or chitosan films further enhances signal stability and selectivity [122, 123]. Thermophilic GDH and rare-earth metal activation have also improved electrochemical performance at elevated temperatures and enhanced electron transfer efficiency [124]. The intrinsic stability and catalytic efficiency of fungal GDHs make them promising components for developing next-generation biosensors for food analysis, clinical diagnostics, and environmental monitoring.

A deeper understanding of GDHs is useful for industries. GDHs can be exploited to simplify downstream processing in fermentation industries. NAD-dependent glutamate dehydrogenase regulates flocculation and cell surface properties in *B. poitrasii*. Modulating GDH activity through substrates or inhibitors alters cell wall composition and aggregation behaviour [125]. The presence of multiple GDH isoenzymes supports nitrogen assimilation and glutamate homeostasis during fermentation by yeast [14]. Fructose-6-phosphate, which serves as a precursor for mannan and chitin biosynthesis, provides a metabolic link between GDH activity and carbon-nitrogen metabolism via the reversible conversion of glutamate and 2-ketoglutarate [28, 39, 126]. 2-ketoglutarate and isophthalate enhance, while glutamate suppresses flocculation in correlation with intracellular NAD-GDH levels [125]. Thus, an understanding of fungal GDHs is useful in fermentation industries.

Ammonia produced through glutamate dehydrogenase activity serves as a precursor for amino acids, nucleotides, and proteins [127]. Industrially, ammonia is widely used in fertilizer production, refrigeration, and as carbon-free chemical feedstock [128]. Glutamate, another key GDH product, functions as a substrate for glutathione synthesis and is commercially used as monosodium glutamate (MSG), an umami flavour enhancer [129, 130]. Beyond primary metabolism, glutamate provides precursor units for non-ribosomal peptide and polyketide biosynthesis, contributing to secondary metabolites such as macrolactams and streptolydigin [131–133]. Fungal GDHs represent ideal candidates for the production of ammonia and L-glutamate due to their high catalytic activity in the reductive amination direction, desirable kinetic characteristics, operational stability, and ease in scalability. These results suggest the potential for the use of fungal GDHs in chemical industries.

Epilogue

Glutamate dehydrogenases in fungi exemplify a unique convergence of metabolic, regulatory, and evolutionary complexity. Beyond their canonical roles in nitrogen metabolism, fungal GDHs regulate a broad range of physiological processes, including morphogenesis, stress adaptation, and secondary metabolism. Advances in molecular and structural characterization have elucidated the catalytic architecture and cofactor interactions that underpin their functional diversity. The integration of biochemical, genetic, and structural data enables the

identification of conserved residues critical for regulation and inhibition, providing an avenue for antifungal drug discovery. The translational applications of fungal GDHs in crop improvement, biosensor development, and metabolic engineering emphasize their biotechnological importance.

Despite the potential for multifaceted applications, our understanding of fungal GDHs has many gaps, as highlighted in this review. Future investigations focusing on allosteric communication, post-translational regulation, and cross-talk with signalling pathways will throw more light on the structure and functions of GDHs in fungal biology and biotechnology.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40694-026-00212-4.

Supplementary Material 1.

Acknowledgements
We are thankful to Unnati Singhania, Karan Patil and Ravish Javed for their help in preparation of illustrations. We thank Gita Madhu and K. P. Madhu for critical reading of this manuscript. HS thank Symbiosis International (Deemed University) for the award of Junior Research Fellowship.

Author contributions
EKP: Conceptualization, Data curation, Resources, Supervision, Writing-Reviewing and Editing; HS: Formal analysis, Methodology, Writing – review & editing. SD: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. NSP: Conceptualization, Supervision, Writing – review & editing.

Funding
Open access funding provided by Symbiosis International (Deemed University). EKP is a recipient of the major research project (MJRP) grant (SIU/SCRI/MJRP- Approval/2024/1397) from the Symbiosis International (Deemed University).

Data availability
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 22 January 2026 / Accepted: 20 April 2026
Published online: 16 May 2026

References

1. Smith EL, Austen BM, Blumenthal KM, Nyc JF. 5 Glutamate Dehydrogenases, Editor(s): Paul D. Boyer, The Enzymes. Volume 11. Academic; 1975. pp. 293–367.
2. Bunik V, Artiukhov A, Aleshin V, Mkrtchyan G. Multiple forms of glutamate dehydrogenase in animals: Structural determinants and physiological implications. Biology (Basel). 2016;5:53. https://doi.org/10.3390/biology5040053
3. Harper CJ, Hayward D, Kidd M, Wiid I, van Helden P. Glutamate dehydrogenase and glutamine synthetase are regulated in response to nitrogen availability in *Myocbacterium smegmatis*. BMC Microbiol. 2010;10:138. https://doi.org/10.1186/1471-2180-10-138
4. Avendaño A, Deluna A, Olivera H, Valenzuela L, Gonzalez A. GDH3 encodes a glutamate dehydrogenase isozyme, a previously unrecognized route for glutamate biosynthesis in *Saccharomyces cerevisiae*. J Bacteriol. 1997;179:5594–7. https://doi.org/10.1128/jb.179.17.5594-5597.1997
5. Peters J, Sypherd PS. Morphology-associated expression nicotinamide adenine dinucleotide-dependent glutamate dehydrogenase in *Mucor racemosus*. J Bacteriol. 1979;137:1134–9. https://doi.org/10.1128/jb.137.3.1134-1139.1979
6. Khale A, Srinivasan MC, Deshpande MV. Significance of NADP/NAD glutamate dehydrogenase ratio in the dimorphic behavior of *Benjaminiella poitrasii* and its morphological mutants. J Bacteriol. 1992;174:3723–8. https://doi.org/10.1128/jb.174.11.3723-3728.1992.
7. Khale-Kumar A, Deshpande MV. Possible involvement of cyclic adenosine 3',5'-monophosphate in the regulation of NADP-/NAD-glutamate dehydrogenase ratio and in yeast-mycelium transition of *Benjaminiella poitrasii*. J Bacteriol. 1993;175:6052–5. https://doi.org/10.1128/jb.175.18.6052-6055.1993
8. Green J, Large PJ. Regulation of the key enzymes of methylated amine metabolism in *Candida boidinii*. J Gen Microbiol. 1984;130:1947–59. https://doi.org/10.1099/00221287-130-8-1947
9. Ferguson AR, Sims AP. The Regulation of Glutamine metabolism in *Candida utilis*: The Inactivation of Glutamine Synthetase. J Gen Microbiol. 1974;80:173–85. https://doi.org/10.1099/00221287-80-1-173
10. Noor S, Punekar NS. Allosteric NADP-glutamate dehydrogenase from *Aspergilli*: Purification, characterization and implications for metabolic regulation at the carbon–nitrogen interface. Microbiol (Reading). 2005;151:1409–19. https://doi.org/10.1099/mic.0.27751-0
11. Choudhury R, Punekar NS. *Aspergillus terreus* NADP-glutamate dehydrogenase is kinetically distinct from the allosteric enzyme of other *Aspergilli*. Mycol Res. 2009;113:1121–6. https://doi.org/10.1016/j.mycres.2009.07.009
12. Vallorani L, Polidori E, Sacconi C, Agostini D, Pierleoni R, Piccoli G, Zeppa S, Stocchi V. Biochemical and molecular characterization of NADP-glutamate dehydrogenase from the ectomycorrhizal fungus *Tuber borchii*. New Phytol. 2002;154:779–90. https://doi.org/10.1046/j.1469-8137.2002.00409.x.
13. Morel M, Buée M, Chalot M, Brun A. NADP-dependent glutamate dehydrogenase: A dispensable function in ectomycorrhizal fungi. New Phytol. 2006;169:179–90. https://doi.org/10.1111/j.1469-8137.2005.01556.x.
14. Mara P, Fragiadakis GS, Gkountromichos F, Alexandraki D. The pleiotropic effects of the glutamate dehydrogenase (GDH) pathway in *Saccharomyces cerevisiae*. Microb Cell Fact. 2018;17:170. https://doi.org/10.1186/s12934-018-1018-4
15. Han T-L, Cannon RD, Gallo SM, Villas-Bôas SG. A metabolomic study of the effect of *Candida albicans* glutamate dehydrogenase deletion on growth and morphogenesis. NPJ biofilms microbiomes. 2019;5:13. https://doi.org/10.1038/s41522-019-0086-5
16. Silao FGS, Ryman K, Jiang T, Ward M, Hansmann N, Molenaar C, Liu N-N, Chen C, Ljungdahl PO. Glutamate dehydrogenase (Gdh2)-dependent alkalization is dispensable for escape from macrophages and virulence of *Candida albicans*. PLoS Pathog. 2020;16:e1008328. https://doi.org/10.1371/journal.ppat.1008328
17. Ma C, Zhao R, Li S-W, Zhao J, Jia Z, Tang L, Song Y, Wang R-J, Yang J, Peng Y-L. Glutamate dehydrogenase MoGDH2 modulates the environmental and host pH to enhance adaptation and virulence of the rice blast fungus *Pyricularia oryzae*. Int J Biol Macromol. 2025;308:142465. https://doi.org/10.1016/j.ijbiomac.2025.142465
18. Thykaer J, Rueksomtawin K, Noorman H, Nielsen J. Disruption of the NADPH-dependent glutamate dehydrogenase affects the morphology of two industrial strains of *Penicillium chrysogenum*. J Biotechnol. 2009;139:280–2. https://doi.org/10.1016/j.jbiotec.2008.12.016.
19. Thykaer J, Rueksomtawin K, Noorman H, Nielsen J. NADPH-dependent glutamate dehydrogenase in *Penicillium chrysogenum* is involved in regulation of beta-lactam production. Microbiol (Reading). 2008;154:1242–50. https://doi.org/10.1099/mic.0.2007/010017-0
20. Pathan EK, Ghormade V, Panwar SL, Prasad R, Deshpande MV. Molecular studies of NAD- and NADP-glutamate dehydrogenases decipher the conundrum of yeast-hypha dimorphism in zygomycete *Benjaminiella poitrasii*. FEMS Yeast Res. 2019;19(8):foz074. https://doi.org/10.1093/femsyr/foz074

21. Pathan EK, Ghormade V, Deshpande MV. Selection of reference genes for quantitative real-time RT-PCR assays in different morphological forms of dimorphic zygomycetous fungus *Benjaminiella poitrasii*. PLoS ONE. 2017;12:e0179454. https://doi.org/10.1371/journal.pone.0179454
22. Amin A, Joshi M, Deshpande MV. Morphology-associated expression of NADP-dependent glutamate dehydrogenases during yeast-mycelium transition of a dimorphic fungus *Benjaminiella poitrasii*. Antonie Van Leeuwenhoek. 2004;85:327–34. https://doi.org/10.1023/B:ANTO.0000020384.26238.48
23. Lu Y, Wu D, Hu J, Peng G, Zhong J, Liu Y, Li J, Gao Q, Yu X-Q. Glutamate dehydrogenase 2 is required for virulence by facilitating fungal growth in the host hemocoel. Virulence. 2025;16. https://doi.org/10.1080/21505594.2025.2591402.
24. Bogáti MS, Pócsi I, Maticsek J, Boross P, Tózsér J, Szentirmai A. NADP-specific glutamate dehydrogenase of *Penicillium chrysogenum* has a homohexamer structure. J Basic Microbiol. 1996;36:371–5. https://doi.org/10.1002/jobm.3620360512
25. DeLuna A, Avendaño A, Riego L, González A. NADP-Glutamate dehydrogenase isoenzymes of *Saccharomyces cerevisiae*. J Biol Chem. 2001;276:43775–83. https://doi.org/10.1074/jbc.M107986200
26. Hudson RC, Daniel RM. L-glutamate dehydrogenases: Distribution, properties and mechanism. Comp Biochem Physiol B. 1993;106:767–92. https://doi.org/10.1016/0305-0491(93)90031-Y.
27. Joshi CV, Pathan EK, Punekar NS, Tupe SG, Kapadnis BP, Deshpande MV. A biochemical correlate of dimorphism in a zygomycete *Benjaminiella poitrasii*: Characterization of purified NAD-dependent glutamate dehydrogenase, a target for antifungal agents. Antonie Van Leeuwenhoek. 2013;104:25–36. https://doi.org/10.1007/s10482-013-9921-5
28. Pathan EK, Kulkarni AM, Prasanna NVL, Ramana CV, Deshpande MV. NADP-dependent glutamate dehydrogenases in a dimorphic zygomycete *Benjaminiella poitrasii*: Purification, characterization and their evaluation as an antifungal drug target, Biochim. Biophys Acta Gen Subj. 2020;1864:129696. https://doi.org/10.1016/j.bbagen.2020.129696
29. LéJohn HB. Enzyme regulation, lysine pathways and cell wall structures as indicators of major lines of evolution in Fungi. Nature. 1971;231:164–8. https://doi.org/10.1038/231164a0.
30. Pavesi A, Ficarelli A, Tassi F, Restivo FM. Cloning of two glutamate dehydrogenase cDNAs from *Asparagus officinalis*: Sequence analysis and evolutionary implications. Genome. 2000;43:306–16. https://doi.org/10.1139/g99-127
31. Andersson JO, Roger AJ. Evolution of glutamate dehydrogenase genes: Evidence for lateral gene transfer within and between prokaryotes and eukaryotes. BMC Evol Biol. 2003;3:14. https://doi.org/10.1186/1471-2148-3-14
32. Benachenhou-Lahfa N, Forterre P, Labedan B. Evolution of glutamate dehydrogenase genes: Evidence for two paralogous protein families and unusual branching patterns of the archaebacteria in the universal tree of life. J Mol Evol. 1993;36. https://doi.org/10.1007/BF00182181.
33. Lomnitz A, Calderon J, Hernandez G, Mora J. Functional analysis of ammonium assimilation enzymes in *Neurospora crassa*. J Gen MicrobioL. 1987;133:2333–40. https://doi.org/10.1099/00221287-133-8-2333
34. Hemmings BA. Phosphorylation of NAD-dependent glutamate dehydrogenase from yeast. J Biol Chem. 1978;253:5255–8.
35. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK. Global analysis of protein localization in budding yeast. Nature. 2003;425:686–91. https://doi.org/10.1038/nature02026.
36. Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, Meyer H.E., Schönfisch B., Perschil I., Chacinska A., Guiard B., Rehling P., Pfanner N., Meisinger C. The proteome of *Saccharomyces cerevisiae* mitochondria. Proc Natl Acad Sci U S A. 2003;100:13207–12. https://doi.org/10.1073/pnas.2135385100
37. Baars JJP, Op den Camp HJM, van Hoek AHAM, van der Drift C, Van Griensven LJLD, Visser J, Vogels GD. Purification and characterization of NADP-dependent glutamate dehydrogenase from the commercial mushroom *Agaricus bisporus*. Curr Microbiol. 1995;30:211–7. . https://doi.org/10.1007/BF00293635
38. Perysinakis A, Kinghorn JR, Drainas C. Biochemical and genetical studies of NADP-specific glutamate dehydrogenase in the fission yeast *Schizosaccharomyces pombe*. Curr Genet. 1994;26:315–20. https://doi.org/10.1007/BF00310495
39. Boles E, Lehnert W, Zimmermann FK. The role of the NAD-dependent glutamate dehydrogenase in restoring growth on glucose of a *Saccharomyces cerevisiae* phosphoglucose isomerase mutant. Eur J Biochem. 1993;217:469–77. https://doi.org/10.1111/j.1432-1033.1993.tb18266.x
40. Orij R, Postmus J, Ter Beek A, Brul S, Smits GJ. *In vivo* measurement of cytosolic and mitochondrial pH using a pH-sensitive GFP derivative in *Saccharomyces cerevisiae* reveals a relation between intracellular pH and growth. Microbiol. 2009;155:268–78. https://doi.org/10.1099/mic.0.022038-0
41. Garnier A, Berredjem A, Botton B. Purification and characterization of the NAD-dependent glutamate dehydrogenase in the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton, Fungal Genet and Biol. 22 (1997) 168–76. https://doi.org/10.1006/fgbi.1997.1004
42. Kersten MASH, Müller Y, Baars JJP, Op den Camp HJM, van der Drift C, Van Griensven LJLD, Visser J, Schaap PJ. NAD+-dependent glutamate dehydrogenase of the edible mushroom *Agaricus bisporus*: Biochemical and molecular characterization. Mol Gen Genet. 1999;261:452–62. https://doi.org/10.1007/s004380050988
43. LéJohn HB, Jackson S. Allosteric interactions of a regulatory nicotinamide adenine dinucleotide-specific glutamate dehydrogenase from *Blastocladiella*. A molecular model for the enzyme. J Biol Chem. 1968;243:3447–57.
44. Alba-Lois L, Segal C, Rodarte B, Valdés-López V, DeLuna A, Cárdenas R. NADP-Glutamate dehydrogenase activity is increased under hyperosmotic conditions in the halotolerant yeast *Debaryomyces hansenii*. Curr Microbiol. 2004;48:68–72. https://doi.org/10.1007/s00284-003-4076-7
45. Campero-Basaldua C, Quezada H, Riego-Ruíz L, Márquez D, Rojas E, González J, El-Hafidi M, González A. Diversification of the kinetic properties of yeast NADP-glutamate-dehydrogenase isozymes proceeds independently of their evolutionary origin. MicrobiologyOpen. 2017;6:e00419. https://doi.org/10.1002/mbo3.419
46. Walvekar AS, Choudhury R, Punekar NS. Mixed disulfide formation at Cys141 leads to apparent unidirectional attenuation of *Aspergillus niger* NADP-glutamate dehydrogenase activity. PLoS ONE. 2014;9:e101662. https://doi.org/10.1371/journal.pone.0101662
47. Hanukoglu I. Rossmann fold: A beta-alpha-beta fold at dinucleotide binding sites. Biochem Mol Biol Educ. 2015;43:206–9. https://doi.org/10.1002/bmb.20849.
48. Rao ST, Rossmann MG. Comparison of super-secondary structures in proteins. J Mol Biol. 1973;76:241–56. https://doi.org/10.1016/0022-2836(73)90388-4.
49. Prakash P, Punekar NS, Bhaumik P. Structural basis for the catalytic mechanism and α-ketoglutarate cooperativity of glutamate dehydrogenase. J Biol Chem. 2018;293:6241–58. https://doi.org/10.1074/jbc.RA117.000149.
50. Cogoni C, Valenzuela L, González-Halphen D, Olivera H, Macino G, Ballario P, González A. *Saccharomyces cerevisiae* has a single glutamate synthase gene coding for a plant-like high-molecular-weight polypeptide. J Bacteriol. 1995;177:792–8. https://doi.org/10.1128/jb.177.3.792-798.1995
51. Moye WS, Amuro N, Rao JK, Zalkin H. Nucleotide sequence of yeast GDH1 encoding nicotinamide adenine dinucleotide phosphate-dependent glutamate dehydrogenase. J Biol Chem. 1985;260:8502–8.
52. Romero M, Guzmán-León S, Aranda C, González-Halphen D, Valenzuela L, González A. Pathways for glutamate biosynthesis in the yeast *Kluyveromyces lactis*. Microbiol (Reading). 2000;146:239–45. . https://doi.org/10.1099/00221287-146-1-239
53. Valenzuela L, Guzman-Leon S, Coria R, Ramirez J, Aranda C, Gonzalez A. A NADP-glutamate dehydrogenase mutant of the petit-negative yeast *Kluyveromyces lactis* uses the glutamine synthetase-glutamate synthase pathway for glutamate biosynthesis. Microbiol. 1995;141:2443–7. https://doi.org/10.1099/13500872-141-10-2443
54. Kinnaird JH, Fincham JRS. The complete nucleotide sequence of the *Neurospora crassa* am (NADP-specific glutamate dehydrogenase) gene. Gene. 1983;26:253–60. https://doi.org/10.1016/0378-1119(83)90195-6
55. Fincham JR, Kinsey JA, Fuentes AM, Cummings NJ, Connerton IF. The *Neurospora* am gene and NADP-specific glutamate dehydrogenase: Mutational sequence changes and functional effects - more mutants and a summary, Genet. Res. 76 (2000) S0016672300004602. https://doi.org/10.1017/S0016672300004602
56. Kinghorn JR, Pateman JA. NAD and NADP L-glutamate dehydrogenase activity and ammonium regulation in *Aspergillus nidulans*. J Gen Microbiol. 1973;78:39–46. https://doi.org/10.1099/00221287-78-1-39
57. Hawkins AR, Gurr SJ, Montague P, Kinghorn JR. Nucleotide sequence and regulation of expression of the *Aspergillus nidulans* gdhA gene encoding NADP dependent glutamate dehydrogenase. Mol Gen Genet. 1989;218:105–11. . https://doi.org/10.1007/BF00330572
58. Macheda ML, Hynes MJ, Davis MA. The *Aspergillus nidulans gltA* gene encoding glutamate synthase is required for ammonium assimilation in the absence of NADP-glutamate dehydrogenase. Curr Genet. 1999;34:467–71. https://doi.org/10.1007/s002940050421
59. Cardoza RE, Moralejo FJ, Gutiérrez S, Casqueiro J, Fierro F, Martín JF. Characterization and nitrogen-source regulation at the transcriptional level of the

gdhA gene of *Aspergillus awamori* encoding an NADP-dependent glutamate dehydrogenase. Curr Genet. 1998;34:50–9. https://doi.org/10.1007/s002940050365
60. Ghogare SS, Jethwa A, Pathan EK. Serum and glycerol regulate yeast-hypha dimorphism in *Benjaminiella poitrasii* by modulating glutamate dehydrogenase gene expression. Ann Microbiol. 2025;75:33. https://doi.org/10.1186/s13213-025-01824-8
61. Uno I, Matsumoto K, Adachi K, Ishikawa T. Regulation of NAD-dependent glutamate dehydrogenase by protein kinases in *Saccharomyces cerevisiae*. J Biol Chem. 1984;259:1288–93.
62. Veronese FM, Nyc JF, Degani Y, Brown DM, Smith EL. Nicotinamide adenine dinucleotide-specific glutamate dehydrogenase of *Neurospora*. I. Purification and molecular properties. J Biol Chem. 1974;249:7922–8.
63. Van Laere AJ. Purification and properties of NAD-dependent glutamate dehydrogenase from *Phycomyces* Spores, J. Gen. Microbiol. 134 (1988) 1597–1601. https://doi.org/10.1099/00221287-134-6-1597
64. Godsora BKJ, Prakash P, Punekar NS, Bhaumik P. Molecular insights into the inhibition of glutamate dehydrogenase by the dicarboxylic acid metabolites. Proteins Struct Funct Bioinform. 2022;90:810–23. https://doi.org/10.1002/prot.26276
65. Godsora BKJ, Das P, Mishra PK, Sairaman A, Kaledhonkar S, Punekar NS, Bhaumik P. Conformational flexibility associated with remote residues regulates the kinetic properties of glutamate dehydrogenase. Protein Sci. 2025;34. https://doi.org/10.1002/pro.70038.
66. Li N, Wang W, Zeng X, Liu M, Li M, Li C, Wang M. Crystal structure of glutamate dehydrogenase 3 from *Candida albicans*. Biochem Biophys Res Commun. 2021;570:15–20. https://doi.org/10.1016/j.bbrc.2021.07.014
67. Landon M, Piszkiewicz D, Smith EL. Sequence of bovine liver glutamate dehydrogenase. II. Sequences of tryptic peptides. J Biol Chem. 1971;246:2374–99. https://doi.org/10.1016/S0021-9258(18)62302-6.
68. Blumenthal KM, Smith EL. Nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase of *Neurospora*. I. Isolation, subunits, amino acid composition, sulfhydryl groups, and identification of a lysine residue reactive with pyridoxal phosphate and N-ethylmaleimide. J Biol Chem. 1973;248:6002–8.
69. Ahn JY, Choi S, Cho SW. Identification of lysine residue involved in inactivation of brain glutamate dehydrogenase isoproteins by o-phthalaldehyde. Biochimie. 1999;81:1123–9. https://doi.org/10.1016/S0300-9084(99)00349-1
70. Lilley KS, Engel PC. The essential active-site lysines of clostridial glutamate dehydrogenase: A study with pyridoxal-5′-phosphate. Eur J Biochem. 1992;207:533–40. https://doi.org/10.1111/j.1432-1033.1992.tb17079.x
71. Lin HPP, Reeves HC. Properties of phosphorylated $NADP^+$-specific glutamate dehydrogenase from *Escherichia coli*. Curr Microbiol. 1992;24:73–9. https://doi.org/10.1007/BF01570901
72. Hernández G, Sánchez-Pescador R, Palacios R , Mora J. Nitrogen source regulates glutamate dehydrogenase NADP synthesis in *Neurospora crassa*. J Bacteriol. 1983;154:524–8. https://doi.org/10.1128/jb.154.1.524-528.1983
73. Hynes MJ. The effects of the carbon source on glutamate dehydrogenase activities in *Aspergillus nidulans*. J Gen Microbiol. 1974;81:165–70. https://doi.org/10.1099/00221287-81-1-165
74. Moore D. Evidence that the NADP-linked glutamate dehydrogenase of Coprinus cinereus is regulated by acetyl-CoA and ammonium levels, Biochimica et Biophysica Acta (BBA) - Enzymology 661 (1981) 247–54. https://doi.org/10.1016/0005-2744(81)90011-5
75. Choudhury R, Noor S, Varadarajalu LP, Punekar NS. Delineation of an *in vivo* inhibitor for *Aspergillus* glutamate dehydrogenase. Enzyme Microb Technol. 2008;42:151–9. https://doi.org/10.1016/j.enzmictec.2007.08.011
76. Choudhury R, Punekar NS. Competitive inhibition of glutamate dehydrogenase reaction. FEBS Lett. 2007;581:2733–6. https://doi.org/10.1016/j.febslet.2007.05.032.
77. Cunliffe D, Leason M, Parkin D, Lea PJ. The inhibition of glutamate dehydrogenase by derivatives of isophthalic acid. Phytochemistry. 1983;22:1357–60. https://doi.org/10.1016/S0031-9422(00)84014-5.
78. Stevens L, Duncan D, Robertson P. Purification and characterisation of NAD-glutamate dehydrogenase from *Aspergillus nidulans*. FEMS Microbiol Lett. 1989;57:173–7. https://doi.org/10.1111/j.1574-6968.1989.tb03294.x
79. Caughey WS, Hellerman L, Smiley JD. L-glutamic acid dehydrogenase; structural requirements for substrate competition; effect of thyroxine. J Biol Chem. 1957;224:591–607.
80. Rogers KS, Boots MR, Boots SG. Molecular interactions of six aromatic competitive inhibitors with bovine liver glutamate dehydrogenase. Biochim et Biophys Acta (BBA) - Enzymol. 1972;258:343–50. https://doi.org/10.1016/0005-2744(72)90225-2.
81. Rogers KS. Molecular interactions of competitive inhibitors with bovine liver glutamate dehydrogenase. J Biol Chem. 1971;246:2004–9.
82. Haberland ME, Chen CW, Smith EL. NAD-specific glutamate dehydrogenase of *Neurospora crassa*. Limited action of trypsin and the presence of two distinct domains. J Biol Chem. 1980;255:7993–8000.
83. Sanwal BD. Diphosphopyridine nucleotide and triphosphopyridine nucleotide linked glutamic dehydrogenases of Fusarium. Arch Biochem Biophys. 1961;93:377–86. https://doi.org/10.1016/0003-9861(61)90281-8.
84. Gonzalez R. The integration of nitrogen and carbon catabolite repression in *Aspergillus nidulans* requires the GATA factor AreA and an additional positive-acting element, ADA. EMBO J. 1997;16:2937–44. https://doi.org/10.1093/emboj/16.10.2937
85. Nair A, Sarma SJ. The impact of carbon and nitrogen catabolite repression in microorganisms. Microbiol Res. 2021;251:126831. https://doi.org/10.1016/j.micres.2021.126831.
86. Portnoy T, Margeot A, Linke R, Atanasova L, Fekete E, Sándor E, Hartl L, Karaffa L, Druzhinina IS, Seiboth B, Le Crom S, Kubicek CP. The CRE1 carbon catabolite repressor of the fungus *Trichoderma reesei*: A master regulator of carbon assimilation. BMC Genomics. 2011;12:269. https://doi.org/10.1186/1471-2164-12-269
87. Sun J, Glass NL. Identification of the CRE-1 cellulolytic regulon in *Neurospora crassa*. PLoS ONE. 2011;6:e25654. https://doi.org/10.1371/journal.pone.0025654
88. Adnan M, Zheng W, Islam W, Arif M, Abubakar Y, Wang Z, Lu G. Carbon catabolite repression in filamentous fungi. Int J Mol Sci. 2017;19:48. https://doi.org/10.3390/ijms19010048.
89. Kunitake E, Uchida R, Asano K, Kanamaru K, Kimura M, Kimura T, Kobayashi T. cAMP signaling factors regulate carbon catabolite repression of hemicellulase genes in *Aspergillus nidulans*. AMB Express. 2022;12:126. https://doi.org/10.1186/s13568-022-01467-x
90. Kapoor M, Vijayaraghavan Y, Kadonaga R, LaRue KEA. NAD (+)-specific glutamate dehydrogenase of *Neurospora crassa*: Cloning, complete nucleotide sequence, and gene mapping. Biochem Cell Biol. 1993;71:205–19. https://doi.org/10.1139/o93-032
91. Dubois E, Grenson M, Wiame J-M. The Participation of the anabolic glutamate dehydrogenase in the nitrogen catabolite repression of arginase in *Saccharomyces cerevisiae*. Eur J Biochem. 1974;48:603–16. . https://doi.org/10.1111/j.1432-1033.1974.tb03803.x
92. Gancedo JM. Yeast carbon catabolite repression. Microbiol Mol Biol Rev. 1998;62:334–61. https://doi.org/10.1128/MMBR.62.2.334-361.1998.
93. ter Schure EG, van Riel NAW, Verrips CT. The role of ammonia metabolism in nitrogen catabolite repression in *Saccharomyces cerevisiae*. FEMS Microbiol Rev. 2000;24:67–83. https://doi.org/10.1111/j.1574-6976.2000.tb00533.x
94. Riego L, Avendaño A, DeLuna A, Rodŕıguez E, González A. GDH1 expression is regulated by GLN3, GCN4, and HAP4 under respiratory growth. Biochem Biophys Res Commun. 2002;293:79–85. https://doi.org/10.1016/S0006-291X(02)00174-2.
95. Rajak N, Shah R, Sharma Y, Rangarajan P. Dual transcriptional and post-transcriptional regulation by the bHLH proteins Rtg1 and RtgX in *Komagataella phaffii*. Nucleic Acids Res. 2026;54. https://doi.org/10.1093/nar/gkaf1475
96. Li G, Gong Z, Dulal N, Marroquin-Guzman M, Rocha RO, Richter M, Wilson RA. A protein kinase coordinates cycles of autophagy and glutaminolysis in invasive hyphae of the fungus *Magnaporthe oryzae* within rice cells. Nat Commun. 2023;14:4146. https://doi.org/10.1038/s41467-023-39880-w
97. Dey T, Rangarajan PN. Carbon starvation-induced synthesis of GDH2 and PEPCK is essential for the survival of *Pichia pastoris*. Biochem Biophys Res Commun. 2021;581:25–30. https://doi.org/10.1016/j.bbrc.2021.10.015
98. Krebs EG, Beavo JA. Phosphorylation-dephosphorylation of enzymes. Annu Rev Biochem. 1979;48:923–59. https://doi.org/10.1146/annurev.bi.48.070179.004423.
99. Hemmings BA. Phosphorylation and proteolysis regulate the NAD-dependent glutamate dehydrogenase from Saccharomyces cerevisiae. FEBS Lett. 1980;122:297–302. https://doi.org/10.1016/0014-5793(80)80460-1.
100. Trotter PJ, Juco K, Le HT, Nelson K, Tamayo LI, Nicaud J, Park Y. Glutamate dehydrogenases in the oleaginous yeast *Yarrowia lipolytica*. Yeast. 2020;37:103–15. https://doi.org/10.1002/yea.3425.
101. West DJ, Tuveson RW, Barratt RW, Fincham JRS. Allosteric effects in nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase from *Neurospora*. J Biol Chem. 1967;242:2134–8. https://doi.org/10.1016/S0021-9258(18)96028-X.

102. Agarwal N, Walvekar AS, Punekar NS. 2-Oxoglutarate cooperativity and biphasic ammonium saturation of *Aspergillus niger* NADP-glutamate dehydrogenase are structurally coupled. Arch Biochem Biophys. 2019;669:50–60. . https://doi.org/10.1016/j.abb.2019.05.018
103. Bartnicki-Garcia S, Nickerson WJ. Nutrition, growth, and morphogenesis of *Mucor rouxii*. J Bacteriol. 1962;84:841–58. https://doi.org/10.1128/jb.84.4.841-858.1962
104. Miller SM, Magasanik B. Role of NAD-linked glutamate dehydrogenase in nitrogen metabolism in *Saccharomyces cerevisiae*. J Bacteriol. 1990;172:4927–35. https://doi.org/10.1128/jb.172.9.4927-4935.1990
105. Pathan EK, Tupe SG, Deshpande MV. : Fungal Differentiation: A model phenomenon to screen antifungal drugs. In: Satyanarayana, T., Deshmukh, S., Johri, B, (eds). Develop. Fungal Biol. Appl. Mycol. Springer Singapore, 2017b: pp. 227–246. https://doi.org/10.1007/978-981-10-4768-8_12
106. Ghormade V, Joshi C, Deshpande MV. Regulation by polyamines: A possible model for signal transduction pathway leading to dimorphism in *Benjaminiella poitrasii*. J Mycol Pl Pathol. 2005;35:442–50.
107. Du C, Lin J, Yang Y, Liu H, Li C, Zhou Y, Li Y, Tang D, Zhao X, Zhu Y, Liu X. Molecular cloning, characterization and function analysis of a GDH gene from *Sclerotinia sclerotiorum* in rice. Mol Biol Rep. 2014;41:3683–93. https://doi.org/10.1007/s11033-014-3233-3
108. Zhou Y, Zhang C, Lin J, Yang Y, Peng Y, Tang D, Zhao X, Zhu Y, Liu X. Overexpression of a glutamate dehydrogenase gene, MgGDH, from *Magnaporthe grisea* confers tolerance to dehydration stress in transgenic rice. Planta. 2015;241:727–40. https://doi.org/10.1007/s00425-014-2214-z
109. Loulakakis KA, Morot-Gaudry JF, Velanis CN, Skopelitis DS, Moschou PN, Hirel B. Roubelakis-Angelakis, Advancements in nitrogen metabolism in Grapevine. In: Roubelakis-Angelakis KA, editor. Grapevine, Mol. Physiol. Biotechnol. Dordrecht: Springer Netherlands; 2009. pp. 161–205. https://doi.org/10.1007/978-90-481-2305-6_7.
110. Zhang H, Liang C, Aoki N, Kawai K, Takane K, Ohsugi R. Introduction of a fungal NADP(H)-dependent glutamate dehydrogenase (*gdhA*) improves growth, grain weight and salt resistance by enhancing the nitrogen uptake efficiency in forage rice. Plant Prod Sci. 2016;19:267–78. https://doi.org/10.1080/1343943X.2015.1133237
111. Abiko T, Wakayama M, Kawakami A, Obara M, Kisaka H, Miwa T, Aoki N, Ohsugi R. Changes in nitrogen assimilation, metabolism, and growth in transgenic rice plants expressing a fungal NADP(H)-dependent glutamate dehydrogenase (*gdhA*). Planta. 2010;232:299–311. . https://doi.org/10.1007/s00425-010-1172-3
112. Tang D, Peng Y, Lin J, Du C, Yang Y, Wang D, Liu C, Yan L, Zhao X, Li X, Chen L, Liu X. Ectopic expression of fungal *EcGDH* improves nitrogen assimilation and grain yield in rice. J Integr Plant Biol. 2018;60:85–8. https://doi.org/10.1111/jipb.12519
113. Du CQ, Lin JZ, Dong LA, Liu C, Tang DY, Yan L, Chen MD, Liu S, Liu XM. Overexpression of an NADP(H) - dependent glutamate dehydrogenase gene, *TrGDH*, from *Trichurus* improves nitrogen assimilation, growth status and grain weight per plant in rice. Breed Sci. 2019;69:429–38. https://doi.org/10.1270/jsbbs.19014.
114. Yan L, Gong Y, Luo Q, Dai G-X, Teng Z, He Y, Wu X, Liu C, Tang D, Ye N, Deng G, Lin J, Liu X. Heterologous expression of fungal *AcGDH* alleviates ammonium toxicity and suppresses photorespiration, thereby improving drought tolerance in rice. Plant Sci. 2021;305:110769. https://doi.org/10.1016/j.plantsci.2020.110769
115. Osuji GO, Mangaroo AS, Reyes J, Bulgin A, Wright V. Biomass enhancement in maize and soybean in response to glutamate dehydrogenase isomerization. Biol Plant. 2003;46:45–52. https://doi.org/10.1023/A:1027324713682
116. Ameziane R, Bernhard K, Lightfoot D. Expression of the bacterial gdhA gene encoding a NADPH glutamate dehydrogenase in tobacco affects plant growth and development. Plant Soil. 2000;221:47–57. https://doi.org/10.1023/A:1004794000267
117. Rocchitta G, Spanu A, Babudieri S, Latte G, Madeddu G, Galleri G, Nuvoli S, Bagella P, Demartis M, Fiore V, Manetti R, Serra P. Enzyme biosensors for biomedical applications: Strategies for safeguarding analytical performances in biological fluids, Sensors 16 (2016) 780. https://doi.org/10.3390/s16060780
118. Basu AK, Chattopadhyay P, Roychudhuri U, Chakraborty R. A biosensor based on co-immobilized L-glutamate oxidase and L-glutamate dehydrogenase for analysis of monosodium glutamate in food. Biosens Bioelectron. 2006;21:1968–72. https://doi.org/10.1016/j.bios.2005.09.011
119. Monosik R, Stredansky M, Tkac J, Sturdik E. Application of enzyme biosensors in analysis of food and beverages. Food Anal Methods. 2012;5:40–53. https://doi.org/10.1007/s12161-011-9222-4.
120. Matos-Peralta Y, Antuch M, Abradelo DG, Bazán-Bravo L, de la Hernández KR. Glutamate dehydrogenase-based electrochemical biosensors: The immobilization method defines sensor selectivity. J Electrochem Soc. 2019;166:B1146–50. https://doi.org/10.1149/2.0281913jes
121. Pasco N, Jeffries C, Davies Q, Downard AJ, Roddick-Lanzilotta AD, Gorton L. Characterisation of a thermophilic L-glutamate dehydrogenase biosensor for amperometric determination of L-glutamate by flow injection analysis. Biosens Bioelectron. 1999;14:171–8. https://doi.org/10.1016/S0956-5663(98)00120-1.
122. Liang B, Zhang S, Lang Q, Song J, Han L, Liu A. Amperometric L-glutamate biosensor based on bacterial cell-surface displayed glutamate dehydrogenase. Anal Chim Acta. 2015;884:83–9. https://doi.org/10.1016/j.aca.2015.05.012.
123. Azmi NE, Ahmad M, Abdullah J, Sidek H, Heng LY, Karuppiah N. Biosensor based on glutamate dehydrogenase immobilized in chitosan for the determination of ammonium in water samples. Anal Biochem. 2009;388:28–32. https://doi.org/10.1016/j.ab.2009.02.005.
124. Guan L, Wu F, Ren G, Wang J, Yang X, Huang X, Yu P, Lin Y, Mao L. Role of rare-earth elements in enhancing bioelectrocatalysis for biosensing with NAD^+-dependent glutamate dehydrogenase. Chem Sci. 2021;12:13434–41. https://doi.org/10.1039/D1SC00193K.
125. Joshi CV, Ghormade V, Kunde P, Kulkarni P, Mamgain H, Bhat S, Paknikar KM, Deshpande MV. Flocculation of dimorphic yeast *Benjaminiella poitrasii* is altered by modulation of NAD-glutamate dehydrogenase. Bioresour Technol. 2010;101:1393–5. . https://doi.org/10.1016/j.biortech.2009.09.052
126. Chattaway FW, Bishop R, Holmes MR, Odds FC, Barlow AJE. Enzyme activities associated with carbohydrate synthesis and breakdown in the yeast and mycelial forms of *Candida albicans*. J Gen Microbiol. 1973;75:97–109. . https://doi.org/10.1099/00221287-75-1-97
127. Voss CM, Arildsen L, Nissen JD, Waagepetersen HS, Schousboe A, Maechler P, Ott P, Vilstrup H, Walls AB. Glutamate dehydrogenase is important for ammonia fixation and amino acid homeostasis in brain during hyperammonemia. Front Neurosci. 2021;15. https://doi.org/10.3389/fnins.2021.646291.
128. Tan H, Sang Z, Tian Y, Peng W, Liu X, Liang J. Ammonia as a green carbon-free fuel: A pathway to the sustainable energy economy. ACS Energy Lett. 2024;9:5120–36. https://doi.org/10.1021/acsenergylett.4c02288.
129. Jinap S, Hajeb P. Glutamate. Its applications in food and contribution to health. Appetite. 2010;55:1–10. https://doi.org/10.1016/j.appet.2010.05.002.
130. Bender DA. Nitrogen metabolism, in: Amino acid metabolism, Third edition, John Wiley & Sons, Ltd, 2012: pp. 1–65. https://doi.org/10.1002/9781118357514.ch1
131. Sieber SA, Marahiel MA. Molecular mechanisms underlying nonribosomal peptide synthesis: Approaches to new antibiotics. Chem Rev. 2005;105:715–38. https://doi.org/10.1021/cr0301191.
132. Olano C, Gómez C, Pérez M, Palomino M, Pineda-Lucena A, Carbajo RJ, Braña AF, Méndez C, Salas JA. Deciphering biosynthesis of the RNA polymerase inhibitor streptolydigin and generation of glycosylated derivatives. Chem Biol. 2009;16:1031–44. https://doi.org/10.1016/j.chembiol.2009.09.015.
133. Moore BS, Hertweck C. Biosynthesis and attachment of novel bacterial polyketide synthase starter units. Nat Prod Rep. 2002;19:70–99. https://doi.org/10.1039/B003939J.

Publisher's Note

Dr. Ejaj K. Pathan Dr. Pathan is an Assistant Professor at Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Pune, India. He completed his doctoral research at CSIR-National Chemical Laboratory on fungal morphogenesis using Benjaminiella poitrasii, identifying glutamate dehydrogenases as potential antifungal targets. He has worked in industry on fungal chitosan-based products and as a Postdoctoral Fellow at IIT Bombay on morphology- citric acid metabolism relationships in Aspergillus niger. At Symbiosis, he leads a research group in molecular mycology and enzymology, focusing on the molecular basis of fungal morphogenesis for biotechnological applications and exploring potential links between fungi and cancer, along with work in protein engineering.

Mr. Himal Sapkota Mr. Sapkota is a Ph.D. scholar at the Symbiosis School of Biological Sciences (SSBS), Symbiosis International (Deemed University), Pune, India. He completed M.Sc. in MLT (Clinical Microbiology) with first rank. His doctoral research focus on exploring human commensal bacteria as sources of glutaminase-free L-asparaginase, for improved and safe treatment of acute lymphoblastic leukaemia.

Dr. Subrata Dasgupta Dr. Dasgupta is a Research Scientist at the RIKEN Center for Integrative Medical Sciences (IMS), Yokohama, Japan. He completed his Ph.D. in Computational Structural Biology at NIT Durgapur, where he investigated the role of conserved water molecules in human monoamine oxidase. As a Postdoctoral Fellow at IIT-Bombay, he explored the interactions between Plasmodium proteins and mosquito receptors through molecular modeling and simulations. At RIKEN IMS, his current research focuses on protein engineering and structural studies to reconstruct evolutionary pathways and design novel functional proteins for diverse applications.

Professor Narayan S. Punekar Professor Punekar is a Retired Professor from the Department of Biosciences and Bioengineering at IIT-Bombay, India. His research focuses on microbial metabolism and enzymology, with extensive work on nitrogen-metabolizing enzymes in fungi, particularly NADP-dependent glutamate dehydrogenase. He has made significant contributions to understanding metabolic regulation and enzyme function in microbial systems. He is the author of the textbook Enzymes: Catalysis, Kinetics and Mechanisms, widely used in enzymology education.