CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2024 Vol.13, pp.124-130/Bhawana and Bhavya
***Research Article** (Open Access)*

PHYLOGENY OF HOMOSERINE DEHYDROGENASE

Bhawana Singh[1] and *Bhavya Jha[2]
[1]*Department of Zoology, RKD College, Patliputra University, Patna- 800020, Bihar, India*
[2]*Department of Zoology, Ganga Devi Mahila Mahavidyalaya, Patliputra University, Patna- 800020, Bihar, India*
**Author for Correspondence: bjha2805@gmail.com*

ABSTRACT
Homoserine dehydrogenase (HSD), a key enzyme in the aspartate pathway, catalyzes reversible conversion of L-aspartate β-semialdehyde to L-homoserine. This enzyme is an attractive anti-fungal and anti-bacterial target. Moreover, HSD is also of industrial interest as it can be exploited for yielding lysine, threonine and methionine. HSD exists as a monofunctional enzyme in some organisms while as a bifunctional Aspartokinase (AK)-HSD in others. It is regulated by the concentration of threonine through feedback inhibition in bifunctional form while may or may not be follow a feedback inhibition pattern in monofunctional form depending upon the source organism. This study based on sequences retrieved from Swiss-Prot database reports a comprehensive analysis of domains present in this enzyme belonging to different organisms and its subsequent effect on the feedback inhibition of the enzyme. The phylogenetic tree traces a pattern of evolution of this enzyme from being monofunctional feedback-insensitive to monofunctional feedback-sensitive and to bifunctional feedback-sensitive. This study provides a better understanding of phylogeny of this very important enzyme.

Keywords: *Homoserine Dehydrogenase, Aspartate Pathway, Homoserine, Domain Architecture, Phylogeny*

INTRODUCTION
Homoserine dehydrogenase (HSD) is a crucial enzyme which contributes to a critical branch point in the aspartate pathway. It furthers the formation of bacterial cell-wall components such as L-lysine and m-DAP as well as leads to biosynthesis of lysine, methionine, threonine, and isoleucine (Azevedo *et al*, 2006; Viola, 2001). The pathway is initiated by phosphorylation of aspartate mediated by the enzyme aspartate kinase (AK). Aspartyl phosphate is converted to L-aspartate-β-semialdehyde which can be metabolized to either L-lysine or L-homoserine (HSE). HSD is responsible for the reversible conversion of L-aspartate-β-semialdehyde to L-HSE. HSE can further be metabolized to yield methionine and threonine (Schroeder *et al*, 2000). The regulation of these enzymes by threonine depends on their structural organization in different organisms. This whole set up is absent in mammals and hence, the associated enzymes especially HSD has been exploited as an attractive target against many pathogenic infections (Bueno *et al*, 2019; Ejim *et al*, 2004; Jacques *et al*, 2001; Yamaki *et al*, 1990). Owing to the allosteric regulation of this enzyme in some organisms, it can be exploited to construct cell factories for large scale production of L-lysine and other HSD related amino acids and derivatives (Liu *et al*, 2024).
Interestingly, HSD has been reported to exist as monofunctional enzyme in some bacteria (Nguyen *et al*, 2020, Jacques *et al*, 2001; Parsot and Cohen, 1988) and yeast (Jacques *et al*, 2001) while as bifunctional enzyme having both HSD activity and AK activity in a few bacteria (Ohshida *et al*, 2018; Angeles and Viola,1990; Thomas *et al*, 1993) and plants (Muehlbauer *et al*, 1994, Paris *et al*, 2002). The enzyme assembly as well as its regulation is also influenced by its domain architecture. The presence of a C-terminal domain in monofunctional HSD enables its feedback inhibition by L-threonine (Navratna *et al*, 2015). All the reported bifunctional enzymes are feedback sensitive. This prompted us to investigate the domain architecture of HSD sequences from different groups available in curated protein database followed by their

evolutionary analysis to have a comprehensive understanding of the phylogeny of this very important enzyme.

MATERIALS AND METHODS

Sequence Retrieval

The Protein database of National Center for Biotechnology Information (NCBI) was searched with term "homoserine dehydrogenase" and filtered using Swiss-Prot as the source database. The search returned 37 protein sequences, of which 29 unique protein sequences with label homoserine dehydrogenase or bifunctional aspartokinase/homoserine dehydrogenase were retrieved for further analysis.

Domain analysis and Phylogenetic analysis

The retrieved sequences were individually submitted to Pfam database (Finn *et al,* 2010) and analyzed for the presence of domains. Sequences were aligned using ClustalW (Chenna *et al*, 2003). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitutions per site. This analysis involved 29 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 946 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al*, 2021). The original tree was exported along with branch lengths and bootstrap values in Newick format which then served as input in iTOL to yield the tree for representation.

RESULTS AND DISCUSSION

Domain analysis and phylogeny of Homoserine dehydrogenase

A search for Homoserine dehydrogenase returned hits from bacteria, eukaryotes and archaea. Interestingly, the eukaryotic hits belonged either to plants or fungi, not to animals. These sequences were labeled either HSDs or AK/HSDs in NCBI database corroborating with the earlier reports that this enzyme is either monofunctional or bifunctional (Navratna *et al*, 2015). This prompted us to explore the domains present in each of them. A summary of the domains in all the 29 sequences is presented in Table1. All the enzymes mandatorily harboured Homoserine_dh domain while the bifunctional AK/HSDs harboured an AA_kinase in addition to the Homoserine_dh domains. NAD_binding_3 domain was also present in all the sequences except for HSD of *Methylobacillus glycogenes 21276*. Archae, Firmicutes and actinobacteria possess monofunctional HSDs. While ß- and ε-proteobacteria have monofunctional HSDs, γ- proteobacteria were observed to harbour either monofunctional or bifunctional enzyme. Interestingly among eukaryotes, while plants have bifunctional AK/HSDs, fungi possess monofunctional HSDs.

The monofunctional HSDs may or may not possess an ACT domain. Presence of ACT enables a feedback regulation by L-threonine (Navratna *et al*, 2015), while its absence leads to a feedback-insensitive enzyme. However, the bifunctional enzyme is always feedback sensitive irrespective of the presence or absence of the ACT domain. A regulatory domain located either between AK and HSD domains or at the C-terminal of the enzyme is responsible for feedback sensitivity (Ohsida *et al*, 2018).

Taken together, the domain architecture analysis mentioned in Table1 and the previously cited relevant literature suggest that the archea harbour feedback insensitive monofunctional HSD, the firmicutes can have feedback insensitive or feedback sensitive monofunctional HSD, actinobacteria and ß-/ε-proteobacteria have feedback sensitive monofunctional HSD, γ- proteobacteria can have feedback sensitive monofunctional HSD or bifunctional AK-HSD, plants have bifunctional AK-HSD.

Table 1: Domain architecture of Homoserine dehydrogenase from different organisms

Sl. No.	Organism (Taxon)	ID	Description	Domain Architecture
1	*Methanocaldococcus jannaschii* (archae)	Q58997	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh
2	*Synechocystis sp.* (cyanobacteria)	P52986	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
3	*Bacillus subtilis* (Firmicutes)	P19582	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
4	*Lactococcus lactis subsp. Lactis* (Firmicutes)	Q9CGD8 P52985	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh
5	*Lactococcus lactis subsp. Cremoris* (Firmicutes)	Q9CGD8 P52985	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh
6	*Corynebacterium glutamicum* (Actinobacteria)	P08499	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
7	*Mycobacterium leprae* (Actinobacteria)	P46806	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
8	*Mycobacterium bovis* (Actinobacteria)	P63630	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
9	*Mycobacterium tuberculosis* CDC1551 (Actinobacteria)	P9WPX0	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
10	*Mycobacterium tuberculosis* H37Rv (Actinobacteria)	P9WPX1	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
11	*Methylobacillus glycogenes* ATCC 21371 (ß- proteobacteria)	P37144	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
12	*Methylobacillus glycogenes* 21276 (ß- proteobacteria)	P37143	Homoserine dehydrogenase	Homoserine_dh
13	*Helicobacter pylori* (**ε**-proteobacteria)	P56429	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
14	*Helicobacter pylori* strain J99 (**ε**-proteobacteria)	Q9ZL20	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
15	*Escherichia coli K12* (**γ**-proteobacteria)	P00561	Bifunctional aspartokinase/ homoserine dehydrogenase 1 (AKHSD1)	AA_kinase, ACT, ACT, NAD_binding_3, Homoserine_dh

16	*Escherichia coli K12* (γ-proteobacteria)	P00562	Bifunctional aspartokinase/ homoserine dehydrogenase 2 (AKHSD2)	AA_kinase, NAD_binding_3, Homoserine_dh
17	*Serratia marcescens* (γ-proteobacteria)	P27725	Bifunctional aspartokinase/ homoserine dehydrogenase 1	AA_kinase, NAD_binding_3, Homoserine_dh
18	*Haemophilus influenza* (γ-proteobacteria)	P44505	Bifunctional aspartokinase/ homoserine dehydrogenase	AA_kinase, ACT, ACT, NAD_binding_3, Homoserine_dh
19	*Buchnera aphidicola* strain APS (γ-proteobacteria)	P57290	Bifunctional aspartokinase/ homoserine dehydrogenase	AA_kinase, NAD_binding_3, Homoserine_dh
20	*Buchnera aphidicola* strain Bp (γ-proteobacteria)	Q89AR4	Bifunctional aspartokinase/ homoserine dehydrogenase	AA_kinase, NAD_binding_3, Homoserine_dh
21	*Buchnera aphidicola* strain Sg (γ-proteobacteria)	Q8K9U9	Bifunctional aspartokinase/ homoserine dehydrogenase	AA_kinase, NAD_binding_3, Homoserine_dh
22	*Pseudomonas aeruginosa* (γ-proteobacteria)	P29365	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh, ACT
23	*Daucus carota* (Plant)	P37142	Bifunctional aspartokinase/ homoserine dehydrogenase, chloroplastic	AA_kinase, ACT, ACT7, NAD_binding_3, Homoserine_dh
24	*Arabidopsis thaliana* (Plant)	O81852	Bifunctional aspartokinase/ homoserine dehydrogenase 2, chloroplastic	AA_kinase, ACT, ACT, NAD_binding_3, Homoserine_dh
25	*Zea mays* (Plant)	P49079	Bifunctional aspartokinase/ homoserine dehydrogenase 1, chloroplastic	AA_kinase, ACT, ACT7, NAD_binding_3, Homoserine_dh
26	*Zea mays* (Plant)	P49080	Bifunctional aspartokinase/ homoserine dehydrogenase 2, chloroplastic	AA_kinase, ACT, NAD_binding_3, Homoserine_dh
27	*Arabidopsis thaliana* (Plant)	Q9SA18	Bifunctional aspartokinase/ homoserine dehydrogenase 1, chloroplastic	AA_kinase, ACT, ACT7, NAD_binding_3, Homoserine_dh
28	*Emericella nidulans* (Fungi)	Q5B998	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh
29	*Saccharomyces cerevisiae* (Fungi)	P31116	Homoserine dehydrogenase	NAD_binding_3, Homoserine_dh

Since we observed a pattern in occurrence of domains in different taxon, it prompted us to explore the phylogeny of HSD/AK-HSD. The 29 sequences were subjected to alignment followed by a phylogenetic analysis (Fig. 1).

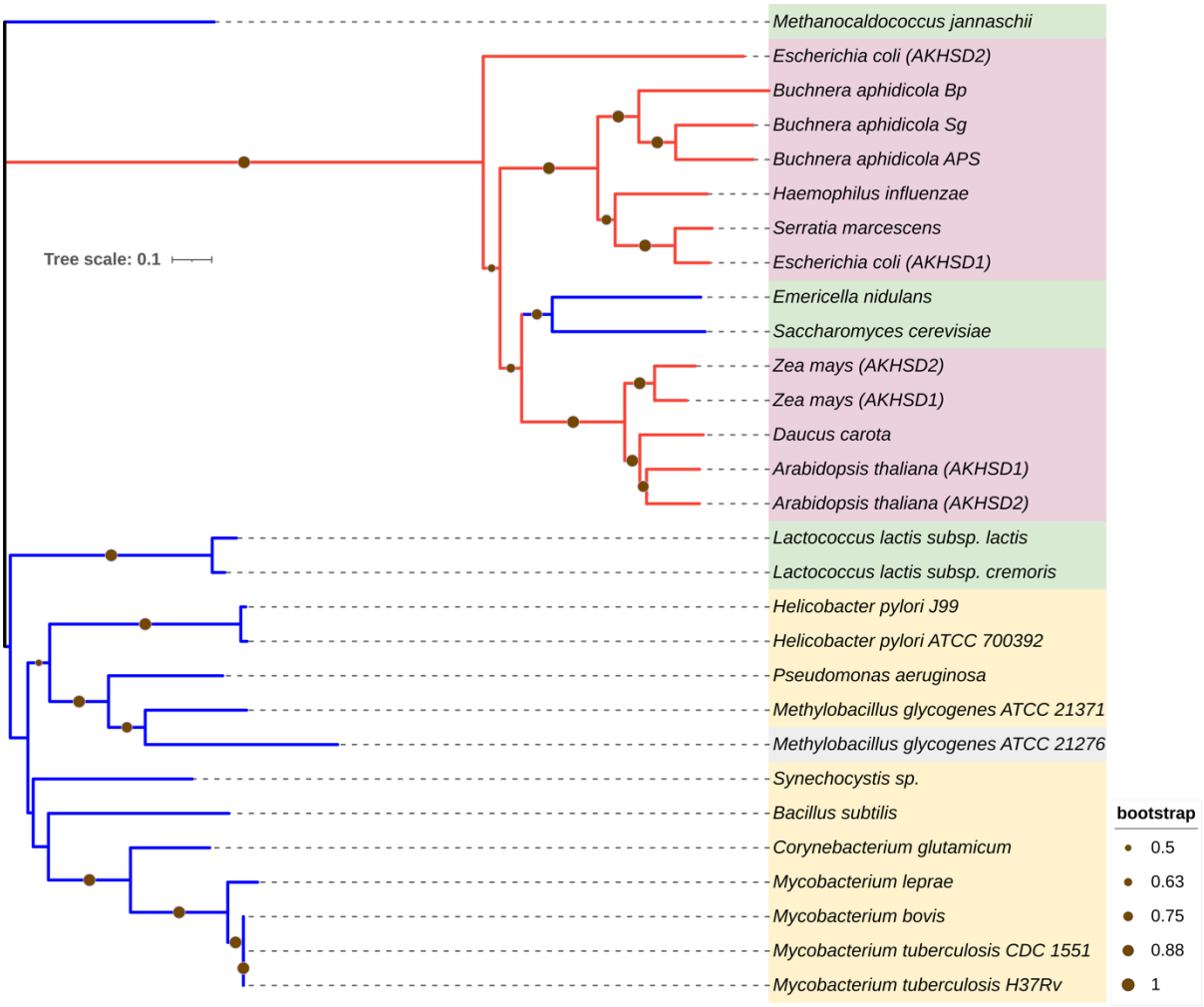

Figure 1: The optimal tree showing evolution of homoserine dehydrogenase in corresponding organisms.

The blue branches depict monofunctional HSDs while the red ones depict the bifunctional AK/HSD. The monofunctional and bifunctional enzyme has a clear divergence except for monofunctional HSD of fungi which is clustered with bifunctional AK/HSDs. Organisms shown in pink, yellow and green backgrounds harbor bifunctional AK/HSD, monofunctional HSD with regulatory domain and unregulated monofunctional HSD, respectively.
The bootstrap values of >0.5 (1000 replicates) are indicated by brown circles on the branches (reference panel at the lowermost right corner of the figure).

The monofunctional HSDs and bifunctional AK-HSDs appear to have a divergent evolution with an exception of fungal HSD. The monofunctional HSDs (shown in blue branches) of cyanobacteria, firmicutes, actinobacteria as well as proteobacteria grouped together to form a larger clade. Within it, proteobacteria and actinobacteria formed exclusive smaller clades. Their HSDs along with HSD of *Synechocystis* (cyanobacteria) are shown in yellow background meaning that they possess an ACT domain making them feedback sensitive. Firmicutes, although grouped together in the larger clade, branch out at smaller level separating *Lactococcus* and *Bacillus subtilis*. *Lactococcus* HSD is shown in green background denoting an ACT lacking, feedback insensitive enzyme while *Bacillus subtilis* HSD is shown in yellow depicting a

feedback sensitive enzyme. Bifunctional HSDs (shown in red branches) of γ- proteobacteria and plants along with monofunctional HSDs of fungi form a large clade. Within it, the eukaryotes and γ- proteobacteria form separate smaller clades. Within the eukaryotes, bifunctional plant HSDs and monofunctional fungal HSDs form separate clades. Notably, the fungal HSDs (green background) lack ACT domain and thus, supposed to be feedback insensitive. The bifunctional HSDs exhibited duplications in *Escherichia coli*, *Zea mays* and *Arabidopsis thaliana*, resulting in two paralogs. Archaeal HSD (*Methanocaldococus jannaschii*) which branched out separately from the common ancestor is also devoid of the ACT domain and hence, feedback insensitive. Overall, for a greater part, the phylogenetic tree matches the evolutionary relationships between the taxonomic groups.

REFERENCES

Angeles TS and Viola RE (1990). The kinetic mechanisms of the bifunctional enzyme aspartokinase-homoserine dehydrogenase I from *Escherichia coli*. *Archives of Biochemistry and Biophysics*, **283**(1), 96-101. https://doi.org/10.1016/0003-9861(90)90617-8

Azevedo RAD, Lancien M and Lea PJ (2006). The aspartic acid metabolic pathway, an exciting and essential pathway in plants. *Amino acids*, **30**, 143-162. https://doi.org/10.1007/s00726-005-0245-2

Bueno PSA, Rodrigues FAV, Santos JL, Canduri F, Biavatti DC, Pimentel AL, Bagatin MC, Kioshima ÉS, de Freitas Gauze G and Seixas FAV (2019). New inhibitors of homoserine dehydrogenase from Paracoccidioides brasiliensis presenting antifungal activity. *Journal of Molecular Modeling*, **25**, 1-9. https://doi.org/10.1007/s00894-019-4221-2

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, and Thompson JD (2003). Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research*, **31**, 3497–3500. https://doi.org/10.1093/nar/gkg500

Ejim L, Mirza IA, Capone C, Nazi I, Jenkins S, Chee GL, Berghuis AM and Wright GD (2004). New phenolic inhibitors of yeast homoserine dehydrogenase. *Bioorganic & Medicinal Chemistry*, **12**(14), 3825-3830. https://doi.org/10.1016/j.bmc.2004.05.009

Finn RD, Mistry J, Tate J, Coggill P, Heger A, Pollington JE, Gavin OL, Gunasekaran P, Ceric G, Forslund K and Holm L (2010). The Pfam protein families database. *Nucleic Acids Research*, **38** (suppl_1), D211-D222. https://doi.org/10.1093/nar/gkp985

Jacques SL, Nieman C, Bareich D, Broadhead G, Kinach R, Honek JF and Wright GD (2001). Characterization of yeast homoserine dehydrogenase, an antifungal target: the invariant histidine 309 is important for enzyme integrity. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, **1544**(1-2), 28-41. https://doi.org/10.1016/S0167-4838(00)00203-X

Liu X, Liu J, Liu Z, Qiao Q, Ni X, Yang J, Sun G, Li F, Zhou W, Guo X and Chen J (2024). Engineering allosteric inhibition of homoserine dehydrogenase by semi-rational saturation mutagenesis screening. *Frontiers in Bioengineering and Biotechnology*, **11**, 1336215. https://doi.org/10.3389/fbioe.2023.1336215

Muehlbauer GJ, Somers DA, Matthews BF, Gengenbach BG (1994). Molecular genetics of the maize (Zea mays L.) aspartate kinase-homoserine dehydrogenase gene family. *Plant Physiology*, **106**(4), 1303-12. https://doi.org/10.1104/pp.106.4.1303

Navratna V, Reddy G, Gopal B (2015). Structural basis for the catalytic mechanism of homoserine dehydrogenase, *Acta Crystallographica D Biological Crystallography***, 71,** 1216–1225. https://doi.org/10.1107/S1399004715004617

Nguyen QT, Ko GS and Yang JK (2020). Molecular and enzymatic features of homoserine dehydrogenase from Bacillus subtilis. *Journal of Microbiology and Biotechnology*, **30**(12), 1905. https://doi.org/10.4014%2Fjmb.2004.04060

Ohshida T, Koba K, Hayashi J, Yoneda K, Ohmori T, Ohshima T, Sakuraba H (2018) A novel bifunctional aspartate kinase-homoserine dehydrogenase from the hyperthermophilic bacterium,

Thermotoga maritima, *Bioscience, Biotechnology and Biochemistry*, **82,** 2084–2093. https://doi.org/10.1080/09168451.2018.1511365

Paris S, Wessel PM and Dumas R (2002). Overproduction, purification, and characterization of recombinant bifunctional threonine-sensitive aspartate kinase-homoserine dehydrogenase from Arabidopsis thaliana. *Protein Expression and Purification*, **24**(1), 105-110. https://doi.org/10.1006/prep.2001.1539

Parsot C and Cohen GN (1988). Cloning and nucleotide sequence of the *Bacillus subtilis* hom gene coding for homoserine dehydrogenase. Structural and evolutionary relationships with Escherichia coli aspartokinases-homoserine dehydrogenases I and II. *Journal of Biological Chemistry*, **263**(29), 14654-14660. https://doi.org/10.1016/S0021-9258(18)68087-1

Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**(4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Schroeder AC, Zhu C, Yanamadala SR, Cahoon RE, Arkus KA, Wachsstock L, Bleeke J, Krishnan HB and Jez JM (2010). Threonine-insensitive homoserine dehydrogenase from soybean: genomic organization, kinetic mechanism, and in vivo activity. *Journal of Biological Chemistry*, **285**(2), 827-834. https://doi.org/10.1074/jbc.M109.068882

Tamura K, Stecher G and Kumar S (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, **38**(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

Thomas D, Barbey R and Surdin-Kerjan Y (1993). Evolutionary relationships between yeast and bacterial homoserine dehydrogenases. *FEBS Letters*, **323**(3), 289-293. https://doi.org/10.1016/0014-5793(93)81359-8

Viola RE (2001). The central enzymes of the aspartate family of amino acid biosynthesis. *Accounts of Chemical Research*, **34**(5), 339-349. https://doi.org/10.1021/ar000057q

Yamaki H, Yamaguchi M, Suzuki H, Nishimura T, Saito H, Yamaguchi H (1990). The mechanism of antifungal action of (S)-2-amino-4-oxo-5-hydroxypentanoic acid, RI-331: the inhibition of homoserine dehydrogenase in Saccharomyces cerevisiae. *Biochemical and Biophysical Research Communication*, **168**, 837–843. https://doi.org/10.1016/0006-291X(90)92397-I

Zuckerkandl E and Pauling L (1965). Evolutionary divergence and convergence in proteins. *In: Evolving Genes and Proteins.* Edited by Bryson V and Vogel HJ (Academic Press, Cambridge, Massachusetts) 97-166. https://doi.org/10.1016/B978-1-4832-2734-4.50017-6