Role of active site loop region in the function of watermelon glyoxysomal malate dehydrogenase

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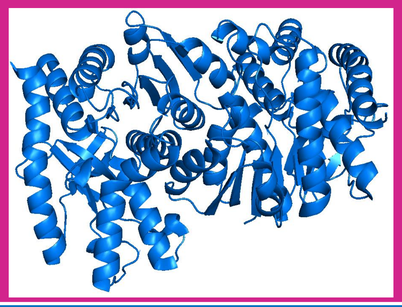

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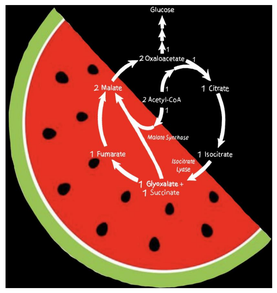
NIH — Shawnee Community College Established 1967

Introduction

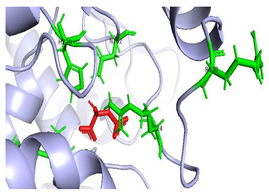
- Malate Dehydrogenase (MDH) is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate using reduction of NAD to NADH

- Glyoxysomes are specialized peroxisomes found in plants and in filamentous fungi.



- During the early germination of watermelon seedlings, the glyoxysomal Malate Dehydrogenase (wgMDH) plays an important role in the glyoxylate cycle, by processing the fat stored in the cotyledons.

- The three conserved arginine residues (R124, R130, R196) shown in green is critical for substrate binding.

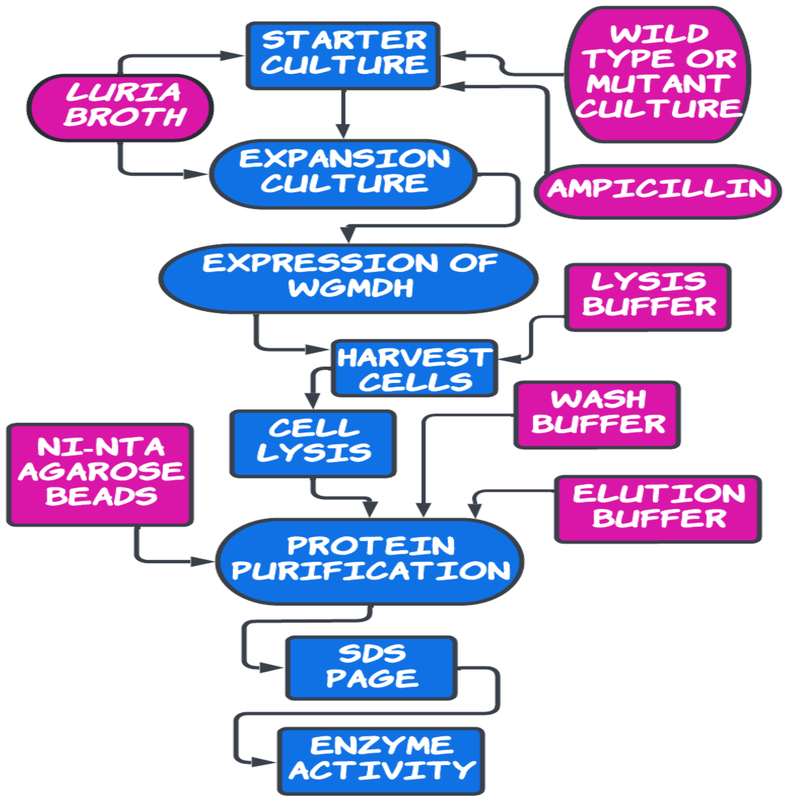


- R130 is part of the active site loop region and is speculated to play a role in catalysis, when the loop swings in and out in presence of the substrate.

- Elucidating the active site loop region function will help develop target drugs against pathogenic organisms such as Mycobacterium tuberculosis and Plasmodium falciparum, or tumor tissues depending upon enhanced metabolism.

- Objective of this study: To investigate the role of mobile loop region in the wgMDH activity by mutating residue R130.

Methodology



Preliminary Results

Fig.1: Sequence alignment of the active site loop region of wgMDH with other MDH with conserved residues

	E.coli_MDH	Human_mMDH	Pig_mMDH	Rat_mMDH	A.thaliana_pMDH	Watermelon_mMDH	Watermelon_gMDH	Cucumber_gMDH
E.coli_MDH	100.00%	58.39%	58.71%	58.39%	57.74%	56.13%	54.52%	55.16%
Human_mMDH	58.39%	100.00%	94.08%	94.38%	56.51%	59.82%	53.70%	55.25%
Pig_mMDH	58.71%	94.08%	100.00%	95.56%	57.40%	59.51%	54.01%	54.94%
Rat_mMDH	58.39%	94.38%	95.56%	100.00%	57.40%	59.82%	53.70%	54.63%
A.thaliana_pMDH	57.74%	56.51%	57.40%	57.40%	100.00%	60.81%	57.87%	57.58%
Watermelon_mMDH	56.13%	59.82%	59.51%	59.82%	60.81%	100.00%	61.63%	61.63%
Watermelon_gMDH	54.52%	53.70%	54.01%	53.70%	57.87%	61.63%	100.00%	96.35%
Cucumber_gMDH	55.16%	55.25%	54.94%	54.63%	57.58%	61.63%	96.35%	100.00%

Fig.2: % identity matrix from uniport of wgMDH sequence with other MDH from different sources.

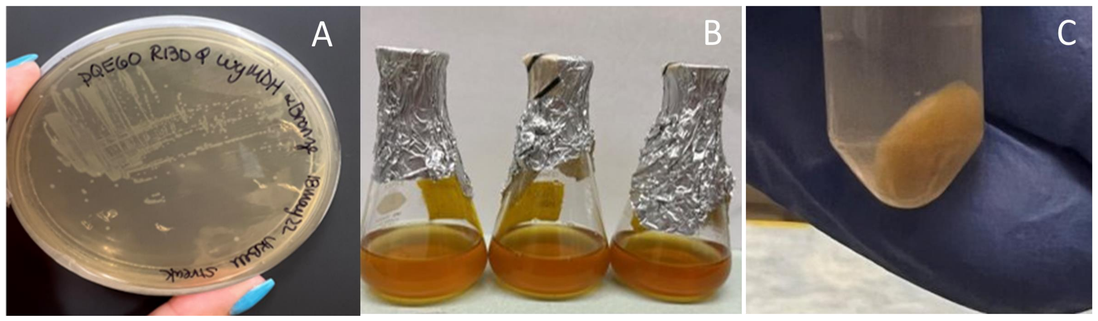


Fig.4: A) Recombinant E. coli carrying wgMDH gene, B) Overnight bacterial culture induced to the production of wgMDH, C) Harvested cell pellet after centrifugation at 3000 g for 15 min

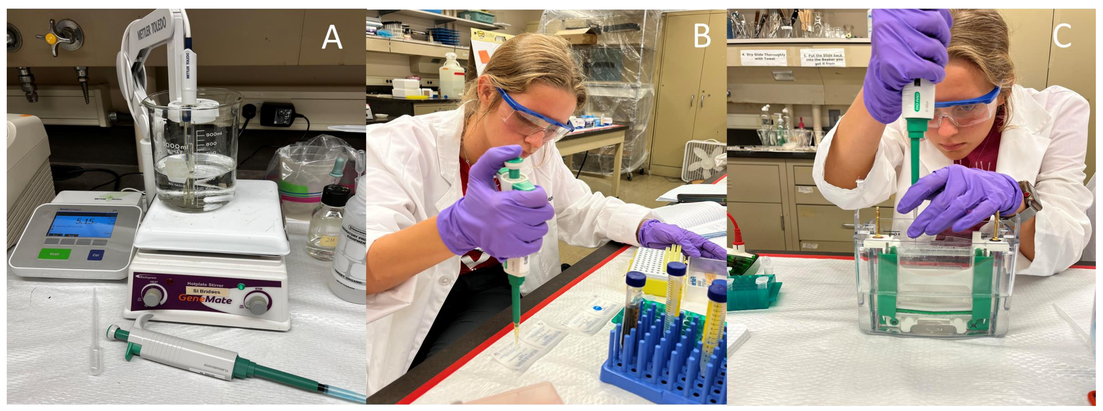
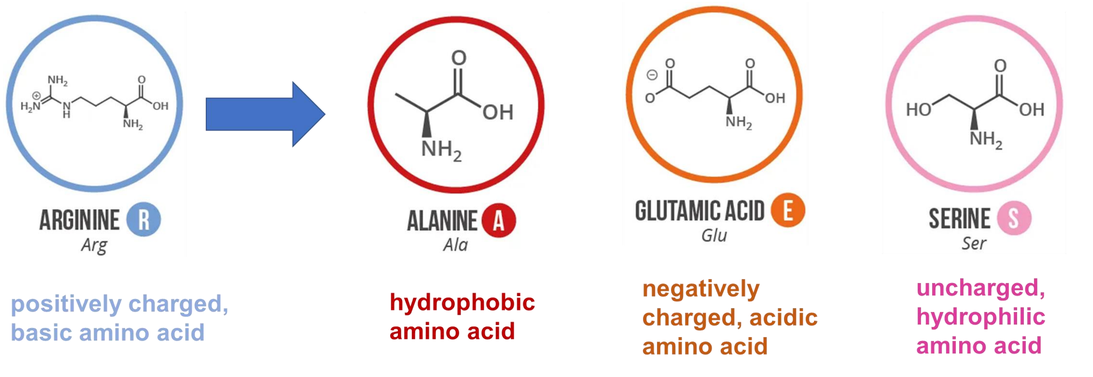


Fig.5: A) Preparation of buffers for cell lysis and purification, B) Protein purification of wgMDH and testing the presence of protein in fractions with Bradford assay, C) Running SDS page at 150V for 30 minutes.

Amino acid substitutions at position R130



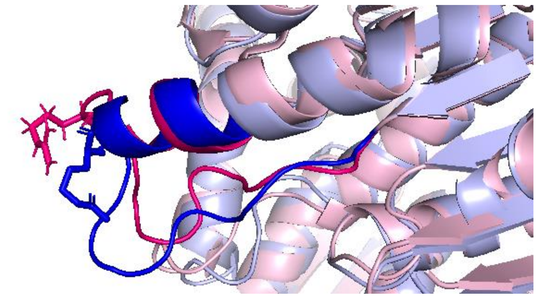


Fig.3: Change in conformation of R130 in loop region open wgMDH (pink) and closed wgMDH (blue)

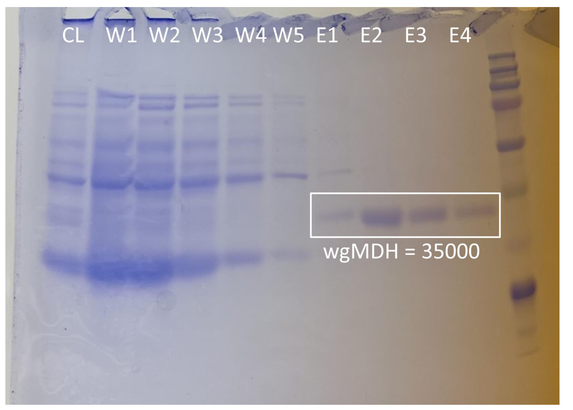


Fig. 6: SDS page of WT wgMDH. Well 1= cell lysate (CL), Well 2-6 = Fractions collected during washing, Well 7-10= Fractions collected during elution, Well 11= Protein standard.

Conclusions/Future

- We were able to successfully express WT wgMDH in E. coli
- Protein purification and SDS page of WT wgMDH confirmed the presence of protein in elution fractions E2, E3, E4 with molecular weight of 35000
- On the other hand, bacterial cultures of R130 wgMDH mutants grew slowly and required longer time before inducing with IPTG
- In the future, protein purification, SDS page of R130 wgMDH mutants, specific activity will be performed to determine if mutation led to loss of protein expression and/or enzyme activity.

References

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Acknowledgements

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