Comparison of the Two Thermophilic and Mesophilic Amylase Enzymes Stability and Structure in Deep Eutectic Solvent (DES)

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Introduction:

Bacillus amyloliquefaciens amylase (BAA) is a mesophilic and *Bacillus licheniformis* amylase (BLA) is a thermophilic enzyme that both are used in industrial microbial production of enzymes as well as fine biochemical. Recently a system for enzyme catalysis was developed using Deep Eutectic Solvents (DESs), mixture of a salt and a hydrogen bond donor. Being non-volatile, thermally stable, biodegradable, cheap, easy to prepare, good solvents for polar substrates and metal salts, favoring synthesis over hydrolysis and suppressing water-induced side reactions make them a suitable solvent over organic solvents. The DES used in this study was prepared by mixing 1:1 ratio of 6 M glycerol and choline chloride. Enzymes activities were measured in the presence of 0-34% DES. BLA activity was increased to 179% of its initial value while BAA retained 65% of its maximum activity. Secondary and tertiary structural changes were analyzed using circular dichroism and fluorescence spectroscopy. Due to the hydrophobic environment of these solvents, tertiary structural changes indicated a compact structure for both BLA and BAA. Circular dichroism analysis showed 53.68% α-helix and 18.46% β-strand for BAA and 69.63% α-helix and 6.64% β-strand for BLA in DESs.

Methods:

Synthesis of DESs:

The DES can be obtained by thermal-mixing procedures. Glycerol-choline chloride(1:1), were mixed with a magnet stirrer at 80 °C for 4-5 h until acolorless clear liquid was formed.

Glycerol+choline chloride

Amylase thermal stability

Thermal stability of amylase were determined at 70,80,90°C . The enzyme was incubated in 15% DES in 50mM of Tris buffer. At different time intervals tubes were chilled on ice for 120 minutes and the residual activity was measured spectrophotometrically.

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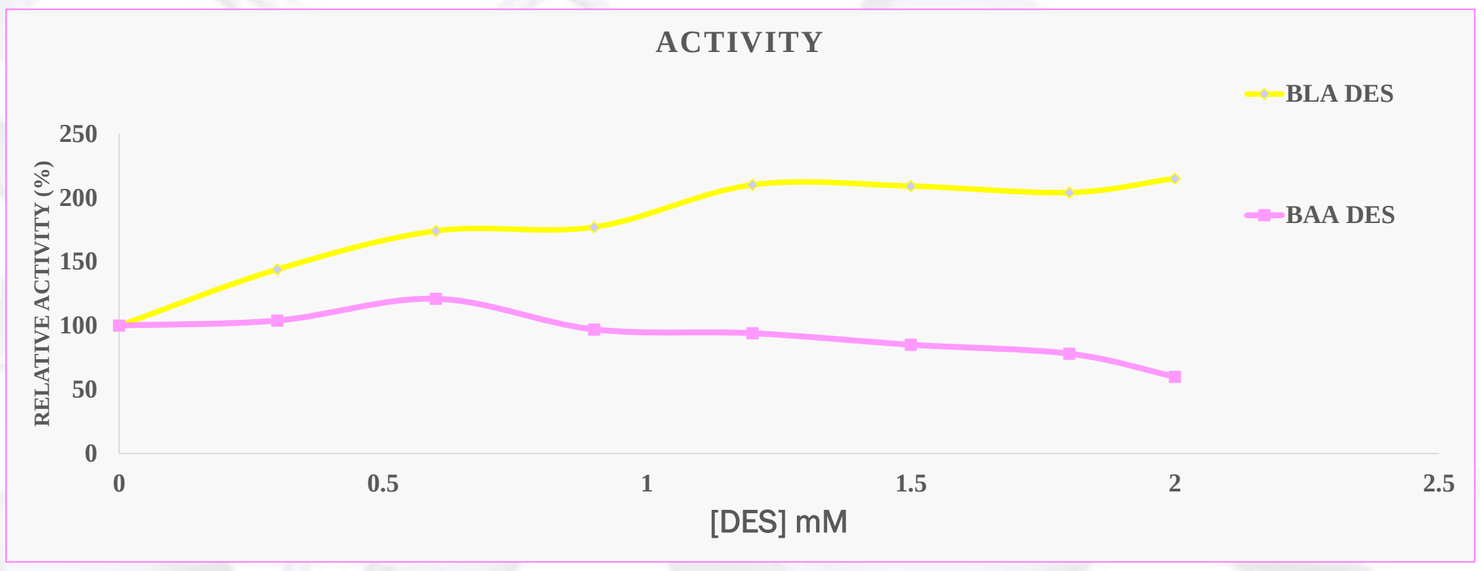
Structural studies

The enzyme tertiary structure was determined in Tris buffer pH=7.5 and 15% DES using fluorescence spectroscopy. The excitation and emission wavelengths were set at 280 and 300-400 nm, respectively.

Results and discussion:



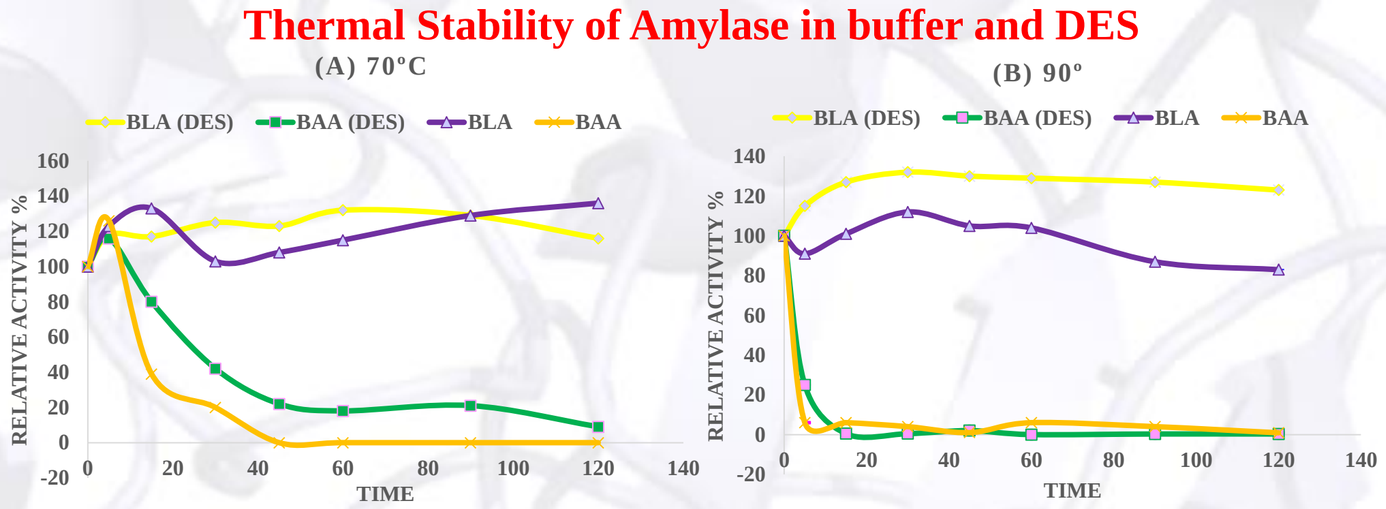


Fig 3: Impact of DESs and buffer on stability of Amylase (A) 70 °C (B) 90 °C

Addition of DES enhanced thermal stability compared to the buffer and enzyme stabilization by DESs seemed to be related to the associated structural changes of the protein.

Fluorescence Intensity

structural changes were analyzed using fluorescence spectroscopy. Due to the hydrophobic environment of these solvents, tertiary structural changes indicated a compact structure for both BLA and BAA.

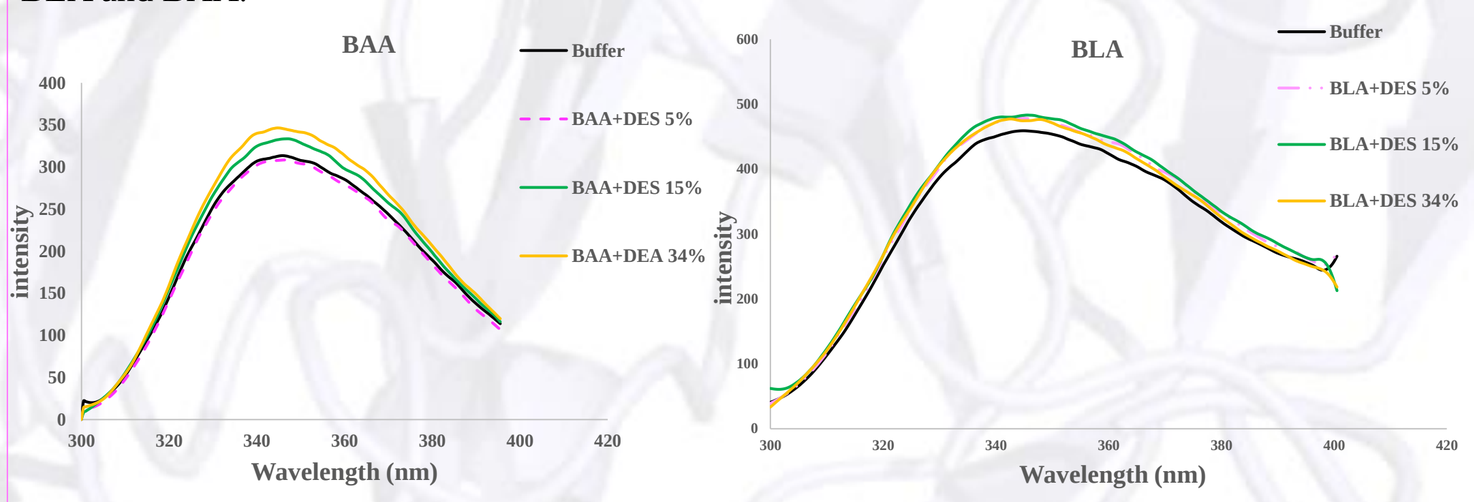
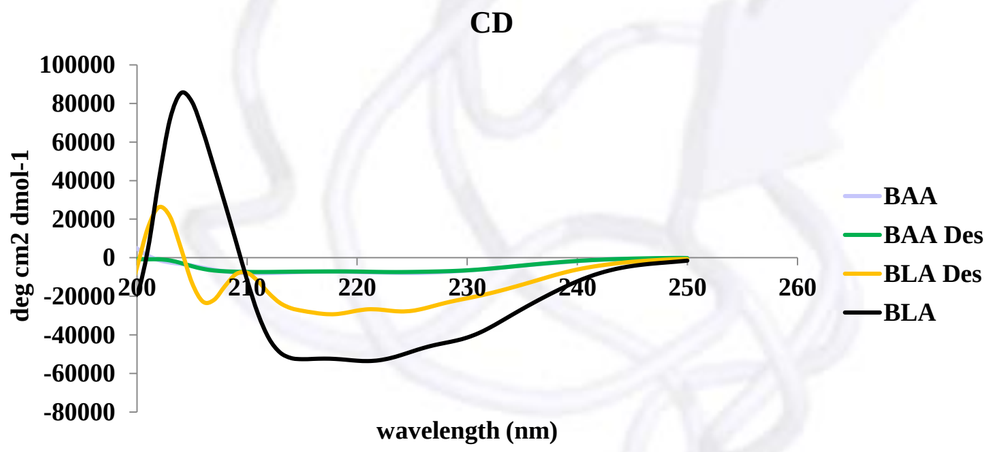


Fig 4: Intrinsic fluorescence spectra of Amylase in buffer and different DESs

Circular Dichroism

Circular dichroism analysis showed 53.68% α-helix and 18.46% β-strand for BAA and 69.63% α-helix and 6.64% β-strand for BLA in DESs.



Conclusion:

Results showed that the thermophilic enzyme (BLA) was significantly more stable than its mesophilic counterpart (BAA) in the presence and absence of DES.
amylase was shown to possess catalytic activity for the systems containing DES. it is also more stable at high temperature in DESs than in buffer.

References:

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