A study on isomerase enzyme improvement for bioprocess demand: pH optima change of L-arabinose isomerase from *Geobacillus strearothermophilus* for D-galactose isomerization

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L-arabinose isomerase from *Geobacillus strearothermophilus* (GSAI; EC 5.3.1.4) has been genetically evolved to increase rate toward D-galactose, which is not a natural substrate. To change the optimal pH of GSAI for D-galactose isomerization (pH optimum at 8.5), we investigated the single point mutations influencing the activity based on the sequences of the previously evolved enzymes, mutations at $Val^{408}V$ and Asn^{475} were determined to be highly influential mutation points for D-galactose isomerization activity. A random mutation was introduced into sites Val^{408} and Asn^{475} (X408V and X475N), and candidates were screened based on nonoptimal pH conditions. Among the mutations of X408V and X475N, mutations of Q408V and R408V were selected. The optimal pH of the both mutations Q408V and R408V was shifted to pH 7.5. At the shifted optimal pH 7.5, the D-galactose isomerization activities of Q408V and R408V were 60 and 30% higher, repectively, than that of the wild type at pH 8.5. Structural information of GSAI would explain the relationship between the amino acid at 408 or 475 and galactose isomerization activity, and the authors are on the way.

References

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