A mutant of *Pseudoalteromonas carrageenovora* arylsulfatase with enhanced enzyme activity and its potential application in improvement of the agar quality

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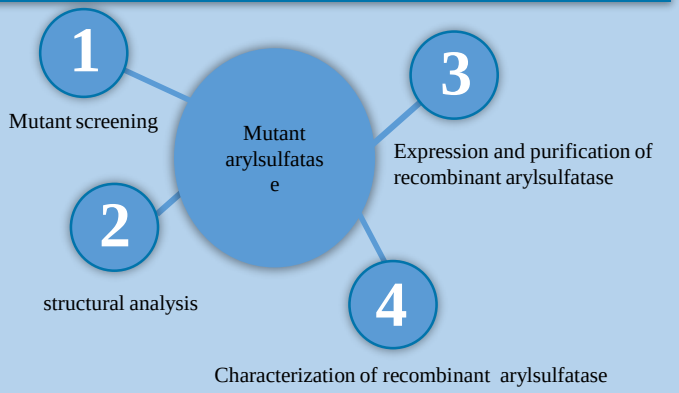
Abstract

Enzymatic desulfation using arylsulfatase provides an attractive approach to improve agar quality. We have previously characterized a functional arylsulfatase from *Pseudoalteromonas carrageenovora*. To further improve its enzymatic performance, we isolated a mutant arylsulfatase of K253Q with improved enzyme activity from a random mutant library. Compared to wild-type arylsulfatase (WT), K253Q showed 33% increase in enzyme activity, with optimal temperature and pH of 55 ℃ and 8.0, respectively. K253Q demonstrated better substrate binding ability with lower Kmvalue. Structure analysis indicated that a combination of the additional hydrogen bond and the enhanced substrate binding affinity could account for the improved enzyme activity of K253Q. K253Q exhibited about 54% sulfate removal against agar, resulting in additional 8% increase in 3,6-AG content and 20% increase in gel strength compared to WT. Scanning electron microscopy showed that K253Q treatment led to a stronger crosslinking structure of agar.

Materials and methods



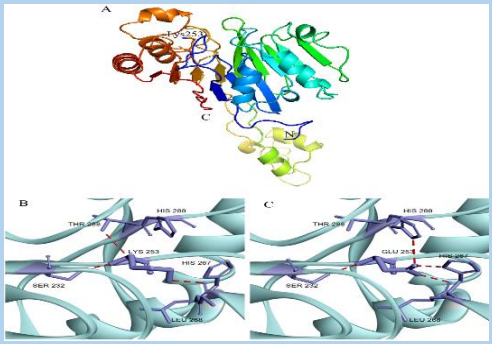


Fig. 2. Arylsulfatase structure analysis. (A) Mutation position of 253 amino acid residue in the three-dimensional model of wide-type arylsulfatase. The sites are represented in sticks. The letters N and C represent the respective termini of the enzyme. Predicted hydrogen bonds in the models of arylsulfatase between amino acid residue 253 and its surrounding residues for WT (B) and K253Q (C). Hydrogen bonds are represented by red dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

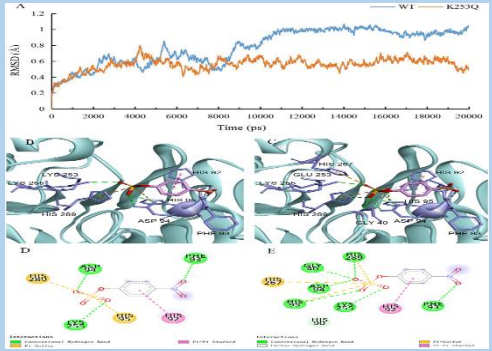


Fig. 3. Molecular dynamics simulation and molecular docking. (A) MD simulation for WT and mutant K253Q arylsulfatases. (B) Molecular docking of WT and pNPS. (C) Molecular docking of K253Q and pNPS. (D) Two-dimensional force analysis diagram for WT and pNPS. (E) Two-dimensional force analysis diagram for K253Q and pNPS.

Table 1 Characteristics of WT and K253Q arylsulfatase.

Enzyme	Relative activity[a] (%)	T_{opt}[b] (° C)	pH_{opt}[c]	K_m (mM)	V_{max} (μmol mg^{-1} min^{-1})
WT	100	55	7.5	0.67	11.87
K253Q	133	55	8.0	0.51	13.06

Results and discussion

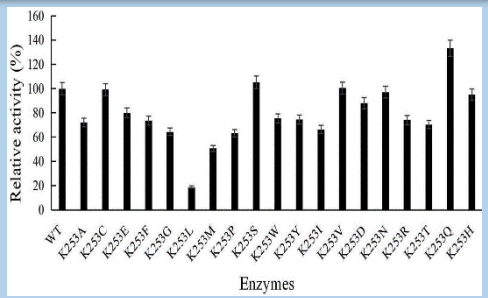


Fig. 1. Effect of amino acid residue at position 253 on the enzyme activity of arylsulfatase. The enzyme activity of WT was defined as 100%. Results are mean ± SD from three independent experiments.

Table 2 Property comparison of commercial agar, enzyme-treated agar and commercial agarose.

Agar samples	Sulfate content (%)	Desulfation ratio (%)	Gel strength (g/cm2)	3,6-AG content (%)
Commercial agar	0.54±0.02	–	833.37±42.85	24.60±1.04
WT-treated agar	0.31±0.01	42.69	892.28±45.71	30.73±0.59
K253Q-treated agar	0.25±0.01	53.91	1057.77±61.64	32.60±1.30
Commercial agarose	–	–	1204.35±52.82	38.88±0.25

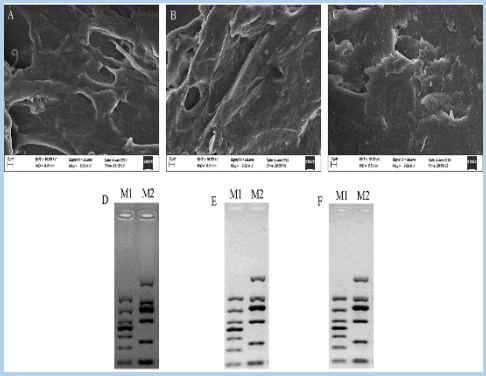


Fig. 4. Scanning electron microscopy analysis and DNA gel electrophoresis assay. Scanning electron microscopy analysis of the commercial agar powder (A), K253Q arylsulfatase-treated agar powder (B) and the commercial agarose powder (C). The EHT was 10.0 kV and the samples were magnified 2000 times. Gel electrophoresis using the gels prepared by the commercial agar powder (D), K253Q arylsulfatase-treated agar powder (E) and the commercial agarose powder (F).

Conclusion

In this study, a mutant arylsulfatase of K253Q with improved enzyme activity was obtained from a mutant library. Biochemical evidence indicated that K253Q had lower Km suggesting better substrate binding ability. Structure modeling revealed the additional hydrogen bond to stabilize the enzyme in concert with the enhanced substrate binding affinity could contribute to the improvement of K253Q enzyme activity. Compared to the WT arylsulfatase, the desulfation efficacy was significantly enhanced imparted by the mutation of K253Q on agar substrate, further reducing the 3,6-AG content and increasing the gel strength of agar. The comparable gel quality of K253Q-treated agar to commercial agarose rendered mutant arylsulfatase K253Q a promising tool in industrial applications for agar quality improvement.