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Novel amyloid-forming protein in *Escherichia coli*

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Motivation and Aim: Amyloids are protein fibrils with a characteristic spatial structure. In previous studies, using a method for the proteomic screening and identification of amyloids (PSIA) [1], we identified 61 proteins of *Escherichia coli* that formed detergent-resistant aggregates *in vivo* and without overproduction [2]. Among these proteins, YghJ was the most enriched with bioinformatically predicted amyloidogenic regions. YghJ is a lipoprotein and important virulence factor of *E. coli* containing the zinc metalloprotease M60-like domain ($YghJ_M$) that is involved in the pathogenesis of the enterotoxigenic strains via mucin degradation in the intestine.

Methods and Algorithms: To analyze amyloid properties of the $YghJ_M$ fibrils *in vitro*, transmission electron, confocal and polarization microscopies of the fibrils were used. For *in vivo* analysis, curli-dependent amyloid generator system (C-DAG), which provides export of the target protein to the surface of bacterial cells, was employed.

Results: We detected detergent-resistant aggregates of $YghJ_M$ by SDS-PAGE and SDD-AGE and confirmed that these aggregates are resistant to α-chymotrypsin protease treatment. We analyzed the fibrillary morphology of the obtained $YghJ_M$ aggregates using transmission electron microscopy. Next, we demonstrated that the $YghJ_M$ aggregates bind Thioflavin-T amyloid-specific dye and exhibit CD-spectra typical for protein aggregates rich in β-sheets. The Congo red stained $YghJ_M$ fibrils demonstrated apple-green birefringence which is considered to be the "gold standard" for verification of the amyloid structure. Finally, we showed that $YghJ_M$ forms amyloid fibrils on the surface of the *Escherichia coli* cells using C-DAG [3].

Conclusion: We demonstrated that $YghJ_M$ forms amyloid fibrils *in vitro* and *in vivo*. Our data on the amyloid properties of the YghJ protein highlight the role of this protein in the pathogenesis and suggest possible mechanism for its functioning.

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