Identification of amyloid-forming proteins in the *Escherichia coli* proteome

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The term ”amyloid” refers to a particular type of unbranched protein fibrils characterized by cross-β structure. More than 30 proteins form pathogenic amyloid fibrils accompanying the development of various incurable disorders such as Alzheimer’s disease. Nevertheless, there are many amyloids involved in different biological processes and called functional. In prokaryotes, functional amyloids control biofilm formation, act as toxin storage systems, facilitate overcoming surface tension, participate in the formation of cell envelope and different surface structures. In *Escherichia coli* amyloid properties have been tested and proven for CsgA curlin, which is a major component of P-type fimbriae, and for OmpC membrane porin. The goal of this work is to identify novel amyloid-forming proteins of *E. coli*.

PSIA (Proteomic Screening and Identification of Amyloids) approach is based on the resistance of amyloid aggregates to treatment with ionic detergents like sodium

dodecyl-sulfate and consists of several steps including purification of detergent-resistant protein fraction, separation and identification of proteins comprising this fraction [1,3]. Using PSIA, 61 detergent-resistant proteins were identified in the *E. coli* proteome [2]. These proteins were analyzed by WALTZ [4] and SARP [5] algorithms. Both algorithms identified YghJ as a potentially amyloidogenic protein. SARP algorithm detected large low-complexity N-rich region in YghJ, which is a particular trait of many amyloid-forming proteins. WALTZ algorithm predicted a higher rate of potentially amyloidogenic regions in YghJ in comparison to other analyzed proteins [2].

YghJ is a secreted lipoprotein involved in the intestinal colonization of enterotoxigenic *E.coli* [6]. YghJ contains N-rich metalloprotease homology domain (1081-1381 aa), which also contains 3 potentially amyloidogenic regions (1165-1170, 1296-1304, and 1325–1330 aa) predicted by WALTZ. We analyzed ability of that domain to form amyloid fibrils *in vitro*. We demonstrated that YghJ (1081-1381 aa) forms detergent-resistant aggregates. The aggregates of YghJ (1081-1381 aa) have the morphology of unbranched fibrils and cause characteristic shift in the fluorescence of Thioflavin-T and apple-green birefringence upon binding with Congo Red dye.

Taken into consideration that full-length YghJ possesses detergent resistance at the physiological level of production, and its metalloprotease domain exhibits main features of amyloid, we may propose that YghJ is a novel amyloid-forming protein in *Escherichia coli*.

Acknowledgements*:* This work was supported by the grants of the President of the Russian Federation (МК-3240.2017.4) and Russian Foundation for Basic Research (16-34-60153). The authors acknowledge Research Park of SPbSU for the opportunity to use equipment of “RRCMCT” and “Chromas”.

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