Title: Pseudomonas aeruginosa amidase: Aggregation in recombinant Escherichia coli

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Abstract: The effect of cultivation parameters such as temperature incubation, IPTG induction and ethanol shock on the production of Pseudomonasaeruginosa amidase (E.C.3.5.1.4) in a recombinant Escherichia coli strain in LB ampicillin culture medium was investigated. The highest yield of solubleamidase, relatively to other proteins, was obtained in the condition at 37 degrees C using 0.40 mM IPTG to induce growth, with ethanol. Our results demonstrate the formation of insoluble aggregates containing amidase, which was biologically active, in all tested growth conditions. Addition of ethanol at 25 degrees C in the culture medium improved amidase yield, which quantitatively aggregated in a biologically active form and exhibited in all conditions an increased specific activity relatively to the soluble form of the enzyme. Non-denaturing solubilization of the aggregated amidase was successfully achieved using L-arginine. The aggregates obtained from conditions at 37 degrees C by Furier transform infrared spectroscopy (FTIR) analysis demonstrated a lower content of intermolecular interactions, which facilitated the solubilization step applying non-denaturing conditions. The higher interactions exhibited in aggregates obtained at suboptimal conditions compromised the solubilization yield. This work provides an approach for the characterization and solubilization of novel reported biologically active aggregates of this amidase.

Author Keywords: Escherichia Coli; FTIR; Protein Aggregates; Recombinant Amidase; Solubilization

KeyWords Plus: Transform Infrared-Spectroscopy; Bacterial Inclusion-Bodies; Active-Site Nucleophile; FT-IR Spectroscopy; Hydromaxic Acids; Monoclonal-Antibodies; Secondary Structure; L-Arginine; Proteins; Solubilization

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