Polish Journal of Veterinary Sciences Vol. 14, No. 1 (2011), 145-146

DOI 10.2478/v10181-011-0022-y

Short communication

Optimization of *Salmonella* Enteritidis recombinant heat shock protein 60 production

K. Rainczak[1], J. Bajzert[1], J. Galli[1], A. Selera[2], A. Wieliczko[2], J. Borkowski[3], T. Stefaniak[1]

[1] Department of Immunology, Physiopathology and Veterinary Prevention,
[2] Department of Epizootiology and Clinic of Birds and Exotic Animals,
Wroclaw University of Environmental and Life Sciences, Norwida 31, 50-375 Wroclaw Wroclaw, Poland
[3] Department of Physiology and Biochemistry, University School of Physical Education, Wroclaw, Poland

Abstract

The aim of the study was to optimize conditions for producing *Salmonella* Enteritidis recombinant heat shock protein 60 (rHsp60). Seven *Escherichia coli* host strains (Rosetta, Turner, C41, C43, Origami, BL21pLys, Rosetta pLys) were transformed by a recombinant plasmid containing Hsp60 gene from S*almonella* Enteritidis, and then cultured and induced by isopropyl-β-D-thiogalactopyranoside (IPTG). The highest *S*. Enteritidis rHsp60 yield was obtained using *E. coli* strain C41. Induction of this strain using IPTG allowed the yield 400 µg of *S.* Enteritidis Hsp60 protein/2L of culture, but by autoinduction the yield exceeded 800 µg/2L.

Key words: rHsp60, *Salmonella* Enteritidis

Introduction

Hsp60 proteins, also named chaperone proteins, prevent the inappropriate holding of cellular proteins caused by temperature or other stress factors (Jäättelä 1999). Hsp60 plays a regulatory role in immune reactions, affecting T-lymphocyte, macrophage and dendritic cell activity (Liossis et al. 1998). Hsp60 antigen was proposed for the production of a subunit vaccine which would induce immune protection against multiple species of bacteria (Moliterno et al. 1995).

The aim of the study was to determine the optimal conditions for *Salmonella* Enteritidis recombinant Hsp60 (rHsp60) production by host *E. coli* strains.

Materials and Methods

Salmonella Enteritidis Hsp60 gene construction: Using PCR reaction, a 1600 bp fragment was amplified by the forward primer H1 (5'GCGAATTCGGCTGCCAAGGATATTC GTTTCGGTG3') and the reverse primer H2 (5'CGGAAGCTTGAAATCCATGCCCGC CCATGC3'), recognized by restriction enzymes (NcoI and XhoI). Plasmid preparation: Plasmid pET 22b(+) containing resistance to ampicillin was used as a vector and the amplified gene Hsp60 from *Salmonella* Enteritidis was inserted into the vector. Bacterial culture: Seven sensitive to ampicillin host strains of *E. coli* [Rosetta, Rosetta pLys, Origami, BL21pLys, C41, C43, Turner (Novagen)] were used as competent cells. Colonies from each strain were cultured separately and checked for Hsp60 yield using SDS-PAGE and the concentration of rHsp60 in a Ni-NTA Sepharose™ column effluent. Experiment 1: The bacteria were induced by isopropyl-β-d-1-thiogalactopyranoside (IPTG, Fermentas). Experiment 2: Competent C41 cells were induced using an autoinduction system (http://www.microbiol-

Correspondence to: T. Stefaniak, e-mail: tadeusz.stefaniak@up.wroc.pl, tel.: 48 71 3205236

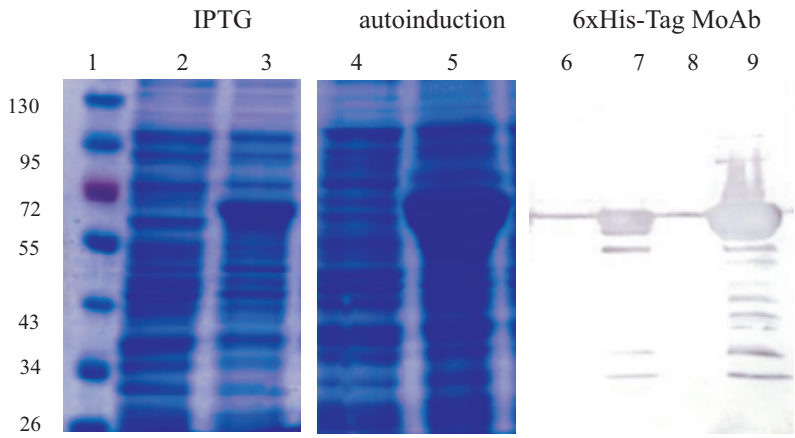

Fig. 1. SDS-PAGE (2-5) and western blotting (6-9) of *E. coli* C41 cells producing *S*. Enteritidis rHsp60 6xHis-Tag monoclonal antibody (1:2000), goat anti Mouse IgG HRPO antibody (1:2000).
1 – Molecular weight standard; 2, 4, 6, 8 – C41cells – before induction; 3, 5, 7, 9 – C41cells – after induction.

ogy.emory.edu/altman/jdaWebSite_v3/p_tet_autoInduction.shtml). Immunological identification: Bacterial proteins were separated by SDS-PAGE and analysed using western blotting. *S.* Enteritidis rHsp60 was detected using monoclonal 6xHis-Tag antibody (Clontech) and goat polyclonal Hsp60 antibody purified using human recombinant GroEL (Sigma) coupled to Sepharose 4B. Chromatography: Affinity chromatography of disintegrated bacterial pellets was performed using Ni-NTA Sepharose™ (Sigma).

Results

Experiment 1: The C41 strain produced the highest levels of *S.* Enteritidis rHsp60. The yield was about 400 µg of affinity purified protein/2L culture). Other strains were excluded because of production of small amounts of Hsp60 (C43, Turner), production of protein of different molecular weight (Bl21pLys) or suspection of virus infection (RosettapLys).

Experiment 2: Using the autoinduction process, the *S.* Enteritidis rHsp60 yield of C41 cells exceeded 800 µg/2L of culture. We found that using this system increased the yield of S. Enteritidis recombinant Hsp60 two-fold compared with the recombinant *E. coli* C41 cell culture induced using IPTG (Fig. 1).

Discussion

We searched for the combination that gave the highest yield of *S.* Enteritis rHrsp60. We selected strain C41(DE3), which originates from BL21(DE3). Its specific features are the ability to express high levels of Hsp60, the lack of formation of inclusion bodies, and the ability to produce toxic proteins without damage to the cell structure. The purified protein was soluble in buffers containing 10% glycerol. In another study by our group, *Histophilus somni* rHsp60 was produced using Turner *E. coli* competent cells induced using IPTG. In contrast to *S*. Enteritidis rHsp60 production, using C41 cells autoinduction was not effective.

The yield of recombinant protein production (Kim et al. 2009) influences costs of future immunization of experimental animals. We would like to check its ability to induce immune protection against the most common facultative pathogens.

Acknowledgements

This work was supported by Ministry of Science and Higher Education, Warsaw, Poland grant N N308 2827 33.

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