Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Efficient Production of Yeast Cu-Zn Superoxide Dismutase in the Periplasm of *Escherichia coli* by Co-expression of Skp Molecular Chaperone

Yogesh Suryawanshi[1]*, Surekha Gupta[2], Hiral Mange[3], Manisha Tripathi[4]

[1]*Guru Nanak Institute of Research and Development, Guru Nanak Khalsa College, Matunga, Mumbai, Maharashtra, India*
[2]*Department of Zoology, Guru Nanak Khalsa College, Matunga, Mumbai, Maharashtra, India*
[3]*Department of Biotechnology, Ramnarain Ruia Autonomous College, Matunga, Mumbai, Maharashtra, India*
[4]*Department of Biotechnology, Patkar-Varde College, Goregaon, Mumbai, Maharashtra, India*

ARTICLE INFO

Article history:
Received: 08 April, 2021
Revised: 22 April, 2021
Accepted: 27 April, 2021
Published: 30 May, 2021

Keywords:
Co-expression, Skp chaperone, Periplasm, Superoxide dismutase.

DOI:
10.25004/IJPSDR.2021.130311

ABSTRACT

Co-expression is a simultaneous expression of two or more proteins. Molecular chaperones are proteins that are naturally produced in the cell and have an essential role in restraining the aggregation of non-native protein production. Co-expression of target protein alongside molecular chaperones is an efficient way to overcome the problems faced during the expression of recombinant proteins. The present study aims to a co-express protein highly useful in cosmetics, superoxide dismutase (SOD), in the periplasm of *E. coli* with molecular chaperone Skp. Superoxide dismutase and Skp were placed under the control of the different promoter and terminator systems to maintain separate expression levels. The co-expression of Skp chaperone on cell growth, SOD protein yield, and SOD enzyme activity were evaluated. Skp co-expression was found effective in all three aspects. The yield of Cu-Zn Superoxide dismutase increased from 30.92mg/L to 52.01mg/L when Skp chaperon is co-expressed. No detectable free SOD subunits were observed on western blot, which shows the method of Skp co-expression could be applied to tackle the problem of unprocessed, free protein subunits while expressing the recombinant protein in *E. coli* periplasm.

Introduction

Escherichia coli is one of the best known and most often used host organisms for cost-effective protein production due to its low cost, simplicity, speed of growth, and widespread availability of bacterial culturing facilities. Various proteins of therapeutic value often contain disulfide bond bridges, which play a crucial role in their folding, stability, and function. However, reducing conditions of *E. coli* cytoplasm can impede disulfide-bond development and render cysteine-containing proteins inclined to incorrect folding and protein accumulation.[1,2] The problem of incorrect disulfide bond formation can be solved by directing the proteins into the periplasm of *E. coli*. It has been demonstrated that the production of large quantities of correctly folded soluble recombinant proteins is possible by targeting them to the periplasm of bacteria.[3,4]

E. coli periplasm provides beneficial properties for protein expression, such as multiple molecular chaperones, quality control mechanisms via the secretion machinery, and the oxidizing environment and folding modulators facile for disulfide bond formation.[2] Regardless of these advantages, recombinant protein secretion in the periplasm is complex, and protein secretion encounters many problems. The most persistent problems are (i) incomplete translocation, (ii) low or undetectable amounts of protein secretion, (iii) variable secretion efficiency depending on the nature of the proteins, and (iv) insufficient capacity of the export machinery.[5,6]

***Corresponding Author:** Yogesh Suryawanshi

Address: Guru Nanak Institute of Research and Development, Guru Nanak Khalsa College, Matunga, Mumbai, Maharashtra, India

Email ✉: yogeshgnkhalsa@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Considering these, various strategies like modification of medium composition, the addition of redox components, and co-expression of chaperones or translocating molecules were devised to optimize folding in the periplasm.[7] Co-expression is a simultaneous expression of two or more proteins. Molecular chaperones are proteins naturally produced in *E. coli* and eukaryotic cells that have an essential role in restraining the aggregation of non-native protein production. Several studies have demonstrated a positive effect of cytoplasmic and periplasmic molecular chaperones co-expression on correct folding of recombinant protein and preventing inclusion body formation in cytoplasm and the periplasm.[1,8,9]

Superoxide dismutase (SOD) is the most powerful antioxidant in the cell and requires a metal cofactor to perform its activity. Different metal ions bound by SOD are iron (Fe), zinc (Zn) copper (Cu), and manganese (Mn). Even though there are various sources and forms of SOD. Yeast derived SOD has a certain advantage as it has better temperature stability. Due to its powerful antioxidant property than most antioxidants, SOD is widely used commercially in cosmetics and personal care products.[10,11]

Skp is an *E. coli* 17-kilo Dalton key periplasmic chaperone that facilitates the correct folding of outer membrane protein intermediates and maintains their solubility. Skp also assists the production of recombinant proteins that are difficult to express. The absence of Skp often leads to protein aggregation in the periplasm, thus indicating the importance of Skp as a periplasmic chaperone in *E. coli*.[12] According to some studies, Skp improves the yield and solubility of recombinant proteins.[13] Skp co-expression helps in decreasing the additional cytoplasmic stress, thus causing better cell physiology.[14] Skp is helpful for expression of periplasmic protein and signal sequence less Skp has also been applied to increase production of active Fab molecule in the cytoplasm of *E. coli* Origami strain.[15] Co-expression of Skp with protein engineering has resulted in high yield secretion of T cell receptors that are otherwise difficult to produce in *E. coli*.[16]

In the current study, the effect of Skp chaperone on the periplasmic expression of superoxide dismutase was determined. Protein expression levels were maintained by placing them under the control of the different promoter and terminator systems in a single vector. Cell growth, SOD protein yield, and SOD enzyme activity were used to determine the effect of Skp chaperone co-expression.

Materials and Methods

Acquiring the Strains and Vector

E. coli K12 was obtained from the Department of Biotechnology, Guru Nanak Khalsa College, Mumbai. *E. coli* DH5α, *E. coli* Rosetta (DE3) were obtained from Microtest Innovations Pvt. Ltd, Bangalore. *E. coli* DH5α was used as cloning host and *E. coli* Rosetta (DE3) as expression host. *Saccharomyces cerevisiae* Cu-Zn SOD was cloned in pET21b earlier as a part of this study.

Primer Designing and PCR

The designed gene construct for *E. coli* Skp in pET21b with PelB SOD protein is shown in Fig. 1. Based on this gene construct, primers for *E. coli* Skp were designed to add T7 terminator, Lac Promoter, Lac Operator, and Ribosome binding site at upstream and Lac Terminator downstream Skp during amplification. Primer designing was done manually and checked using pDRAW32 software. The Skp was decided to amplify in two-step PCR, first by amplifying Skp gene from *E. coli* genomic DNA and joining with Lac promoter, Lac operator, and rbs region at 5' end of the gene and lac terminator XhoI restriction site at 3' end of the gene. This is done with the help of forward primer 2 (FP_2) and Skp reverse primer (Skp_R) primers mentioned in Table 1. In the second PCR, the EcoRI restriction site and T7 terminator were attached at 5' end to the product amplified in the first step. Forward primer 1 (FP_1) and Skp reverse primer (Skp_R) primers were utilized for the second step. PCR conditions for both the steps were initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, annealing at 46°C for 45 seconds,

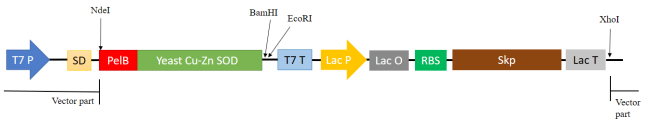

Fig. 1: PelB SOD with Skp gene construct. T7p: T7 promoter, SD: Shine-Dalgarno sequence, T7 T: T7 terminator, Lac P: Lac promoter, Lac O: Lac operator, RBS: Ribosome binding site, Lac T: Lac terminator.

Table 1: Designed primers for Skp cloning.

Primer	*Primer name*	*Sequence (5' – 3')*	*Length*
Forward primer 1	FP_1	GGATCCATGGTGCGAAGAATTCCTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGTTTA-CACTTTATGCTTCCGGCTCGTATGTT	100bp
Forward primer 2	FP_2	TTTACACTTTATGCTTCCGGCTCGTATGTTGTGGGAATTGTGAGCGGATAACAATTCCTTAACTTTAAGAAGGAGA-TATAATGAAAAAGTGGTTATTAGC	98bp
Reverse primer	Skp_R	TGGATCGTATCTCGAGGGCATGATGCGACGCTTGTTCCTGCGCTTTGTTCATGCCGTTATTTAACCTGTTTCAG-TA	76bp

extension at 72°C for 60 seconds, final extension at 72°C for 7 minutes, 35 cycles.

Cloning

Genomic DNA extraction from *E. coli* DH5α was done using GeneI Genomic DNA extraction kit (cat. no. 106276) according to manufacturer's instructions. All PCR amplifications were done using DFS-Taq DNA Polymerase (Bioron). PCR product and pET21b (with Cu-Zn SOD) vector were digested separately with FastDigest EcoRI and XhoI (Thermo Scientific). These digested products were ligated using T4 DNA Ligase (1 U/µL) (Invitrogen) and transformed in *E. coli* DH5α using the calcium chloride method developed by A. Y. Chang et. al.[17] The positive clones were confirmed using PCR utilizing gene-specific primers and restriction enzyme digestion. The plasmid was isolated from a positive clone and transformed into *E. coli* Rosetta (DE3) expression host. SureSpin Plasmid Mini Kit (Genetix Biotech Asia Pvt. Ltd, cat. no. NP-37107) was used for all plasmid purification. According to the manufacturer's instructions, all purifications from agarose gels were performed using the gel extraction kit (Bio Basic, cat. no. BS353). All agarose gel electrophoresis was done at 100V using horizontal gel electrophoresis apparatus (TechnoSource).

Fermentation

Fed-batch fermentation was carried out on a Sartorius BLite 5L fermenter with parameters optimized at shake flask level. The initial media volume was set at 3 liters. The media was supplemented with 0.001% of Cu-Zn solution. 100µg/mL of ampicillin and 50µg/mL of chloramphenicol were added to the media. Media was then inoculated with 300mL (10%) of overnight grown *E. coli* Rosetta (DE3) with PelB SOD and Skp culture in LB broth. Fermenter temperature was maintained at 30°C, and pH was maintained at 7.2. The temperature was maintained by the automated system available in the fermenter console. pH, dissolved oxygen, and glucose concentrations were controlled manually. Dissolved oxygen was maintained above 30%, and glucose concentration was maintained above 250mg/L. Samples were collected after every hour, and pH, glucose concentration and OD_{600nm} were checked. When OD_{600nm} reached the mid-log phase (1.2), culture was added with 0.4M NaCl, 0.4M L-arginine, and 0.5M sorbitol. The culture was induced with 0.05mM IPTG. A mixture of 50% dextrose and 30% yeast extract was used as a feeding agent, and culture was supplemented with 500mL of total feed. Glucose concentration was monitored with Accu-chek Active Glucometer Kit. The fermenter was harvested at the 10^{th} hour after induction. The broth was collected and stored at 4°C and processed within 10 hours for periplasmic protein isolation. Superoxide dismutase was extracted from periplasm using Tris Sucrose EDTA (TSE) extraction method developed earlier.[11]

SOD Activity Determination

According to the manufacturer's guidelines, superoxide dismutase activity was determined from samples extracted from periplasm by a commercially available assay kit (SOD Assay kit Sigma, cat. no. 19160). Unknown SOD concentrations (U/mL) were determined using an inhibition curve obtained with bovine Cu-Zn SOD (Sigma, cat. no. S7571).

Western Blot Analysis

After growing for 10 hours after induction, periplasmic proteins were extracted from *E. coli* Rosetta (DE3) with Cu-Zn SOD without and with Skp co-expression. Samples were separated on 12% SDS-poly-acrylamide gel in non-reducing conditions, and proteins from the gel were transferred to a nitrocellulose membrane. The membranes were blocked by incubating overnight at 4°C with 1.5% BSA prepared in TBST (Tris-buffered saline having 0.05% Tween-20) and washed thrice with TBST. The membrane was incubated with anti-SOD1 rabbit antibodies (StressMarq Biosciences Inc, cat. no. SPC-115) at 1:1000 for 2 hours at room temperature. After washing thrice with TBST, the membrane was then incubated for 1 hour at room temperature with alkaline phosphatase conjugated Goat Anti-Rabbit IgG secondary antibodies (Abcam, cat. no. ab97048) at 1:10000. The membrane was developed with the BCIP/NBT Liquid Substrate System (Sigma-aldrich, cat. no. B1911).

Purification of SOD

10mL of periplasmic protein isolated protein fraction was packed inside the dialysis membrane with 10kDa molecular weight cut-off, and the membrane was dipped in 50mM phosphate buffer (pH 7.4). The setup was placed at room temperature on a magnetic stirrer. The buffer was replaced after every two hours for 24 hours. Two-step purification of Cu-Zn SOD was carried using the modified

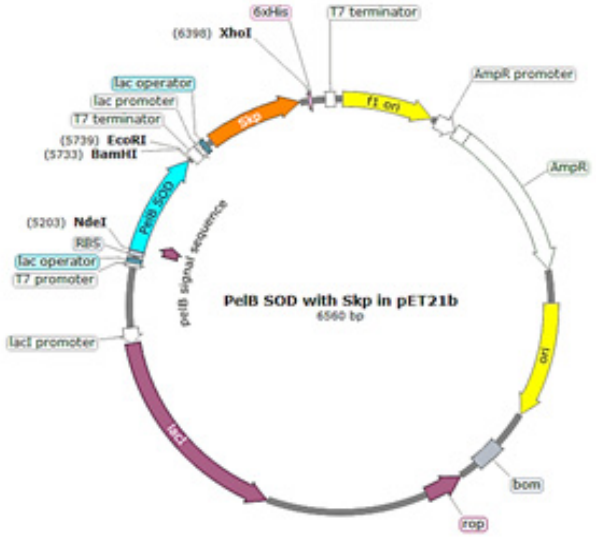

Fig. 2: Vector map pET21b with PelB SOD and Skp.

method initially developed by Petkar Medha B *et al.*[18]. The first step of purification was done manually utilizing the gravity flow column. DEAE-cellulose (Cellufine) column with 2 mL column volume (CV) was taken and washed with 20 CVs of distilled water. The column was then equilibrated with 5 CVs of binding buffer, i.e., 50mM phosphate buffer (pH 7.4). Then, 10mL of periplasmic protein isolated sample containing Cu-Zn SOD was applied to DEAE-cellulose column. 5 CVs of binding buffer were applied, and flow-through was collected. Elution of the protein was done with step gradient, first with 2 CVs of 100mM NaCl containing 50 mM phosphate buffer (pH 7.4), and later with 2 CVs of 300 mM NaCl containing 50mM phosphate buffer (pH 7.4). SOD activity was checked for all the collected fractions. Fraction, showing SOD activity after DEAE-C column purification, was loaded on SDS-PAGE gel and stained with Coomassie brilliant blue staining. Fractions having SOD activity collected in DEAE purification were loaded onto a Sephadex G-75 (GE Healthcare) column equilibrated with 50mM phosphate buffer (pH 7.4), and elution was done with the same buffer. SOD activity was checked for all the collected fractions. Fraction showing the highest SOD activity after Sephadex column purification was loaded on SDS-PAGE gel and stained with silver staining.

Ultra Performance Liquid Chromatography (UPLC)

Cu-Zn SOD was separated by Reverse phase UPLC using a modified method formerly developed by Steen V. Petersen *et al.*[19]Cu/Zn superoxide dismutase (Cu/Zn-SOD] Bovine Cu-Zn SOD is used as a standard. Concentration of standards used were 5, 10, 20, 40, 60, 85, and 100ng/mL. Samples were diluted to fall within the range and acidified with the addition of trifluoroacetic acid (TFA). The separation was carried out on reversed-phase UPLC (Waters) on the ZORBAX C8 column (2.1 mm x 50 mm; Agilent). Bound Cu-Zn SOD was eluted by a segmented linear-gradient increasing the concentration of solvent B (90% acetonitrile/ 0.08% TFA) in solvent A (0.1% TFA) from 0% to 30% in 2.5 minutes (6% B/ 0.5 min) followed by 0.5% B/0.5 min from 30% to 60%. The column was operated at room temperature at a flow rate of 0.5 mL/min.

Results

Cell Growth

The cloned vector (Fig. 2) was transformed in an expression host and expressed at a lab-scale fermenter. The growth pattern for the cultures without and with Skp chaperon was checked and it was observed that culture with Skp chaperone co-expression showed improvement in OD_{600nm}. This confirms that Skp chaperone co-expression helps in increasing cell viability and cell number (Fig. 3).

SOD Activity

When tested for SOD activity after fermentation, the SOD activity increased from 6.85 U/mL to 12.9 U/mL for Cu-Zn SOD when Skp chaperone is co-expressed (Fig. 4).

Periplasmic protein expression

Samples collected during fermentation were subjected to periplasmic protein isolation. When run on SDS-PAGE, periplasmic proteins were detected. An increase in the expression was observed for Cu-Zn SOD protein on the gel (Fig. 5).

Western Blot

The presence of unprocessed, free SOD subunits was observed in the western blot analysis for samples isolated

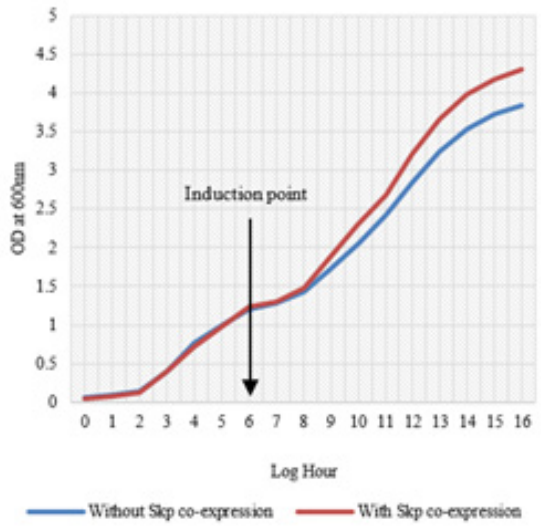

Fig. 3: Growth pattern of *E. coli* Rosetta (DE3) PelB Cu-Zn SOD during lab-scale fermentation without and with Skp co-expression.

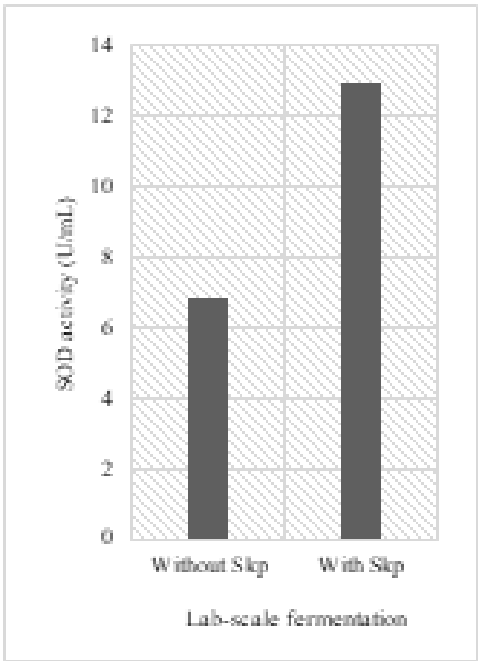

Fig. 4: SOD activity comparison with and without co-expression of Skp.

from periplasm (Fig. 6a). Skp chaperone co-expression was done to solve it. In western blot analysis done after Skp co-expression, only a single band for SOD dimer at ~31.4 kDa was observed with the absence of free SOD subunits (Fig. 6b).

Purification of SOD

Equilibration buffer eluted unbound proteins on the DEAE cellulose column. The bound proteins with Cu-Zn SOD were eluted through a step gradient method using 100 mM and 300 mM NaCl with 50 mM phosphate buffers at pH 7.4. In the SOD activity determination, fractions 11-14 showed significant SOD activity and were used in further purification. The highest SOD activity was observed in fraction 13. In the SDS-PAGE analysis of the fraction, SOD protein was observed in the fractions 10–14 (Fig. 7). Fractions from the first purification with Cu-Zn SOD were loaded on a Sephadex G-75 gel chromatography column for second-step purification. SOD activity was observed for fractions between 9 to14, and maximum activity was observed in fraction 11. A purified Cu-Zn SOD band was observed on SDS-PAGE stained with silver staining (Fig. 8).

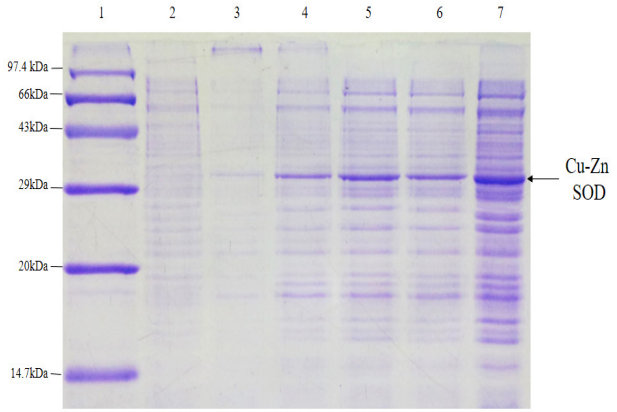

Fig. 5: Periplasmic protein SDS-PAGE. 15% polyacrylamide gel – Non-reducing. Lane 1: Medium range protein marker, Lane 2: Before induction samples, Lane 3: 2nd-hour sample, Lane 4: 4th-hour sample, Lane 5: 6th-hour sample, Lane 6: 8th-hour sample, Lane 7: 10th-hour sample.

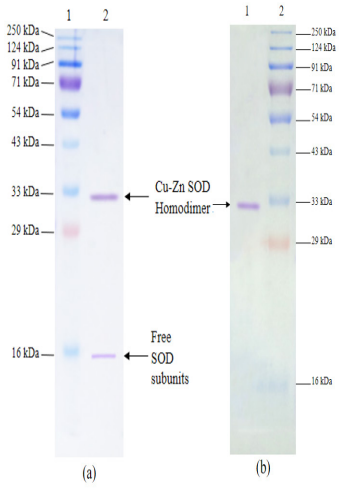

Fig. 6: Western blot analysis of periplasmic protein fractions. (a) Cu-Zn SOD expression without Skp chaperon co-expression and (b) Cu-Zn SOD expression with Skp chaperon co-expression.

UPLC

To determine unknown Cu-Zn SOD from the sample, UPLC was carried out. A set of known concentrations of bovine Cu-Zn SOD was used to obtain a standard curve (Fig. 9) with

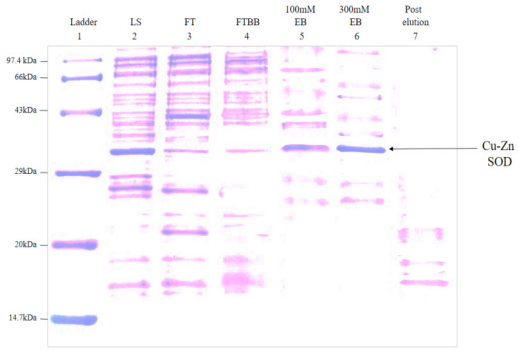

Fig. 7: SDS-PAGE of DEAE-C fractions. LS: loading solution, FT: flow through (1-5 combined), FTBB: Flow through binding buffer (1-5 combined), 100mM EB: 100mM NaCl elution buffer (1 & 2 combined), 300mM EB: 300mM NaCl elution buffer (1 & 2 combined), Post elution (1-3 combined).

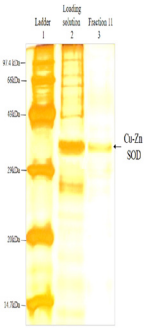

Fig. 8: SDS-PAGE of Sephadex purified fraction.

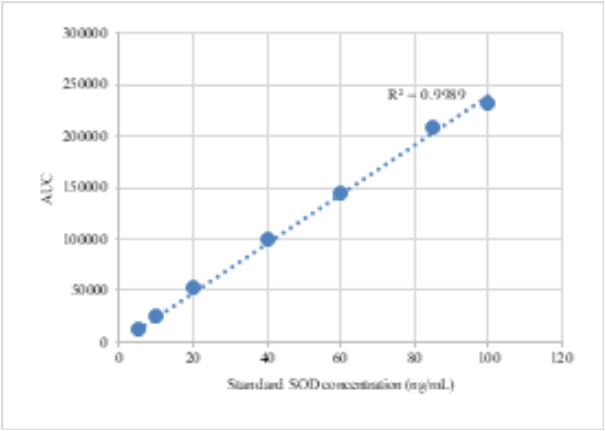

Fig. 9: Standard curve obtained with SOD standards.

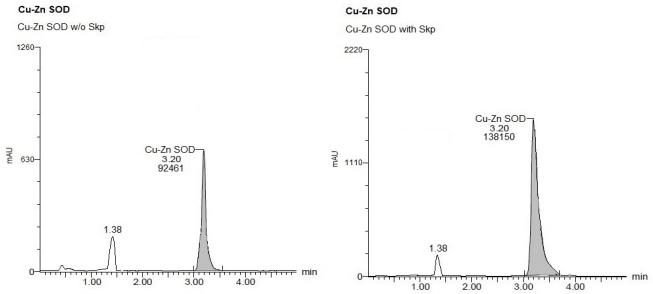

Fig. 10: Chromatograms for Samples (a) Cu-Zn SOD without (w/o) Skp co-expression (b) Cu-Zn SOD with Skp co-expression.

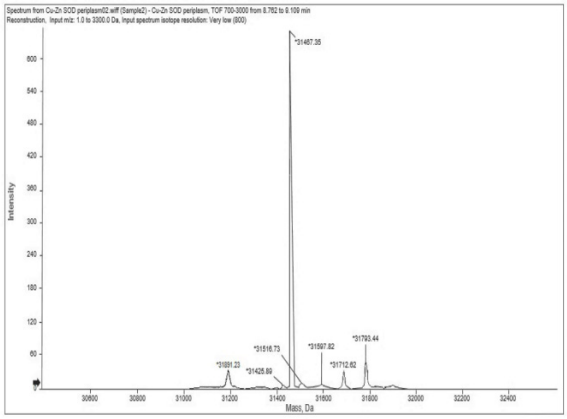

Fig. 11: Mass spectrometry cloned Cu-Zn SOD.

area obtained for Cu-Zn SOD from samples extrapolated to find unknown concertation. The concertation for Cu-Zn SOD with co-expression of Skp chaperone was found to be higher (Fig. 10). The yield of Cu-Zn SOD without and with co-expression of Skp chaperone was determined using concertation of SOD in the samples. The yield of Cu-Zn Superoxide dismutase was found to be 30.92mg/L without Skp chaperone co-expression, while yield increased to 52.01mg/L when Skp chaperone is co-expressed.

Mass Spectrometry

Mass spectrometry analysis was carried out to determine the molecular weight of expressed Yeast Cu-Zn Superoxide dismutase in E. coli periplasm. The molecular weight for Yeast Cu-Zn Superoxide dismutase was found to be 31.46 kDa (Fig. 11).

Discussion

Molecular chaperones act as a folding catalyst and co-expression of such molecules improve–very significantly in many cases–the yield of numerous heterologous proteins in *E. coli*. Although there is still no cure for folding-related difficulties, nor even a decided method to find out the ideal candidate(s) for co-expression with a protein of interest, scientists are gradually turning to chaperone co-expression methods that are accessible commercially and non-commercially as to look to solve folding bottlenecks.

Skp is one of the critical chaperones to tackle various issues related to periplasmic protein expression. Skp co-expression was found effective in processing free Cu-Zn SOD subunits present in the periplasm in this work. Co-expression of the Skp helped improve cell viability and cell number, observed during lab-scale fermentation. The activity of Cu-Zn SOD doubled when Skp chaperone is co-expressed. This proves the benefits of Skp co-expression during Cu-Zn SOD periplasmic expression in *E. coli*.

Acknowledgement

We thank Guru Nanak Institute of Research and Development (GNIRD), Guru Nanak Khalsa College, Matunga, Mumbai, for infrastructure support and kind co-operation throughout the research process.

We appreciate the financial support from Dr. Babasaheb Ambedkar Research and Training Institute (BARTI), Pune.

We thank Microtest Innovations Pvt. Ltd, Bangalore, and Department of Biotechnology, Guru Nanak Khalsa College, Mumbai for strains and vectors.

References

1. O'Reilly AO, Cole AR, Lopes JLS, Lampert A, Wallace BA. Chaperone-mediated native folding of a β-scorpion toxin in the periplasm of Escherichia coli. Biochim Biophys Acta - Gen Subj. 2014;1840(1):10-15.
2. Rodriguez C, Nam DH, Kruchowy E, Ge X. Efficient Antibody Assembly in E. coli Periplasm by Disulfide Bond Folding Factor Co-expression and Culture Optimization. Appl Biochem Biotechnol. 2017 Oct 10;183(2):520–529.
3. Jalalirad R. Production of antibody fragment (Fab) throughout Escherichia coli fed-batch fermentation process: Changes in titre, location and form of product. Electron J Biotechnol. 2013;16(3):1–8.
4. Mergulhão FJM, Summers DK, Monteiro GA. Recombinant protein secretion in Escherichia coli. Biotechnol Adv. 2005;23(3):177–202.
5. Suryawanshi Y, Gupta S. Optimization and comparative studies can help in selecting best signal peptide for periplasmic expression of target protein in gram-negative bacteria. Int J Recent Sci Res. 2020;11(1):37011–37018.
6. Kolaj O, Spada S, Robin S, Gerard JG. Use of folding modulators to improve heterologous protein production in Escherichia coli. Vol. 8, Microbial Cell Factories. 2009. p. 9.
7. Schäffner J, Winter J, Rudolph R, Schwarz E. Cosecretion of Chaperones and Low-Molecular-Size Medium Additives Increases the Yield of Recombinant Disulfide-Bridged Proteins. Appl Environ Microbiol. 2001 Sep;67(9):3994–4000.
8. Humphreys DP, Weir N, Lawson A, Mountain A, Lund PA. Co-expression of human protein disulphide isomerase (PDI) can increase the yield of an antibody Fab' fragment expressed in Escherichia coli. FEBS Lett. 1996;380(1–2):194–197.
9. Mamipour M, Yousefi M, Hasanzadeh M. An overview on molecular chaperones enhancing solubility of expressed recombinant proteins with correct folding. Vol. 102, International Journal of Biological Macromolecules. Elsevier B.V.; 2017. p. 367–375.
10. Gopal RK, Elumalai S. Industrial Production of Superoxide Dismutase (SOD): A Mini Review. J Probiotics Heal. 2017;05(03).
11. Suryawanshi Y, Gupta S, Patel S. Optimization of Media and Growth Conditions Improve Overall Quality of Yeast Superoxide Dismutase in Escherichia coli. IJPBS. 2020;10(4):133–142.
12. Ow DS, Lim DY, Morin Nissom P, Camattari A, Wong V V. Co-expression of Skp and FkpA chaperones improves cell viability

and alters the global expression of stress response genes during scFvD1.3 production. Microb Cell Fact. 2010 Apr 13;9(1):22.
13. Sonoda H, Kumada Y, Katsuda T, Yamaji H. Effects of cytoplasmic and periplasmic chaperones on secretory production of single-chain Fv antibody in Escherichia coli. J Biosci Bioeng. 2011 Apr;111(4):465–470.
14. Narayanan N, Chou CP. Physiological improvement to enhance Escherichia coli cell-surface display via reducing extracytoplasmic stress. Biotechnol Prog. 2008;24(2):293–301.
15. Levy R, Weiss R, Chen G, Iverson BL, Georgiou G. Production of correctly folded fab antibody fragment in the cytoplasm of Escherichia coli trxB gor mutants via the coexpression of molecular chaperones. Protein Expr Purif. 2001 Nov;23(2):338–347.
16. Maynard J, Adams EJ, Krogsgaard M, Petersson K, Liu CW, Garcia KC. High-level bacterial secretion of single-chain αβ T-cell receptors. J Immunol Methods. 2005;306(1–2):51–67.
17. Chang AY, Chau VW, Landas JA, Pang Y. Preparation of calcium competent Escherichia coli and heat-shock transformation. JEMI Methods. 2017;1(June):22–25.
18. Petkar MB, Pillai MM, Kulkarni AA, Bondre SH, Rao KRSS. Purification and characterization of superoxide dismutase isolated from sewage isolated E. coli. J Microb Biochem Technol. 2013;5(4):102–106.
19. Petersen S V., Oury TD, Valnickova Z, Thøgersen IB, Højrup P, Crapo JD, et al. The dual nature of human extracellular superoxide dismutase: One sequence and two structures. Proc Natl Acad Sci U S A. 2003;100(SUPPL. 2):13875–13880.

HOW TO CITE THIS ARTICLE: Suryawanshi Y, Gupta S, Mange H, Tripathi M. Efficient Production of Yeast Cu-Zn Superoxide Dismutase in the Periplasm of *Escherichia coli* by Co-expression of Skp Molecular Chaperone. Int. J. Pharm. Sci. Drug Res. 2021;13(3):311-317. **DOI:** 10.25004/IJPSDR.2021.130311