Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.3.623-630

Optimisation of induction and purification protocols of recombinant 22.6kDa tegumental protein of *Schistosoma spindale* in prokaryotic vector#

Nikitha Shajan[1], M.N Priya[1*], Bindu Lakshmanan[1], Amrutha Anand[1], Ambily R.[2], M.A Pradeep[3] and Asha Rajagopal[1]

[1]Department of Veterinary Parasitology, [2]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651, Kerala Veterinary and Animal Sciences University, Kerala, India, [3]Central Marine Fisheries Research Institute, Kochi

Citation: Nikitha, S., Priya, M.N., Bindu, L., Amrutha, A., Ambily, R., Pradeep, M.A. and Asha, R. 2024. Optimisation of induction and purification protocols of recombinant 22.6kDa tegumental protein of Schistosoma spindale in prokaryotic vector. *J. Vet. Anim. Sci.* **55** (3):623-630

Received: 09.04.2024 Accepted: 16.05.2024 Published: 30.09.2024

Abstract

Schistosomosis is a prevalent zoonotic parasitic disease affecting both humans and animals on a global scale, with an estimated 165 million cattle and 200 million people impacted worldwide. Eventhough, serological methodologies designed for the identification of specific antibodies targeting parasitic antigens are esteemed for their high sensitivity, there are criticisms due to their incapacity to reliably indicate active infection, inability to correlate with the intensity of infection and lack of specificity. Enhancing the specificity of serological assays presents a significant challenge, primarily attributable to the identification and synthesis of specific antigens. In addressing these limitations, recombinant technology with specific immunogenic proteins as candidate antigens emerges as a viable alternative. In this study, induction of 22.6 kDa recombinant tegument protein of Schistosoma spindale was achieved using 0.6 mM concentration of IPTG at 37°C for four hours. Nickel chelating affinity chromatography was employed for protein purification, yielding maximum protein concentration at 75mM elution. Subsequently, the dialysis technique was employed to remove contaminants, while lyophilisation method was employed for protein concentration. The protein concentration post-dialysis was measured at 0.220 mg/mL, while lyophilisation resulted in a concentration of 2mg/mL.

Keywords: *22.6 kDa tegumental protein, Schistosoma spindale, dialysis, lyophilisation*

Schistosomosis poses a significant public health and veterinary concern in numerous tropical and subtropical regions worldwide (Vercruysse and Gabriel, 2005). Schistosomosis is widely acknowledged as the primary helminthic disease affecting domestic animals in Africa and Asia (Sumanth *et al.,* 2004; Islam *et al.,* 2011). The prevalence of *Schistosoma spindale* in south India, as evidenced by abattoir surveys (Lakshmanan *et al.,* 2011; Sudhakar *et al.,* 2016), often remains unnoticed in field conditions, particularly in areas characterised by low endemicity and infection intensity.

Schistosomosis has not received much attention it deserves, despite its economic impact on producing animals in terms of morbidity, mortality, poor reproductive performance, reduced productivity and overall drain of vitality (McCauley

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University

**Corresponding author: mnpriya@kvasu.ac.in, Ph.9847801988*

et al., 1984). Out of 19 species of schistosomes described worldwide, 10 species naturally infect cattle. *S. spindale* and *S. indicum*, which inhabit the mesenteric veins and *S. nasalis*, which parasitises the nasal veins, are the most common species causing ruminant schistosomosis in India (Agrawal and Southgate, 2000). Consequently, there has been significant research emphasis on the development of enhanced and sensitive diagnostics aimed at identifying light infections and facilitating large-scale screening studies (Torre Escudero *et al.,* 2012; Xu *et al.,* 2014). Timely diagnosis, effective treatment and exploring the possibilities of vaccination are the key control strategies for this infection. The multi-faceted control approach and the search for diagnostic and vaccine candidates capable of eliciting an effective immune response is still in its early stages.

The tegument of schistosomes serves as a multifunctional interface crucial for various physiological processes and host interactions. Studies have elucidated its involvement in signal transduction, nutrition, excretion, osmoregulation, and immune evasion and modulation (Han *et al.,* 2009; Mulvenna *et al.,* 2010; Fonseca *et al.,* 2012). Moreover, the antigenicity of numerous tegumental proteins has been well-established. Recent advancements have led to the development of recombinant peptide antigens such as rSj23, rSm21.6, rSj29 and rSb22.6, showcasing their potential for diagnosing both animal and human schistosomosis (Li *et al.,* 2012; Torre-Escudero *et al.,* 2012; Ren *et al.,* 2017; Lv *et al.,* 2016). Evaluation of the 22.6 kDa tegument protein from both *S. bovis* and *S. mansoni* revealed its potential as a valuable epidemiological tool for surveillance purposes. The 22.6 kDa tegument protein of schistosomes lacks glycosylation, thereby minimising the risk of cross-reactions with related helminths (Pacifico *et al.,* 2006; Torre-Escudero *et al.,* 2012).

The pET 28 b (+) vector system are commonly used for the expression of proteins. This system is one of the most widely used expression systems in *Escherichia coli* mainly due to its very high expression levels as the target protein can represent more than 50 per cent of the total cell protein few hours after induction (Rosano and Ceccarelli, 2014). The objective of this study was to develop an optimization strategy aimed at enhancing the efficiency of the induction, purification and concentration processes for the 22.6 kDa tegument protein, with the ultimate goal of utilizing it in the development of diagnostic tools.

Materials and methods

Induction of recombinant protein

For protein expression, 22.6 kDa tegument gene was inserted into the pET 28 b (+) expression cloning vector and then transformed to competent *E. coli* BL21 cells (Sambrook and Russel, 2001). In order to ascertain the optimal culture conditions for maximizing protein synthesis in *E. coli* BL21, various parameters were investigated, including growing temperatures set at 37°C, inducer concentrations ranging from 0 mM to 1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) (0mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, and 1 mM) and post-induction incubation periods spanning from 30 min to five hours.

For optimization, bacterial clones with the insert were grown in 5 mL LB Kanamycin (100 mg/mL) broth overnight at 37^{0}C under constant shaking at 200 rpm in a shaker incubator. Then 1 mL of that broth was inoculated in 50 mL of LB medium. The culture was kept in shaker incubator until an OD_{600} of 0.6 was attained. The culture was then distributed into sterile tubes containing 5 mL in each. Further, different concentrations of IPTG (Sigma-Aldrich) *viz*., 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM and 1 mM were added to each tube and the cultures were further incubated under shaking at 37^{o}C for five hours. The aliquots of 1 mL from IPTG induced culture was collected at 30 min and then from one to five hours at one hour interval. After that 2 mL each of induced LB broth was poured into tubes, pelleted at 10000 x g for 10 minutes, resuspended in 50 µL sterile distilled water and kept at -20^{o}C for assessing the induced total cellular protein (TCP). To that 5 µL cell lytic B cell reagent (Sigma) was added, incubated at room temperature for five minutes and centrifuged at 10000 x g at room temperature for five minutes. Supernatant which contained the soluble fraction of expressed protein was preserved at -20^{o}C until use (Priya, 2019). The soluble portion was analysed using Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Purification of recombinant 22.6 kDa tegument protein (rSs22.6)

The polyhistidine (6X-His) tagged fusion protein, designated as rSs22.6 was purified under native conditions by Nickel chelating affinity chromatography using Ni-NTA agarose column (Qiagen, Germany). Briefly, Two millilitre of Ni-NTA agarose was added to the column, allow the agarose to settle then ethanol in the agarose was allowed to flow through the column. To that about 5 millilitre of 10 mM lysis buffer (50 mM Sodium dihydrogen phosphate, 300 mM Sodium chloride and 10 mM Imidazole; pH-8) was added and was allowed to flow through the column. After that 10 mL of 10 mM lysis buffer was added to the column which was also allowed to pass through. Then sample supernatant containing soluble fraction of the expressed protein was added to the column followed by addition of 5 mL of each 20 mM, 50mM and 60 mM wash buffer (50 mM Sodium dihydrogen phosphate, 300 mM Sodium chloride and Imidazole; pH-8), respectively and collected as unbound fraction. Further, 5 mL of 20 mM elution buffer (50 mM Sodium dihydrogen phosphate, 300 mM Sodium chloride and Imidazole; pH-4.5) was passed through the column, followed by 5 mL of 30 mM, 50 mM, 150 mM and 250 mM of elution buffer respectively and

each eluted fractions were collected separately. The purity of different elutes of the recombinant protein was analysed using SDS-PAGE (Priya, 2019). The protein concentration of elute was estimated by Nanodrop (Nanodrop 2000, Thermo Scientific, USA) and the protein was stored at -20°C till further use.

Dialysis of recombinant 22.6 kDa tegument protein

The 22.6 kDa protein, previously purified, underwent dialysis utilizing 12 cm cellulose membrane tubing with a 12 kDa cut-off (Sigma-Aldrich, USA). The membrane was primed for optimal function by soaking in lukewarm water for an hour. Subsequently, one end of the tubing was securely tied and 5 ml of the protein sample was introduced before sealing the other end. Dialysis occurred against 1 litre of phosphate buffered saline (PBS) at pH 7.5, conducted for 2 hours at 4ºC on a magnetic stirrer. Following buffer replacement, the protein underwent an overnight dialysis at 4ºC on a magnetic stirrer, spanning 16 hours. Post-dialysis, the protein's pH was verified and it was then stored at -20ºC according to the protocol outlined by Andrew *et al.* (2001) with minor adjustments.

Concentration of recombinant 22.6 kDa tegument protein by lyophilisation

The lyophilisation process for the rSs22.6 protein involved following steps, 750 µl of dialyzed protein was distributed into 1.5 mL eppendorf tubes. These tubes were then subjected to rapid freezing at -120 ºC utilizing an Operon cold trap specifically designed for this purpose. Following the freezing step, the tubes containing the frozen protein samples were transferred to a centrifuge (Operon MSVQ-20) connected to a glass chamber, ensuring proper tube balance before initiating the vacuum. After approximately eight hours of lyophilisation under vacuum conditions, the protein underwent desiccation to remove moisture. Subsequently, the lyophilised protein was reconstituted in 50 µl of phosphate-buffered saline (PBS) solution to facilitate storage and handling, according to the protocol outlined by Matejtschuk, (2007) with minor adjustments. The concentration and quality of the lyophilised protein were then assessed using a Nanodrop (Nanodrop 2000, ThermoScientific, USA) to ensure suitability for further experimental procedures.

Results and discussion

In this, we cloned the gene encoding 22.6 kDa tegument protein of *S. spindale* into the histidine-tagged pET-28 b (+) vector and transformed the resulting plasmid into the *E. coli* strain BL21. In numerous expression studies, *E. coli*-based prokaryotic systems have been preferred due to the organism's well-studied nature, rapid multiplication, high cell density formation, strong acceptance of exogenous DNA, and widespread availability. Torre-Escudero *et al*. (2012) utilised pSC-A cloning vector for cloning the 22.6 kDa tegument protein coding gene of *S. bovis* followed by subcloning into the pGEX-4T-1 expression vector. However, Zhang *et al*. (2012) utilised the PMD19-T cloning vector and pET28a expression vector for cloning a calcium-binding tegumental protein in *S. japonicum*.

The post induction examination of expression for the 22.6 kDa recombinant protein (Fig. 1) in the present study revealed a rise in protein levels when cells were grown with IPTG (0.6 and 0.8 mM) compared to conditions without induction. Protein production slightly increased within the initial 4 hours, peaked around 4-5 hours post-induction and showed no significant rise after overnight incubation. Protein concentration was determined using Nanodrop, with an average concentration of 0.323 mg/ml. Recombinant protein expression can be attained either through self-induction or with the addition of an inducer. The raising of the inducer concentration typically results in enhanced expression yields, higher levels of expression may also lead to the formation of inclusion bodies (Atroshenko *et al.,* 2024). Isopropyl β-D-1-thiogalactopyranoside (IPTG) stands out as one of the most commonly utilized and efficient inducers (Marbach and Bettenbrock, 2012). The range of IPTG concentrations for inducing gene expression spans from 0.005 to 5 mM (Xie *et al.,* 2003). Elevated IPTG levels can result in a notable decrease in growth rate and the production of bacterial proteases that degrade foreign proteins. Larentis *et al.* (2014) demonstrated improved expression of mature rPsaA in *E. coli BL21* through optimization, employing an IPTG concentration ten times lower than the standard dosage. High concentrations of IPTG have been found to hinder the growth of *E. coli* (Jeong and Lee, 1999; Einsfeldt *et al.,* 2011; Malik *et al.,* 2016). Decreasing the IPTG concentration from 1.2 to 0.3 mM led to an increase in the yield of the soluble form of recombinant bovine sex-determining region Y protein (Soleymani and Mostafaie, 2019). Similarly, during the expression of leptospiral

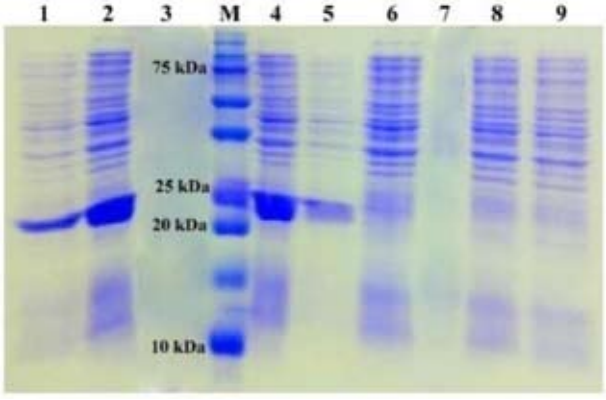

Fig. 1: **SDS PAGE- Crude extract of *E. coli* BL21 induced for 4 hour**

Lane 1: Induction by 1 mM IPTG; Lane 2: Induction by 0.8 mM IPTG; Lane 4: Induction by 0.6 mM IPTG; Lane 5: Induction by 0.4 mM IPTG; Lane 6: Induction by 0.2 mM IPTG; Lane 8 & 9: Induction by 0 mM IPTG (Uninduceed) Lane M- Protein Marker

protein, the lowest investigated IPTG concentration of 0.1 mM yielded the highest quantity of soluble proteins and the best cell growth (Larentis *et al.,* 2014). In investigations examining the impact of IPTG concentrations ranging from 0.25 to 1.25 mM, the optimal expression of the receptor activator of nuclear factor-κB was found to occur at 0.3 mM IPTG (Papaneophytou *et al.,* 2013). Similarly, in a study assessing IPTG concentrations (0.25, 0.5, 1 or 2 mM) on the expression levels of the thioredoxin fusion with the epithelial cell adhesion molecule's extracellular domain, the highest protein yield was attained with 0.5 mM IPTG (Rasooli and Hashemi, 2019).

Zhang *et al.* (2012) employed a comparable IPTG concentration for inducing the expression of the calcium-binding tegument protein in *S. japonicum* (SjTP22.4). Concentration of 1mM IPTG was also employed for inducing protein expression in schistosomes (McManus *et al.,* 2002; Peng *et al.,* 2008; Lopes *et al.,* 2009; Xiong *et al.,* 2013; Zhang *et al.,* 2015; Lv *et al.,* 2016). Many protein expression studies have employed a final concentration of 0.1 mM IPTG (Zhang *et al.,* 1998; Cai *et al.,* 2008), whereas Torre-Escudero *et al*. (2012) utilised a concentration of 0.5 mM IPTG for inducing the expression of the 22.6 kDa tegument protein in *S. bovis.*

The purification of newly expressed polyhistidine (6X-His) tagged tegument protein of *S. spindale* (rSs22.6) in the present study was carried out by Nickel chelating affinity chromatography using Ni-NTA agarose column under native conditions with imidazole based elution buffers. Recombinant protein purification is essential as it plays a crucial role in separating target proteins from cellular impurities, aiding in precise characterization and enabling diverse downstream applications in research. While numerous protocols have emerged in recent years for the isolation of recombinant proteins, many rely on labor-intensive and error-prone manual procedures. Utilising a polyhistidine tag for the expression and purification of recombinant proteins represents a prevalent strategy aimed at obtaining large quantities of highly purified proteins (Kokpinar *et al.,* 2006). Before purification is initiated, several factors should be taken into account to enhance precision and efficiency. The volume of buffer used is also pivotal for successful purification, as excessive elution buffer may dilute the protein concentration, while inadequate volumes could result in incomplete elution (Meng *et al.,* 2008). Obtaining a sufficient quantity of pure protein is essential for its study or utilization as an antigen in diagnostics or immunization. Nonetheless, achieving high-level production of prokaryotic recombinant proteins in *E. coli* can pose challenges and may not always be straightforward. The optimization of the protein purification process, particularly during the stages of binding, washing and elution, is crucial for overcoming these challenges. The N-terminal His-tag enabled an effective Ni-NTA affinity purification, yielding soluble recombinant protein free from contaminants. Optimization of the purification process involved adjustments during binding, washing and elution stages, where extending the duration and repetitions of the binding process was anticipated to enhance target protein binding to the Ni-NTA resin, while increased washing was expected to yield proteins with reduced impurities (Gharakhani *et al*., 2023). The imidazole concentration, acting as a competitive agent in the elution of histidine-tagged proteins owing to its structural similarity to histidine, exerts influence over the elution process. It was observed during the elution step that elevating the imidazole concentration and adjusting the pH of the elution buffer near the protein's isoelectric point resulted in higher protein concentration and purity. Furthermore, pH adjustment is essential to maintain protein solubility and prevent degradation (Gharakhani *et al.,* 2023).

In the present study, the recombinant protein was purified using Ni-NTA column with approximately 100 per cent purity. Among the different concentrations of elution buffers, the most pure and concentrated fraction of rSs22.6 was observed in the 75mM concentration as it was seen as a thicker band in the 22.6 kDa position (Fig. 2). The concentration of recombinant tegument protein was estimated to be 0.235mg/mL. Similar protocols were adopted for purification of many recombinant proteins of schistosomes (Zhang *et al*., 2012; Qiu *et al*., 2013; Zhang *et al*., 2015; Lv *et al*., 2016). Xiong *et al.* (2013) performed the purification of the dysferlin tegument protein of *S. japonicum* utilising Ni-NTA His-Bind resin, followed by urea-based dialysis. McManus *et al.* (2002) purified recombinant paramyosin of *S. japonicum* (rec-Sj-97) through Nickel chelating affinity chromatography, followed by anion exchange chromatography, while Damasceno *et al.* (2017) employed anion-exchange chromatography, followed by hydrophobic interaction chromatography, for purifying the GST-tagged recombinant *S. mansoni* antigen.

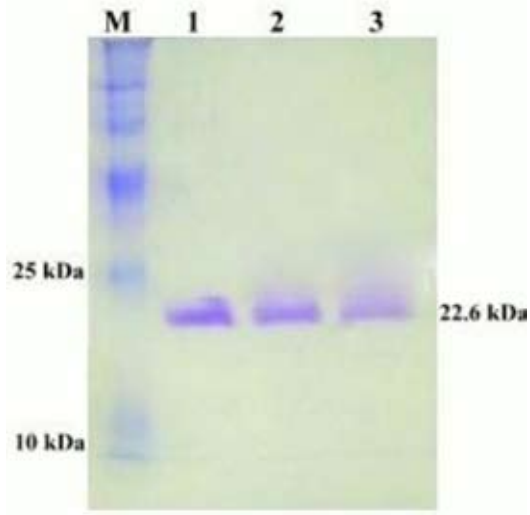

Fig. 2: SDS PAGE- Elution of induced soluble rSs22.6

Lane M- Protein marker; Lane 1: Elution by 75mM Imidazole, Lane 2: Elution by 100 mM Imidazole, Lane 3: Elution by 150 mM Imidazole

To enhance the purity of the newly expressed protein under study, dialysis was carried out. Purification of recombinant proteins is a critical step in their production, impacting their quality, stability and functionality. Dialysis has emerged as a versatile and widely used technique for protein purification, offering advantages such as gentle handling, minimal sample loss, and compatibility with various buffer systems. Following dialysis of the 22.6 kDa protein using carbonate bicarbonate buffer, the protein's pH transitioned from 4.4 to 9.6, with a resulting concentration of 0.220 mg/mL. One of the primary benefits of dialysis is the gentle and selective removal of contaminants, such as salts, small molecules and denaturants, from protein solutions (Ludwig *et al*., 2019). Unlike chromatography-based methods that rely on specific interactions between the target protein and immobilised ligands, dialysis relies on simple diffusion across a semi-permeable membrane, making it suitable for purifying a wide range of proteins without the need for customized affinity ligands (Wiltzius and Sieker, 2019). Moreover, dialysis is a scalable and cost-effective purification method that requires minimal equipment and consumables, making it accessible to researchers and industry professionals with varying resource constraints (Lilie *et al*., 2000). Dialysis can be performed using various configurations, including traditional batch dialysis, continuous flow dialysis and membrane-based systems such as dialysis tubing or dialysis cartridges (Lilie *et al*., 2000). In batch dialysis, the protein sample is placed in a dialysis bag or cassette and immersed in a large volume of dialysis buffer, allowing contaminants to diffuse out of the bag while retaining the protein of interest. Membrane-based dialysis systems offer advantages such as higher throughput, improved control over dialysis conditions, and compatibility with automated purification platforms (Eswari and Naik, 2020). Dialysis is often employed as a final polishing step to remove residual contaminants and exchange the protein into a physiologically relevant buffer for downstream applications such as structural studies, enzymatic assays, and drug formulation (Ludwig *et al*., 2019). Moreover, dialysis enables the refolding of denatured or aggregated proteins by gradually diluting denaturants and promoting correct folding under native conditions, making it an indispensable tool for protein renaturation and recovery (Wiltzius and Sieker, 2019). However, one common challenge that exist in dialysis is the slow diffusion rate of large proteins or protein complexes, which may require prolonged dialysis times or optimization of dialysis conditions to achieve efficient purification (Lilie *et al*., 2000). Moreover, the choice of dialysis membrane pore size and material must be carefully considered to prevent protein adsorption, fouling, or denaturation, which can compromise purification efficiency and yield (Wiltzius and Sieker, 2019). Additionally, the scalability of dialysis-based purification methods may be limited by the available membrane surface area and buffer volume, necessitating process optimization and automation for large-scale protein production (Juza *et al*., 2000).

Post-lyophilisation and reconstitution of the rSs22.6 kDa tegument protein led to an estimated concentration of 2mg/mL serving the purpose of stabilising and preserving the protein for future applications. The concentration of expressed recombinant proteins is a critical step in protein purification processes, impacting downstream applications in various fields such as pharmaceuticals, biotechnology, and research. Lyophilisation or freeze-drying, has emerged as a favoured method for protein concentration due to its ability to effectively remove water without compromising protein stability. Lyophilisation offers several advantages over alternative concentration methods. Firstly, it allows for the removal of water from protein solutions without subjecting them to harsh conditions such as high temperatures or organic solvents, which can denature proteins. This gentle dehydration process helps to maintain the native structure and biological activity of recombinant proteins, preserving their functionality for downstream applications (Geeraerd *et al*., 2000). Secondly, lyophilisation facilitates the long-term storage of concentrated protein samples by stabilising them in a dry state, thereby extending their shelf life and minimizing the need for repeated purification cycles (Cleland *et al*., 1993). Additionally, lyophilisation is scalable and compatible with automation, making it suitable for high-throughput protein production workflows in both academic and industrial settings (Sharma *et al*., 2021). However, several challenges must be addressed to ensure its successful application for protein concentration. One major consideration is the selection of appropriate cryoprotectants to prevent protein aggregation and maintain stability during freeze-drying (Carpenter *et al*., 2002). The choice of cryoprotectant depends on factors such as protein structure, concentration and intended use. Moreover, optimizing lyophilisation conditions, including freezing rate, drying time and temperature, is crucial to prevent damage to the protein structure and ensure uniform drying (Pansare and Patel, 2019). Furthermore, the cost associated with equipment and consumables for lyophilisation can be significant, particularly for large-scale production, necessitating economic evaluations to determine feasibility (Williams *et al*., 2013). In biopharmaceutical production, lyophilisation is routinely employed for the formulation of protein-based drugs, including monoclonal antibodies and vaccines, to enhance stability and prolong shelf life (Pikal *et al*., 2000). Thus, concentration by lyophilisation represents a valuable strategy for enhancing the yield and stability of expressed recombinant proteins. By effectively removing water while preserving protein structure and activity, lyophilisation enables the production of high-quality protein preparations. Addressing challenges such as cryoprotectant selection and process optimization is essential to maximize the benefits of lyophilisation for protein concentration. Continued research and innovation in lyophilisation techniques hold promise for further advancing recombinant protein production and its applications in various scientific disciplines.

Conclusion

Optimisation of the expression conditions, such as inducer concentration significantly impacts recombinant protein expression, yielding enhanced yields and quality. Notably, varying IPTG concentrations have been shown to affect both growth and protein expression levels in *E. coli*, with lower concentrations often proving optimal for soluble protein production. Purification techniques, particularly Ni-NTA affinity chromatography, play a vital role in. obtaining highly purified proteins, facilitating downstream applications. Moreover, post-purification processes like dialysis and lyophilisation serve to enhance protein purity and stability, essential for various research and biotechnological endeavors.

Acknowledgements

The authors gratefully acknowledge the grants received from Kerala State Council for Science Technology and Environment (Fund no. Council (P) order no. No.411/2021/KSCSTE dated 08/10/2021) and technical support of Kerala Veterinary and Animal Sciences Univesity, Pookode, Kerala, India for conducting the research.

Conflict of interest

The authors declare that is no potential conflict of interest

References

Agrawal, M. C. and Southgate, V. R. 2000. *Schistosoma spindale* and bovine schistosomosis. *J. Vet. Parasitol.* **14**: 95-107.

Andrew, S.M., Titus, J.A. and Zumstein, L., 2001. Dialysis and concentration of protein solutions. *Curr. Protoc. Toxicol.* **10**: A-3H.

Atroshenko, D.L., Sergeev, E.P., Golovina, D.I. and Pometun, A.A. 2024. Additivities for Soluble Recombinant Protein Expression in Cytoplasm of *Escherichia coli. Fermentation* **10**: 120.

Cai, P., Bu, L., Wang, J., Wang, Z., Zhong, X. and Wang, H. 2008. Molecular characterization of *Schistosoma japonicum* tegument protein tetraspanin- 2: Sequence variation and possible implications for immune evasion. *Biochem. Biophys. Res. Commun.* **327:** 197-202.

Carpenter, J.F., Chang, B.S., Garzon-Rodriguez, W. and Randolph, T.W. 2002. Rationale design of stable lyophilised protein formulations: theory and practice in "Rationale Design of stable protein formulations-theory and practice" (J.F. Carpenter and M.C. Manning eds.) Kluwer Academic/Plenum publishers, New York, pp. 109-133

Cleland, J.L., Powell, M.F. and Shire, S.J. 1993. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation. *Crit. Rev. Ther. Drug Carr. Syst.* **10**: 307-377.

Damasceno, L., Ritter, G. and Batt, C.A. 2017. Process development for production and purification of the *Schistosoma mansoni* Sm14 antigen. *Protein Expr. Purif.* **134**: 72-81.

Eswari,J.S.and Naik, S.2020. A critical analysis on various technologies and functionalized materials for manufacturing dialysis membranes. *Mat. Sci. Energy Technol*. **3**: 116–126

Einsfeldt, K., Júnior, J.B.S., Argondizzo, A.P.C., Medeiros, M.A., Alves, T.L.M., Almeida, R.V. and Larentis, A.L. 2011. Cloning and expression of protease ClpP from *Streptococcus pneumoniae* in *Escherichia coli*: study of the influence of kanamycin and IPTG concentration on cell growth, recombinant protein production and plasmid stability. *Vaccine* **29**: 7136-7143.

Fonseca, C.T., Carvalho, G.B.F., Alves, C.C. and DeMelo, T.T. 2012. Schistosoma Tegument Proteins in Vaccine and Diagnosis Development: An Update. *J. Parasitol. Res.* doi:10.1155/2012/541268.

Geeraerd, A.H., Herremans, C.H. and Van Impe, J.F. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *Int. J. Food Microbiol.* **59**: 185-209.

Gharakhani, M., Ghasemi, M.F., Khaki, P., Esmaelizad, M. and Tebianian, M. 2023. Improvement the expression and purification of Loa22: a lipoprotein with OmpA domain from pathogenic *Leptospira serovars*. *Iran. J. Microbiol.* 15: 674.

Han, Z.G., Brindley, P.J., Wang, S.Y., Chen, Z., 2009. Schistosoma genomics: new perspectives on schistosome biology and host-parasite interaction. Annual review of genomics and human genetics **10:** 211-240. doi: 10.1146/annurev-genom-082908-150036

Islam, M.N., Begum, N., Alam, M.Z. and Mamun, A.A. 2011. Epidemiology of intestinal schistosomiasis in ruminants of Bangladesh. *J. Bangladesh Agric. Univ*. **9**: 221-228.

Jeong, K.J. and Lee, S.Y. 1999. High-level production of human leptin by fed-batch cultivation of recombinant Escherichia coli and its purification. *Appl. Environ. Microbiol.* **65**: 3027-3032.

Juza, M., Mazzotti, M. and Morbidelli, M. 2000. Simulated Moving Bed Chromatography and Its Application to Chirotechnology. *Trends Biotechnol.* **18**:108-18. DOI:10.1016/S0167-7799(99)01419-5.

Kokpinar, O., Harkensee, D., Kasper, C., Scheper, T., Zeidler, R., Reif, O.W. and Ulber, R. 2006. Innovative modular membrane adsorber system for high‐throughput downstream screening for protein purification. *Biotechnol. Prog.* **22**: 1215-1219.

Lakshmanan, B., Rauoof, A., Fawaz, M. and Subramanian, H. 2011. Abattoir survey of *Shistosoma spindale* infection in thrissur. *J. Vet. Anim. Sci*. **42**: 53-54.

Larentis, A.L., Nicolau, J.F.M.Q., Esteves, G.D.S., Vareschini, D.T., de Almeida, F.V.R., dos Reis, M.G., Galler, R. and Medeiros, M.A. 2014. Evaluation of pre-induction temperature, cell growth at induction and IPTG concentration on the expression of a leptospiral protein in *E. coli* using shaking flasks and microbioreactor. *BMC research notes* **7**:.1-13.

Li, Y., Wang, L., Fang, R., Nie, H., Zhou, Y. and Zhao, J. 2012. Establishment and evaluation of an iELISA using the recombinant membrane protein LHD-Sj23for the serodiagnosis of *Schistosoma japonicum* infection in cattle in China. *Vet Parasitol*. **188**: 247-254.

Lilie, H., Schwarz, E., Rudolph, R. and Hilgenfeld, R. 2000. Controlled reshuffling of a protein folding intermediate by rational compound design: a dialysis study. *J. Mol. Biol.* **297**: 1091-1102.

Lopes, D.O., Paiva, L.F., Martins, M.A., Cardoso, F.C., Rajão, M.A., Pinho, J.M., Caliari, M.V., Oliveira, R.C., Mello, S.M., Leite, L.C.C. and Oliveira, S.C. 2009. Sm21.6 a novel EF-hand family protein member located on the surface of *Schistosoma mansoni* adult worm that failed to induce protection against challenge infection but reduced liver pathology. *Vaccine*. **27**: 4127-4135.

Ludwig, C., Claassen, M., Schmidt, A. and Aebersold, R. 2019. Estimation of absolute protein quantities of unlabeled samples by selected reaction monitoring mass spectrometry. *Mol. Cellular Proteomics.* **18**: 1027-1036.

Lv, C., Hong, Y., Fu, Z., Lu, K., Cao, X., Wang, T., Zhu, C., Li, H., Xu, R., Jia, B., Han, Q., Dou, X., Shen, Y., Zhang, Z., Zai, J., Feng, J. and Lin, J. 2016. Evaluation of recombinant multi-epitope proteins for diagnosis of goat schistosomiasis by enzyme-linked immunosorbent assay. *Parasites Vectors*. **9**: 135-145.

Malik, A., Alsenaidy, A.M., Elrobh, M., Khan, W., Alanazi, M.S. and Bazzi, M.D. 2016. Optimization of expression and purification of HSPA6 protein from Camelus dromedarius in *E. coli. Saudi J. Biol. Sci.* **23**: 410-419.

Marbach, A. and Bettenbrock, K. 2012. Lac operon induction in Escherichia coli: Systematic comparison of IPTG and TMG induction and influence of the transacetylase LacA. *J. Biotechnol.* **157**: 82-88.

McCauley, E.H., Majid, A.A. and Tayeb, A. 1984. Economic evaluation of the production impact of bovine schistosomiasis and vaccination in Sudan. *Preventive Vet. Med*. **6**: 735-754.

McManus, D.P., Wong, J.Y.M., Zhoub, J., Cai, C., Zeng, Q., Smyth, D., Li, Y., Kalinna, B. H., Duke, M.J. and Yi, X. 2002. Recombinant paramyosin (rec-Sj-97) tested for immunogenicity and vaccine efficacy against *Schistosoma japonicum* in mice and water buffaloes. *Vaccine*. **20**: 87.

Meng, J., Walter, J.G., Kökpinar, Ö., Stahl, F. and Scheper, T., 2008. Automated microscale His‐tagged protein purification using Ni‐NTA magnetic agarose beads. *Chem. Eng. Technol.* **31**: 463-468.

Mulvenna, J., Moertel, L., Jones, M.K., Nawaratna, S., Lovas, E.M., Gobert, G.N., Colgrave, M., Jones, A., Loukas, A., McManus, D.P., 2010. Exposed proteins of the Schistosoma japonicum tegument. *Int. J. Parasitol.* **40**: 543-554. doi: 10.1016/j.ijpara.2009.10.002

Pacifico, L.G., Fonseca, C.T., Chiari, L., Oliveira, S.C., 2006. Immunization with Schistosoma mansoni 22.6 kDa antigen induces partial protection against experimental infection in a recombinant protein form but not as DNA vaccine. *Immunobiology 211*: 97-104.

Pansare, S. and Patel, S.M. 2019. Lyophilization Process Design and Development: A Single-Step Drying Approach. *J. Pharm. Sci.* **108**: 1423-1433. *https://doi.org/10.1016/j.xphs.2018.11.021*

Papaneophytou, C.P., Rinotas, V., Douni, E. and Kontopidis, G. 2013. A statistical approach for optimization of RANKL overexpression in *Escherichia* coli: purification and characterization of the protein. *Protein Expr. Purif.* **90**: 9-19.

Peng, S.Y., Lee, K.M., Tsaihong, J.C., Cheng, P.C. and Fan, P.C. 2008.Evaluation of recombinant fructose-1,6-bisphosphate aldolase ELISA test for the diagnosis of *Schistosoma japonicum* in water buffaloes. *Res. Vet. Sci*. **85**: 527-533.

Pikal, M.J., Dellerman, K.M., Roy, M.L. and Riggin, R.M.

2000. The effects of formulation variables on the stability of freeze-dried human growth hormone. *Pharm. Res.* **17**: 551-558.

Priya, M.N., 2019. Development and evaluation of recombinant protein based elisa for the diagnosis of intestinal schistosomosis. PhD thesis. Kerala Veterinary and Animal Sciences Univesity.

Qiu, C., Fu, Z., Shi, Y., Hong, Y., Liu, S., and Lin. J. 2013. A retinoid X receptor (RXR1) homolog from *Schistosoma japonicum*: Its ligand-binding domain may bind to 9-cis-retinoic acid. *Mol. Biochem. Parasitol.* **188:** 40-50.

Rasooli, F. and Hashemi, A. 2019. Efficient expression of EpEX in the cytoplasm of *Escherichia coli* using thioredoxin fusion protein. *RPS* **14**: 554-565.

Ren, C.P., Liu, Q., Liu, F.C., Zhu, F.Y., Cui, S. X., Liu, Z., Gao, W.D., Liu, M., Ji. Y.S. and Shen, J. J. 2017. Development of monoclonal antibodies against Sj29 and its possible application for schistosomiasis diagnosis. *Intl. J. Infectious Dis.* **61**:74-78.

Rosano, G.L. and Ceccarelli, E.A. 2014. Recombinant protein expression in *Escherichia coli*: Advances and challenges. *Front. Microbiol.* doi: **10**. 3389/ fmicb.2014.00172

Sambrook, J. and Russell, D.W. 2001. *Molecular Cloning: A laboratory manual.* (3rd Ed), Cold Spring Harbor Laboratory Press, New York. 2100p.

Sharma, A., Dikshitkumar Khamar, D., Sean Cullen, S., Ambrose Hayden, A. and Helen Hughes, H. 2021. Innovative Drying Technologies for Biopharmaceuticals. *Int. J. Pharm.* 609: 121115. *https://doi.org/10.1016/j.ijpharm.2021.121115*

Soleymani, B. and Mostafaie, A. 2019. Analysis of methods to improve the solubility of recombinant bovine sex determining region Y protein. *RBMB* **8**: 227.

Sudhakar, K., Murthy, G. S. S. and Rajeshwari, G. 2016. An abattoir study of bovine visceral schistosomiasis in Telengana state, India. *Int. J. Agric. Sci.* **8**: 3205-3208.

Sumanth, S., D'Souza, P.E. and Jagannath, M.S. 2004. A study of nasal and visceral schistosomosis in cattle slaughtered at an abattoir in Bangalore, South India. *Rev. Sci. Tech. Off. Int. Epiz*. **23**: 937-942.

Torre-Escudero, E., Román, R.M., Sánchez, R.P., Barrera, I., Siles-Lucas, M. and Oleaga, A. 2012. Molecular cloning, characterization and diagnostic performance of the *Schistosoma bovis* 22.6 antigen. *Vet. Parasitol*. **190**: 530-540.

Vercruysse, J. and Gabriël, S. 2005. Immunity to schistosomiasis in animals: an update. *Parasite Immunol.* **27:** 289-295.

Williams, R.O., Reynolds, T.D., and Andrews, G.P. 2013. Biopharmaceutical Considerations in Freeze-Drying: Effects of Annealing on Protein Stability During Freeze-Drying. In Pharmaceutical Freeze-Drying Technology. pp. 129-144. CRC Press.

Wiltzius, J.J. and Sieker, L.C. 2019. Continuous dialysis and protein crystal growth. *J. Cryst. Growth.* **106**: 380-386.

Xie, L., Hall, D., Eiteman, M.A. and Altman, E.J.A.M. 2003. Optimization of recombinant aminolevulinate synthase production in *Escherichia coli* using factorial design. *Appl. Microbiol. Biotechnol.* **63**: 267-273.

Xiong, Y., Ai, D., Meng, P., Wei, M., Hong, Y., Zhang, M., Huang, L., Fu, Z., Shi, Y. and Lin, J. 2013. Cloning, expression, and preliminary characterization of the dysferlin tegument protein in *Schistosoma japonicum. Parasitol. Int.* **62**: 522–529.

Xu, X., Zhang, Y., Lin, D., Zhang, J., Xu, J., Liu, Y.M., Hu, F., Qing, X., Xia, C., Pan, W., 2014. Serodiagnosis of Schistosoma japonicum infection: genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China. *Lancet. Infect. Dis.* **14**: 489-497. doi: 10.1016/S1473-3099(14)70067-2

Zhang, Y., He. Y., He, L., Zong, H. Y. and Cai, G. B. 2015. Parasitology molecular cloning and characterization of a phospholipidhydroperoxide glutathione peroxidase gene from a blood fluke *Schistosoma japonicum*. *Mol. Biochem. Parasitol.* **203**: 5-13.

Zhang, Y., Taylor, M.G. and Bickle, Q.D. 1998. *Schistosoma japonicum* myosin:cloning, expression and vaccination studies with the homologue of the *S. mansoni* myosin fragment IrV-5. *Parasite Immunol*. **20:** 583-594.

Zhang, Z., Xu, H., Gan, W., Zeng, S. and Hu, X. 2012. *Schistosoma japonicum* calcium-binding tegumental protein SjTP22.4 immunization confers praziquantel schistosomulumicide and antifecundity effect in mice. *Vaccine*. **30**: 5141-5150. ■