

GJ1158: A Salt-Inducible Host for Eco-friendly Expression of Recombinant Proteins

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ABSTRACT

The demand for recombinant protein expression is on the rise, especially for therapeutic and medical uses. However, the production of these proteins presents a significant challenge in biotechnology. The choice of expression system is critical, as it greatly influences downstream processing. *E. coli* is considered the most suitable bacterial system due to its rapid growth, ease of handling, low cost, and well-understood genetics. Although IPTG is commonly used for induction in *E. coli*, it is both environmentally harmful and expensive. In contrast, GJ1158, an *E. coli* strain with the T4 bacteriophage, uses an osmoresponsive operator that allows cell induction with NaCl. This makes it a cost-effective, readily available, and ecofriendly inducer. This review explores the suitability of GJ1158 as an expression host for recombinant proteins.

KEYWORDS: Recombinant proteins, GJ1158, Salt-inducible system, Inclusion bodies, Growth curve, Ecofriendly.

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INTRODUCTION

The production of recombinant proteins is the basis for modern biotechnology and covers areas as diverse as in therapeutics, diagnostics, vaccines, and industrial enzymes. *E. coli* is a popular bacterial system due to its fast growth, ease of manipulation and low cost. Nonetheless, standard IPTG induction is cost and environmentally expensive. A colorless and environmentally friendly salinity-inducible *E. coli* derivative with NaCl-controlled proU operon and a new controllable protein expression system called the cosmid type of genetic tool GJ1158 improves such inconveniences.

The market for recombinant proteins has experienced unprecedented growth in recent decades, reflecting its key role in biopharmaceuticals, industrial enzymes, diagnostics and basic research [14]. Recombinant proteins are of paramount importance for the manufacture of therapeutics, the production of vaccines and the provision of accurate diagnostic tools [22]. As the cornerstone of modern biotechnology, the production of recombinant proteins requires host systems capable of delivering high production volumes with correct protein folding, stability and functionality [40].

Conventional expression systems like *Escherichia coli* (*E. coli*), yeast strains (*Saccharomyces cerevisiae*, *Pichia pastoris*), insect cells, and mammalian cells have been used extensively owing to their thoroughly characterized genetic background and amenability to being scaled up [10]. While these systems are useful, they are prone to experiencing some significant limitations like protein misfolding, aggregation, inefficient post-translational modification, and long cultivation processes [38]. Apart from this, increasing environmental pressure to ensure the sustainability of these systems has necessitated the development of new alternatives that align with the ideals of green biotechnology [42].

GJ1158 was recognized in this case as a potential salt-inducible host organism that offers a new avenue for recombinant protein production [6]. Salt-inducible systems are designed to be cultured under high salinity conditions, utilizing novel stress response pathways that enhance protein expression and stability [30]. GJ1158 is characterized by its strength and versatility, especially in saline environments [5]. Its ability to function in such conditions minimizes the risk of contamination and simplifies cultivation protocols, making it a potential candidate for use in processes where sustainability and resource optimization are paramount. One of the distinguishing features of GJ1158 is its salt stress-responsive protein expression system [48]. This feature not only facilitates the highly controlled production of proteins, but also environmentally friendly biotechnological processes by reducing dependence on chemical inducers and freshwater consumption. Growth in salt or brackish water significantly reduces the environmental impact. This review addresses the biological and biotechnological promise of GJ1158 as a salt-inducible host platform for recombinant protein production. It addresses its physiological characteristics, genetic engineering promise, and environmental sustainability promise for hosting eco-friendly processes of production [42]. Moreover, the broader implications of employing salt-inducible systems in reducing carbon footprint and improving resource utilization are addressed. Through a critical assessment of current developments and research prospects, this review attempts to place GJ1158 as a game-changing instrument of sustainable recombinant protein production[3].

Mammalian Expression System:

Mammalian cells surpass all other recombinant protein-expression methods when it comes to licensed biopharmaceuticals based on recombinant proteins. They can express large and complex recombinant proteins. However, in this system, the introduction of the gene and the selection of the clone take longer than in microbial systems. Important steps in the development of cell lines include transfection, vector and transfection selection, and cell line selection [50].

Insect Expression System:

In its most basic form, the traditional baculovirus expression vector is a recombinant baculovirus containing a foreign nucleic acid sequence, typically a cDNA, that encodes a protein of interest and is transcriptionally regulated by the polyhedrin promoter. The polyhedrin locus of the viral genome is modified to contain a chimeric gene that replaces the non-essential wild type polyhedrin gene. This chimeric gene consists of the polyhedrin promoter and a foreign protein coding sequence. When the recombinant virus infects cultured insect cells or larvae (caterpillars), high-level transcription of the foreign cDNA occurs in the very late stages of infection. The target protein is then produced by translating the resultant mRNA [33].

Yeast Expression System:

Yeasts are excellent hosts for the expression of recombinant proteins due to their rapid growth, ease of genetic modification, low-cost growth media requirements, complete genome sequences, and ability to produce post-translational modifications (PTMs). Among the various yeast species used for producing recombinant biopharmaceuticals, *P. pastoris* and *S. cerevisiae* are the most employed expression systems [4].

Viral Expression System:

A rapid and effective method for producing proteins in plants involves agroinfiltration of plant leaves with binary vectors containing a gene of interest within a plant viral vector [6]. While the genetic engineering of plant viral vectors for heterologous gene expression began in the early 1980s, recent advances in molecular biology and plant virology have significantly enhanced and refined these vectors [27]. Today, recombinant protein production is commonly achieved using the baculovirus expression vector system (BEVS). This baculovirus is primarily used as a vector for expression in systems [20].

Bacterial Expression System:

Bacterial hosts are simple to work with, cheap to cultivate, and quick to produce recombinant proteins. *E. coli* continues to be the most desirable host for the synthesis of heterologous proteins, although many alternative species and expression methods are now available for recombinant protein production. Because of its low cost, well-known biochemistry and genetics, quick growth, and high productivity, a bacterial expression host system, often *E. coli*, is the preferred host for recombinant proteins [11]. Early in the 1980s, the FDA approved the first recombinant insulin produced by *E. coli* for the treatment of diabetes, opening the way and serving as a foundation for the creation of following recombinant therapies. *E. coli* is a primary workhorse for the manufacture of recombinant therapies since it is responsible for producing one-third of these approved protein therapies [].

Table 1: Comparison of different Recombinant protein expression systems.

Characteristics	Mammalian	Insect	Yeast	Viral	Bacterial
1. Protein complexity	Large and complex proteins	Unspecified protein complexity	Unspecified protein complexity	Unspecified protein complexity	Unspecified protein complexity
2. Time to produce	Slow	Rapid	Rapid	Rapid	Rapid
3. Cost	Unspecified Cost	Unspecified Cost	Low Cost	Unspecified Cost	Low Cost
4. Genetic modification	Transfection, vector and cell line selection	Recombinant baculovirus vector	Ease of genetic modification	Agroinfiltration with binary vectors	Simple to work with and allows high yield recombinant protein production
5. Common usage	Licensed biopharmaceuticals	High-level transcription of cDNA	Recombinant biopharmaceuticals	Protein production in plants	Recombinant therapies

Salt-Inducible Expression Mechanism

The proU operon is an osmoresponsive promoter activated under high salinity. It regulates the expression of genes involved in osmoprotectant uptake. In GJ1158, this system drives the expression of T7 RNA polymerase in the presence of NaCl, allowing for targeted induction of genes placed downstream of the T7 promoter. This results in high protein yield with reduced inclusion body formation [52].

T7 promoter and its associated RNA polymerase help the recombinant protein fraction making up 50% of the total cellular protein, that too with a high level of specific activity [10]. IPTG, which is typically used to induce a lac promoter, is expensive and harmful

to ecology [33]. Thus, IPTG has an impact on both the efficiency of a process and the quality of the final product [50]. A temperature shift is also a problem that leads to the formation of inclusion bodies [16]. Instead, GJ1158 could be a better system to work with. For the proteins expressed by various workers (Table 2), the genes have been cloned downstream of the T7 promoter for the expression. This has found to decrease the levels of inclusion body development. Osmoresponsive promoters need salts like NaCl for induction [3]. It has been demonstrated that hyperosmolarity enhances the expression of numerous proteins, not just in *E. coli*, but also in insect and mammalian expression system [20].

Many studies have already been published on comparative protein yields, in various expression systems. For example, for the expression of a variant of staphylokinase, the yield of the enzyme was reported to be similar from *E. coli* BL21, yeast and GJ1158, without losing any of its functional features [35]. In the case of a cationic antimicrobial peptide (cAMP), in comparison to IPTG-inducible BL21, NaCl-inducible GJ1158 shows higher expression. Further, as the inducer is cheaper and easily accessible in case of GJ1158, it seems to be a better system than BL21 [12]. The production of the peptide in soluble form using GJ1158 made purification simple as there is less inclusion body formation. The expression of three different therapeutic proteins in a variety of *E. coli* strains including BL-21(DE3), BL-21(DE3) pLys S, BL-23(DE3) Rosetta, and BL-21(DE) GJ1158. Researchers reported that GJ1158 had less aggregation of protein and a higher yield [35]. The inclusion bodies within cells are less likely to occur in proteins produced by NaCl induction in GJ1158. Furthermore, it is possible that by carrying out the NaCl induction at lower growth temperatures, the tendency for inclusion body formation by specific proteins might be diminished even further. Dermatoxanthin (DPT) is a non-collagenous extracellular matrix protein that is expressed in a variety of human tissues and has important roles in each one [29]. DPT has recently been mentioned in reports as a potential treatment for metabolic diseases. This protein's diverse biotechnological applications and further investigation into its function will benefit from cost-effective recombinant synthesis [29]. Thus, in this investigation, the manufacture of DPT proteins was carried out utilizing a straightforward prokaryotic system of *E. coli* GJ1158, which has been successful in producing many eukaryotic proteins, including erythropoietin and streptokinase [35]. Erythropoietin (EPO), a glycoprotein hormone, is most frequently used to treat anaemia. It controls the synthesis of red blood cells in mammals; this recombinant protein is transformed into GJ1158 and get expressed successfully [36]. White spot syndrome virus (WSSV)'s VP28 gene was cloned into the pRSET B expression vector. *Escherichia coli* GJ1158 was used to express the VP28 protein as a 6-histidine tag protein after NaCl induction. The researchers found that there is less inclusion body formation in GJ1158 than BL21 host [48]. The overexpression of lethal factor of *B. Anthracis* gene is expressed in GJ1158 [42]. Osmotically induced multifunctional fusion protein SRH (SAK-RGD-Hirulog), which was generated from *E. coli* GJ1158, is a promising thrombolytic agent and maintained its multifunctionality in animal models [14]. The soluble version of the multifunctional fusion protein (SAK-RGD-Hirulog) was expressed in GJ1158 with a yield of 0.27g/L, which is greater than the total host protein by 55%. The yield is close to the figures reported for a similar construct made from yeast and *E. coli* BL21 (DE3) [21]. With the addition of IPTG the growth of cells declined and leads to 'inclusion body' formation. To overcome this problem, the workers have overexpressed TNF- α scfv in GJ1158 by considering its metabolic advantages [39].

Table 2. List of Proteins that have been expressed in GJ1158 host.

Sr. No.	Protein Name (Expressed in GJ1158)	Reference
1.	Human Erythropoietin	[38]
2.	Erythropoietin	[36]
3.	Streptokinase	[36]
4.	Necrosis Factor Receptor	[36]
5.	β -Galactosidase	[30]
6.	Tzs protein	[24]
7.	Dermatopontin	[29]
8.	Msak – RGD – Hirulog	[21]
9.	Cationic Antimicrobial Peptide	[32]
10.	Anthrax lethal factor gene (LF protein)	[42]
11.	Nucleocapsid protein based IgM-ELISA	[25]
12.	Staphylokinase Variant with Reduced Reocclusion	[35]
13.	Staphylokinase Variant	[22]
14.	Anti TNF α scfv	[39]

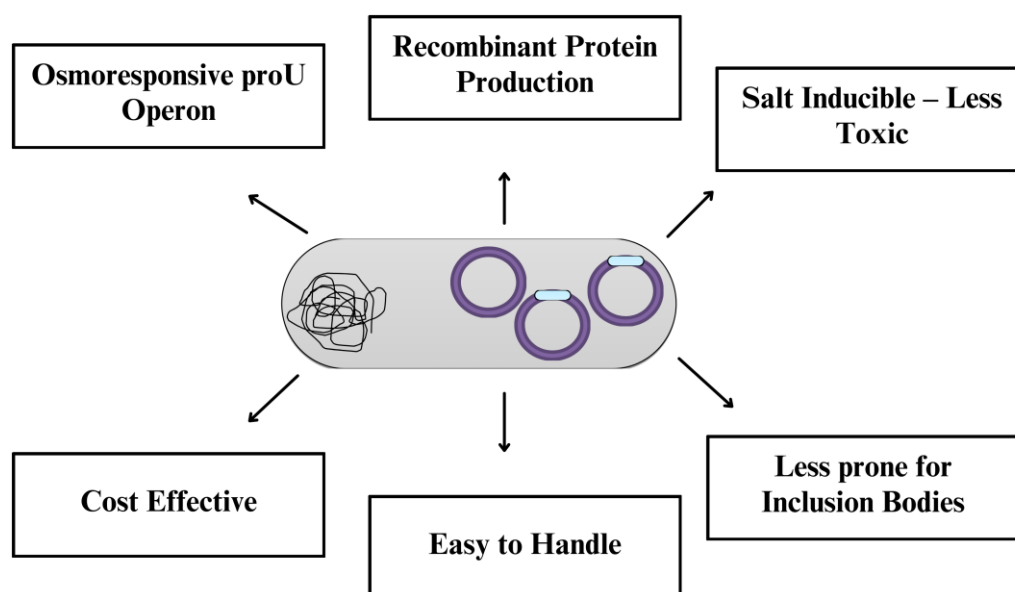


Figure 1: Showing the Benefits of Using Host GJ1158

Figure 1 is showing the Benefits of using host GJ1158, including it has Osmoresponsive operator system, due to NaCl induction the system makes cost effective and ecofriendly, no. of recombinant protein has been produced with the help of GJ1158 and comparably less prone to inclusion body production.

- This figure summarizes the advantages of using the GJ1158 host system for recombinant protein production:
- Recombinant Protein Production: The system supports the production of various recombinant proteins.
- Salt Inducible – Less Toxic: The induction system is based on salt (NaCl), which is less toxic compared to other chemical inducers.
- Osmoresponsive proU Operon: The operon used for induction is responsive to osmotic changes, making it efficient and controlled.
- Less prone to Inclusion Bodies: The system is designed to reduce the formation of inclusion bodies, which are aggregates of misfolded proteins that can complicate purification processes.
- Cost Effective: The system is economical in terms of operation and production costs.
- Easy to Handle: The GJ1158 system is user-friendly and easy to manipulate, making it suitable for various laboratory and industrial applications.

These tables and figures collectively highlight the effectiveness, efficiency, and advantages of the GJ1158 host system in the context of molecular biology and recombinant protein production.

DISCUSSION:

Largely, for recombinant *E. coli* the choice of induction is IPTG, but it is ecologically hazardous. A salt-inducible *E. coli* strain called GJ1158, a modified version of BL21 (DE3), has been used to express several recombinant proteins and it has its own advantages. It contains an osmoresponsive proU operon used for induction. It has already been used to express many recombinant proteins eg (Table 2). This review aims to explore the suitability of GJ1158 as an expression host for recombinant proteins.

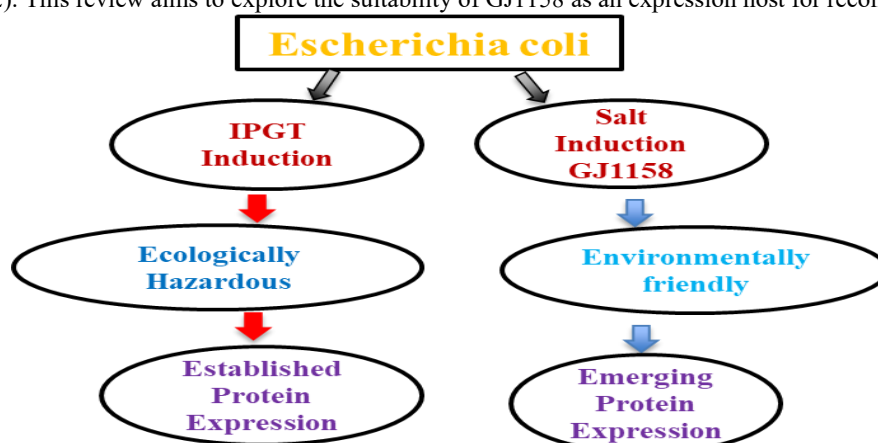


Figure 2: Comparing salt-inducible and IPTG *E. coli* strains

Some disadvantages are also there for the expression of recombinant proteins in the *E. coli*/bacterial expression system .

1. However, because it is a prokaryotic-based system, heterologous eukaryotic proteins synthesised are not appropriately changed. Additionally, it can be challenging to assist the secretion of highly expressed proteins. premature translational termination are examples of translation errors caused by uncommon codon bias [19].
2. The low ability of *E. coli* to produce serine/threonine/tyrosine protein kinases, an essential component of eukaryotic phosphoproteins, is thought to be a major drawback. As a result, making soluble recombinant proteins continues to be superior to the in vitro refolding process [50].

CONCLUSION

Advancement in rDNA technologies leads to the production of recombinant proteins for various purposes. These proteins can be used as vaccines, drugs, agricultural products etc. For over-expressing these recombinant proteins, bacterial host is mostly preferred. GJ1158 is easy manipulating with cost-effective, fast growing and eco-friendly inducing system. This features made GJ1158 as a suitable host for the expression of various proteins.

GJ1158 is a novel and ecologically friendly host for recombinant protein production, through the removal of IPTG, minimizing inclusion bodies, and having high yield, it provides a suitable alternative for research and industrial use. Further discovery of its capabilities in co-expression schemes and industry may expand its use.

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DECLARATION OF ETHICS:

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