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Recombinant Protein Manufacturing Using Actinomycetes as Host Cells

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Abstract

Actinobacteria are highly sought-after for use as cell factories or bioreactors in the pharmaceutical, agricultural, industrial, and environmental sectors. The commercial production of these proteins as recombinants and the biochemical and structural characterization of key proteins have been made possible by the genome sequencing of numerous species of actinomycetes. In this regard, better expression vectors that may be used with actinomycetes are required. As discussed in this article, recent developments in gene expression systems, knowledge of the intracellular environment, and identification and characterization of plasmids have made it possible to create workable recombinant expression systems in actinomycetes.

Keywords: Actinomycetes, recombinant protein, plasmid, yeast, promoter, gene

INTRODUCTION

The number of open reading frames with unidentified activities has increased as a result of the whole genome sequencing of more than 100 different organisms. It's critical to collect significant quantities of recombinant proteins in order to biochemically and structurally characterise such proteins. The most popular host bacteria for the mass synthesis of recombinant proteins is the Gram-negative-proteobacterium Escherichia coli. However, due to issues with insolubility, cytotoxicity, post-translational changes, or ineffective translation, it is challenging to express and isolate all of the proteins in E. coli.

In an effort to circumvent these problems, prokaryotic and eukaryotic cells have developed host-vector systems other than E. coli. Gram-positive bacteria with low G+C concentration, such as Lactococcus lactis and Bacillus spp., are often used to poured expressed proteins into the culture media [1–5]. In some cases, proper protein folding is aided by secretion, which prevents the local accumulation of recombinant proteins [6]. The commercial expression of prokaryotic and eukaryotic proteins uses Bacillus brevis, which has a very high capacity for protein secretion. Recombinant proteins can also be secreted by Lactococcus lactis, and these proteins can then be employed directly in food applications [5]. The production of proteins that go through post-translational changes is particularly beneficial in eukaryotic cells like yeast cells, insect cells, or immortalised cell lines [7].

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REVIEW OF LITERATURE

Actinomycetes (Gram-positive bacteria with a high G + C concentration), notably in the industrial fields, have garnered an expanding amount of interest in the genera Streptomyces, Rhodococcus, Corynebacterium, and Mycobacterium. They show prospective benefits for producing important secondary metabolites for industry and medicine, for fermenting amino acids, and for bioconversion

procedures. Actinomycetes have also seen the development of a number of host-vector systems [8, 9], while further advancements are still required to give highly inducible and tightly regulated promoters, broad-host range vectors, and high recombinant protein producibility. It has recently been reported that this type of bacteria's host-vector systems have improved, making it possible to produce large amounts of recombinant proteins with tightly controlled promoters [10–12]. The host-vector systems, in particular expression vectors, in actinomycetes are reviewed here, along with the system's advantages and potential applications in the future. We can draw attention to two standout qualities of actinomycetes as host cells. They first display a distinct metabolic variety and enzymatic capacities. The products they produce as secondary metabolites are valuable for industrial and medicinal applications [13], and the enzymes themselves are also helpful. For instance, some Rhodococcus spp. are utilised in the industrial synthesis of acrylamide [15] while Streptomyces spp. produce a variety of antibiotics [14]. Actinomycetes have historically established their host-vector systems to acquire such enzymes in high quantities and/or to control the metabolic pathway involved in the synthesis of antibiotics [8, 9]. Second, it is anticipated that actinomycetes will have a different intracellular environment from traditional host cells like E. coli. There hasn't been a host cell discovered yet that can globally express all the proteins in significant amounts. In order to maximise the chances of finding the best expression settings or host cell, it is crucial to offer a diversity of host-vector systems (expression systems).

For high-level protein production, it is crucial to choose the right promoter, and generally speaking, an inducible promoter is preferred over a constitutive promoter [8,10]. TipA [11,12], the acetoamidase gene [16,17], and the nitrilase gene [10] all had well-researched promoters. The expression vector harbouring the actI/actIII promoter was induced during the transition from the growth to the stationary phase in order to successfully produce polyketido synthases in S. coelicolor [18]. This vector's derivative can be employed with different actinomycetes [19], broadening the expression system's potential uses. Novel strong constitutive promoters were found in M. smegmatis using a genomic library connected to promoterless green fluorescent protein and a fluorescence-associated cell sorting method [20]. On the other hand, a mutation in the repressor gene prevents the constitutively generated temperature-sensitive repressor protein from suppressing the target gene expression at a high temperature [21, 22].

STREPTOMYCES

S. lividans has been extensively employed as a potential host for both cytoplasmic and secretory protein production among the Streptomyces spp. because it lacks the restriction systems that are frequently present in other Streptomyces and prevent the genetic modification of host cells [23]. A very low level of endogenous extracellular proteolytic activity is also present in S. lividans [23].

An expression vector that works in many Streptomyces species was recently reported by Herai et al. [10]. The vector transports the nitrilase gene promoter (PnitA) from the nocardioform actinomycete Rhodococcus rhodochrous J1 [15]. Only when the inducer -caprolactam is present is the expression significantly and tightly regulated. These researchers expressed a variety of bacterial genes and estimated that up to 396 mg of the target protein, making up to 40% of the total soluble protein, was produced per litre of culture media (e.g., the Streptomyces inducible expression system had thus far expressed a maximum of 38 mg of the protein per litre of the culture media [10,24]). The creation of proteins from sources other than bacteria or secreted proteins was not mentioned in the report, though. It is possible to enhance this hyperinducible expression vector such that it may express higher eukaryotic proteins and secrete proteins. Additionally, this vector might speed up the development of natural product gene clusters like those found in enediyne antibiotics and genome mining [25].

Streptomyces has been identified as the perfect host for the synthesis of secreted proteins in an increasing number of research during the past few years. Signal peptides are intensively investigated using mutagenesis techniques because they play a key role in increasing the effectiveness of secreted

protein synthesis [26]. Eukaryotic proteins have been effectively generated by a number of Streptomyces secretion systems [27–29]. S. lividans served as an effective host cell for the productive production of human CD4 in its soluble form. Utilizing pLTI-CD4 that contains the gene promoter and secretion signals for the S. longisporus serine protease inhibitor, more than 300 mg of protein was generated per litre of culture [27].

RHODOCOCCUS

Since the initial report on the E. coli-Rhodococcus shuttle vector [28], many cloning vectors have been created for Rhodococcus, and more recently, adaptable expression vectors have been built [11,12]. The levels of protein expression from these vectors are lower than those from the E. coli expression system (a maximum of 10 mg of protein per litre of culture media). However, because some Rhodococcus cells are psychrotrophic, proteins can be produced in Rhodococcus over a wide temperature range, from 4°C to 35°C [11,12]. When the thiostrepton-inducible tipA promoter was used, several micrograms of mouse protein per litre of culture media could be generated even at 4°C [11]. Most of the up to recently developed recombinant protein expression techniques are only effective between 10°C and 37°C. For instance, E. Recombinant protein expression has been done in a range of growth temperatures similar to that of coli, a mesophilic bacterium that can grow between 18 and 37 degrees Celsius [29]. It is well-known that producing recombinant proteins frequently requires a lower temperature [30]. Several mouse proteins were incompatible with E. At 4°C, Rhodococcus allowed the expression of E. coli [11]. Due to the ability of such proteins to have their enzymatic functions repressed, expression at lower temperatures is predicted to be efficient in creating proteins that harm the host cell.

The mycolic acid makeup of the host cells in R. erythropolis makes it difficult to disrupt the cell walls, therefore a method for the alteration of the host cells is required to make the recombinant-protein extraction processes easier [31]. The scientists discovered lysozyme-sensitive mutants that can be lysed by the addition of 12.5 g ml-1 lysozyme, but the wild type is resistant to more than 1 mg ml-1 lysozyme. Organic solvents are just one of the many dangerous compounds that Rhodococcus species are resistant to. Combining the expression system with this distinctive trait of Rhodococcus spp. results in a highly effective bioconversion process.

CONCLUSION

When expressing recombinant proteins, it is generally advised to use host cells that are phylogenetically close to (ideally identical) the origin of the protein of interest. This is caused by the cells' similar redox states, similar codon usage patterns, compatibility with translational machinery and molecular chaperones, and/or other factors. Therefore, utilising higher eukaryotes as hosts for the expression of higher eukaryotic proteins is perfect in theory but frequently produces low yields, is costly, and requires a lot of effort.

Actinomycete host-vector systems are appropriate for expressing actinomycete proteins as well as proteins from closely related organisms and higher eukaryotes. The host-vector system in actinomycetes still has to be improved, particularly with regard to modifying host cells. This includes making foreign genes more stable and simple to maintain (for example, by integrating plasmids), as well as eliminating host proteins that impede synthesis (for example, by knocking off proteases), either at the gene level or when proteins are extracted.

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