# Optimization of expression host for Thermomyces Lanuginosus lipase

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**Abstract**

In today's bio-based industry, lipase-catalyzed processes hold eminent commercial worth, yet their use is restricted owing to low yields, high production cost, inconsistent reproducibility, and poor performance in native form. Cloning and expression of multiple lipase genes in various systems have been investigated in order to produce enzymes for the food and detergent industries more cheaply since the development of recombinant DNA technology. The identification of novel lipases is still hampered by the rather difficult expression of these enzymes. The expression of lipases still requires a case-to case optimization. However, the unbiased choice of the appropriate promoter system and host for a specific protein of interest remains difficult. Here, we concisely expressed TLIP (Lipase from *Thermomyces lanuginosus*; mainly used in the detergent industry) in the frequently used conventional and alternative host systems, with their unique features, along with different promoters (T5, T7, aprE and hp4d) to produce recombinant products. Screening of expression was done among both prokaryotic ( *Escherichia coli, Bacillus subtilis*) and eukaryotic ( *Yarrowia lipolytica*) hosts consisting of both intracellular and secretory expression. *E. coli* (BL21 Shuffle) and *Y. lipolytica* (extracellular) were found to be the best expression systems for lipase production.



**Optimization of expression host for *Thermomyces Lanuginosus* lipase**

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**Abstract**

In today's bio-based industry, lipase-catalyzed processes hold eminent commercial worth, yet their use is restricted owing to low yields, high production cost, inconsistent reproducibility, and poor performance in native form. Cloning and expression of multiple lipase genes in various systems have been investigated in order to produce enzymes for the food and detergent industries more cheaply since the development of recombinant DNA technology. The identification of novel lipases is still hampered by the rather difficult expression of these enzymes. The expression of lipases still requires a case-to case optimization. However, the unbiased choice of the appropriate promoter system and host for a specific protein of interest remains difficult. Here, we concisely expressed TLIP (Lipase from *Thermomyces lanuginosus* ; mainly used in the detergent industry) in the frequently used conventional and alternative host systems, with their unique features, along with different promoters (T5, T7, aprE and hp4d) to produce recombinant products. Screening of expression was done among both prokaryotic (*Escherichia coli, Bacillus subtilis* ) and eukaryotic (*Yarrowia lipolytica* ) hosts consisting of both intracellular and secretory expression. *E. coli* (BL21 Shuffle) and *Y. lipolytica* (extracellular) were found to be the best expression systems for lipase production.

**Keywords:** ***Thermomyces Lanuginosus*; lipase; gene expression; specific activity**

## Introduction

With increasing information about the machinery of gene expression regulation and developments in the field of biotechnology, all the currently available gene expression systems will be improved functionally, and one or more novel systems will emerge. Nevertheless, just like a two-edged sword, some disadvantages of certain systems cannot be avoided totally. The need for novel-expression host platforms has increased with the increase in the number of gene-targets used in various industrial applications. Literature shows various expression platforms that are already in use. These platforms vary from prokaryotes/ bacteria (Baneyx, 1999; Rosano & Ceccarelli, 2014), eukaryotes like yeast (Cregg, Cereghino, Shi, & Higgins, 2000; Malys, Wishart, Oliver, & McCarthy, 2011), filamentous fungi (Visser et al., 2011) to higher complex systems like baculovirus/ insects and mammals (Joshi et al., 2021; Kesidis et al., 2020; Leal Filho et al., 2019). But when considering a handy expression system, both economic and qualitative characteristics have to be considered. Usually, in the industry, enzyme production depends upon factors like use of inexpensive media, less cost of production and high yield of product. Depending on the purpose, suitable host systems needs to be selected. With the use of recombinant DNA technology, cloning and expression of gene of interest in appropriate and suitable host systems like bacteria/ yeast/ plant/ algae/ insect will circumvent the difficulties caused during the production of enzymes. Prokaryotic and eukaryotic expression systems fall under the two broad groups. In general, host systems like bacteria, yeast, and filamentous fungus are easier to manage and exhibit productive host platforms than insects and mammalian cells. Because of this, they are more suited for industrial uses than insects and mammalian cells, which are mostly employed in the manufacture of medications (Altmann, Staudacher, Wilson, & März, 1999; Borrelli & Trono, 2015; Streatfield, 2007). The survey conducted in Scopus and the ISI Web of Knowledge may be utilised to obtain information on the microbial species typically employed for producing heterologous proteins of recombinant lipases (Borrelli & Trono, 2015) as shown in Figure 1. The result shows that among prokaryotes, *Escherichia coli* is the most widely used and exploited host for heterologous expression of lipolytic enzymes, whereas among eukaryotes, *Pichia pastoris* is being used. Lipases expressed in *E. coli* account for a little more than half of all the other recombinant lipases and are used for structural and functional studies. Whereas, for industrial applications, yeast and fungal systems especially *Pichia* are being preferred due to ease with post translational modifications and high rate of protein production.

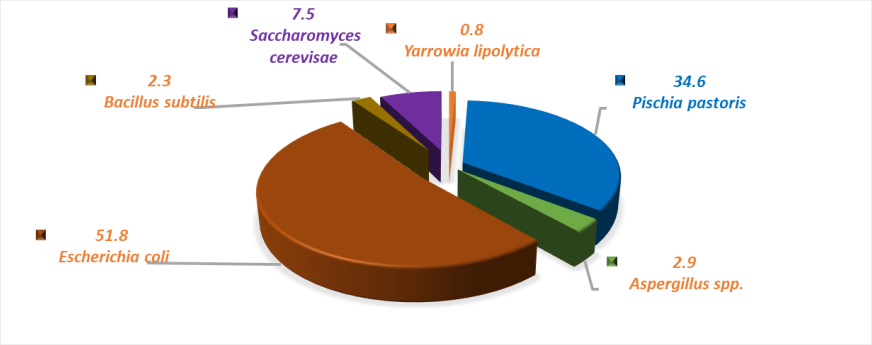


***Figure 1:*** *Percentage of lipases that are expressed heterologously on microbial hosts.*

Generally, prokaryotic hosts are mostly used for heterologous protein expression. This is mainly due to the fact that they are easier to handle, have short growth times, and reach high cell densities in inexpensive media (Rosano & Ceccarelli, 2014; Sezonov, Joseleau-Petit, & D'Ari, 2007). Additionally, their genetic makeup has been thoroughly investigated, leading to the commercial availability of several cloning vectors and mutant host strains. However, they do have significant drawbacks, the majority of which are caused by the bacterial hosts' inability to carry out post-translational changes, such as appropriate protein folding, phoshorylation and glycosylation, and proper formation of disulphide bridges (Adrio & Demain, 2010). Therefore, when

post-translational modifications are not required, a bacterial strain serves as the ideal host for the creation of a recombinant protein. The most often used host among bacteria is *E. coli* . Commercially available *E. coli* strains with a variety of molecular toolboxes (such as vectors, promoters, tags, etc.) can be used to set up an expression system that is suitable for the high-yield generation of desired recombinant proteins (Rosano & Ceccarelli, 2014; Terpe, 2006).*B. subtilis* is the second most researched and used prokaryotic organism for the generation of recombinant proteins after *E. coli.* As shown in Figure 1, *B. subtilis* is a bacterial host that has rarely been used for heterologous expression of lipases. Only bacterial lipases from the *Bacillus, Pseudomonas, and Staphylococcus* species make up the 2.3% of enzyme produced in*Bacillus* species. By fusing the protein with an N-terminal signal sequence, *B. subtilis* , unlike *E. coli* , can produce the protein at high quantities straight into the medium.

Among eukaryotes, the most widely used heterologous system for producing economically important recombinant proteins is *P. pastoris.* In comparison to *S. cerevisiae* , it exhibits lower levels of hyperglycosylation and greater expression levels. (Byrne, 2015; Valero, 2018). Many lipases from the yeast strains of *Candida* and fungal strains of *Rhizopus, Yarrowia, Aspergillus, Rhizomucor* and*Thermomyces* are expressed in these hosts. For e.g. CALB, CRL, ROL, YlLip2, etc., which are being used in the biodiesel industry. Among the filamentous fungi, *Aspergillus* species, *A. oryzae* has been frequently used for recombinant expression of lipases like CALB, ROL, TLL and FHL, thus accounting for 2.9 % of all the expressed lipases (Rosano & Ceccarelli, 2014). Technical advantages in this system include site-specific integration, increase in copy number and post-translation modifications. Currently, *P.pastoris* (Valero, 2018) and *Schizosaccharomyces pombe* , a fission yeast (Benko, Elder, Li, Liang, & Zhao, 2016) are being the most used yeast systems for heterologous protein expression besides *S. cerevisae* . Many non-conventional yeasts like *Y.lipolytica* are also being used as expression hosts in the industry because of the fact that they have rapid growth in low cost medium, appropriate post translational modifications and no endotoxin production (Gomes, Byregowda, Veeregowda, & Balamurugan, 2016).

Despite the large number of microbial lipases identified, there still exists a desperate need for a standardized method of their expression which also depends on the type of enzyme studied or case-to case study. Economic production of enzymes for industrial processes makes the selection criteria of the host/vector expression system critical. The lipase from *Thermomyces laguginosus* (TLIP) is1, 3-specific, basophilic and noticeably thermostable enzyme, commercially available in both soluble and immobilized form. Initially this enzyme catered the food industry, but later it found applications in many different industrial areas, from biodiesel production to fine chemicals (mainly in enantio and regioselective or specific processes) (Fernandez-Lafuente, 2010). All these practical applications require large quantities of enzymes. As a result, there is an urgent need to achieve high levels of lipase expression in commonly used industrial strains. In spite of being exploited commercially, its expression profile has not been studied properly. Its characterization data is readily available online which can aid in the hunt for a suitable expression host. Also, these hosts can be further used for expression of other lipases. Thus, based on the above-mentioned advantages, we selected *E. coli, B. subtilis* and*Y. lipolytica* for optimization of heterologous expression of TLIP using different promoters, cloning hosts and expression hosts accordingly.

**Materials and methods:**

*Strains, plasmids, culture media and materials:*

All heterologous host strains and plasmids used in the present study are summarized in Table 1. *E. coli* XL1 Blue (Promega, USA) was used for the general cloning procedure. The vectors used for general cloning purpose were pET23a, pQE30 (*E. coli* ) (Addgene, USA), pBES (*B. subtilis* RIK1285) (Takara Bio, USA) and pYLEX1, pYLSC1 (Yeastern Biotech, Taiwan).

Microbial strains used for expression of recombinant protein were*E. coli* - BL21 (DE3), BL21 (DE3) pLysS (Codon plus) and Shuffle T5 express (New England Biolabs, France); *Bacillus subtilis* RIK1285 (Takara Bio, USA) and *Yarrowia lipolytica* (Yeastern Biotech, Taiwan).

*E. coli* was grown in two different media: Luria Bertini (Himedia Labs, India) and Auto Induction Media

(Himedia Labs, India). *B. subtilis* was grown in LB medium. Yeast Extract-Peptone-Dextrose (YPD) plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 20 g/L agar were used for general growing of the yeast strains. Minimal selection medium for *Y. lipolytica* contained Yeast Nitrogen Base without amino acids (Himedia Labs, Mumbai).

Restriction enzymes, Taq DNA polymerase and T4 DNA ligase were all purchased from New England Biolabs (Hertfordshire, UK) while the primers were obtained from Eurofins (Bangalore, India). The purification kits (Columns and Ni-NTA resin) were bought from Qiagen (Valencia, CA, USA).

***Table 1**: Constructs and strains used in this study.*

| Strain | Strain features | Vector | Vector features promoter/inducer | Fusion tag and location |
|---|---|---|---|---|
| E. coli | E. coli | E. coli | E. coli | E. coli |
| *XL1 Blue* | Allows blue-white color screening | pET23a pQE30 | T7 lac / IPTG T5 lac / IPTG | n-His N-t n-His C-t |
| BL21 (DE3) | Deficient in both lon and ompT proteases | pET23a | T7 lac / IPTG | n-His N-t |
| BL21 (DE3) pLysS (Codon plus) | High stringency, reduces basal expression level | pET23a | T7 lac / IPTG | n-His N-t |
| SHuffle T5 express | Allows disulfide bond formation in the cytoplasm | pQE30 | T5 lac / IPTG | n-His C-t |
| B. subtilis | B. subtilis | B. subtilis | B. subtilis | B. subtilis |
| *B. subtilis RIK1285* | Allows secretory expression in the media | pBES | *aprE* */Constitutive* | - |
| Y. lipolytica | Y. lipolytica | Y. lipolytica | Y. lipolytica | Y. lipolytica |
| *Y.lipolytica* Po 1 g | Heterologous protein expression, either intracellular or extracellular | pYLEX1 pYLSC1 | hp4d /Constitutive hp4d /Constitutive | - - |

n-His, Histidine tag; N-t, N-terminal; C-t, C-terminal; +, positive expression; -, no expression.

*Cloning procedures:*

Cloning and transformation procedures were performed according to established techniques and supplier's manuals. For cloning in *E. coli* , the gene of TLIP lipase from *T. lanuginosus* was amplified and cloned in vector pQE30 using the reverse primer R.PQE and the forward primers F.PQE, in pET23a using the primers F. PET and R.PET and in pBES using the primers F.PBES and R.PBES. Similarly, TLIP was also amplified and cloned in vectors pYLEX1 and pYLSC1 using the respective primers described in Table 2.

***Table 2:** Primers used in this study.*

| **Primers** | **Sequence ( 5'3')** | **Restriction site** |
|---|---|---|
| E. coli | E. coli | E. coli |
| F.PQE | TTAATT*GGATCC*ATGCGTAGCAGCCTGGTGCTGTTCTTTG | BamHI |
| R.PQE | ATATAT*AAGCTT*TTACAGGCAGGTGCCGATCAGGCCGAAA | HindIII |
| F.PET | TTAATT*CTCGAG*ATGCGTAGCAGCCTGGTGCTGTTCTTTG | XhoI |

| Primers | Sequence ( 5'3') | Restriction site |
|---|---|---|
| R.PET | ATATAT*CATATG*TTACAGGCAGGTGCCGATCAGGCCGAAA | NdeI |
| B. subtilis | B. subtilis | B. subtilis |
| F. PBES | ATATAT*GGTACC*ATGCGTAGCAGCCTGGTGCTGTTCTTTGT | KpnI |
| R. PBES | TATATA*AGATCT*TTACAGGCAGGTGCCGATCAGGCCGAAAT | XbaI |
| Y. lipolytica | Y. lipolytica | Y. lipolytica |
| SF. LEX | *AATG*CGTAGCAGCCTGGTGCTGTTCTTTGTGAGCGC | PmlI |
| SR. LEX | ATAT*GGTACC*TTACAGGCAGGTGCCGATCAGGCCGAAA | KpnI |
| SF. LSC | ATAT*GGCC*GTTCT*GGCC*ATGCGTAGCAGCCTGGTGCTGTTC | SfiI |
| SR. LSC | ATAT*GGTACC*TTACAGGCAGGTGCCGATCAGGCCGAAA | KpnI |

The amplifications (protocol not provided here) were carried out in a thermocycler (C1000 Biorad, United States). The PCR products were purified and ligated with the vectors pET23a, pQE30, pBES pYLEX1 and pYLSC1 and then cloned in *E. coli* XL1 Blue for screening of positive clones which were later expressed in their expression vectors as shown in table 1. All these recombinant plasmids were verified by sequencing (data not shown).

*Protein expression and purification:*

*Intracellular:*

The constructs were introduced into their corresponding host. In the case of *E. coli* , different strains (Table 1) were transformed using following the manufacturer's protocol. For the expression screening, several clones were grown overnight in 5 mL of LB medium with the corresponding antibiotic at 37°C and 200 rpm. To induce the expression, 0.5 mM of IPTG was added to the culture.

*Extracellular:*

In the case of *B. subtilis* (Takara Bio, USA), the transformation was done by following the protocol provided in the kit manual (Murayama, Akanuma, Makino, Nanamiya, & Kawamura, 2004). The positive clones obtained, were incubated in LB media containing kanamycin (10 µg/ml) at 30 for 24-48 hours. In the case of *Y. lipolytica* , (Yeastern Biotech, Taiwan), the plasmid was linearized using NotI restriction endonuclease to direct its integration at the pBR322 docking platform of the recipient *Y. lipolytica* Po1g strain (Madzak, Tréton, & Blanchin-Roland, 2000). Yeasts were transformed by the lithium acetate method and Leu+ transformants were selected on minimal YNB medium. The cultivation of *Y. lipolytica* yeasts was carried out on YPD medium (g/l): yeast extract - 10; peptone - 10; glucose - 10. YPD agar medium comprised 1.5% of agar. The cultivation of yeast transformants was carried out on YNB-N5000 (g/l) medium: nitrogen base without amino acids - 6.7; glucose - 10; agar - 16. For the expression screening, several clones were grown overnight in 5 mL of YPD medium with the corresponding at 30°C and 200 rpm.

*Lipase Purification:*

For intracellular cell expression, the cells were harvested after 24 hours by centrifugation (9000 rpm, 10 min, 4°C) and re-suspended in 5 mL lysis buffer (10 mM Tris HCl, pH 8, with 300 mM NaCl). It was then crushed with liquid nitrogen and then sonicated. The cell free suspension was then loaded on a pre-equilibrated 2 ml Ni-NTA-Agarose column (Qiagen, Venlo, Netherlands). Bound proteins were released by the elution buffer (10 mM Tris HCl, pH 8, 300 mM NaCl, 1M Imidazole). Protein estimations in elution fractions were done by Bradford's assay, and the purified enzymes were analysed by Laemmli 12% SDS PAGE to assess the purity and molecular weight.

Similarly, for extracellular expression, the supernatant fraction was concentrated on 10kDa Macrosep Centrifugal device (Pall Corporation, United States) checked for protein expression using 12% SDS-PAGE gels followed by staining with Coomassie R-250 followed with activity with pNP-esters.

*Activity analysis:*

The protein was quantified by measuring UV absorbance at 280 nm based on the molar extinction coefficient (15.045 $mM^{-1}cm^{-1}$). Lipase activity was determined by measuring the initial hydrolysis rate of pNP-dodecanoate hydrolysis. For this, 10 mM stock solutions of pNP-dodecanoate was prepared in 100% isopropanol. Assays were performed with 0.25 mM substrate and ˜1 µg each of the protein fractions in the total reaction volume of 2 ml. Released p-NP after hydrolysis was continuously measured at 410 nm in UV-2550 (Shimadzu, Kyoto, Japan) spectrophotometer coupled to an accessory TCC-240A to control the temperature of the glass cuvette at 50 °C.

**Results and discussion:**

*Cloning and protein expression:*

The lipase from *Thermomyces lanuginosus* has already been expressed in *P. pastoris* (Fang et al., 2014; Fernandez-Lafuente, 2010; Zheng, Guo, Song, & Li, 2011; Xu et al., 2023; Yang et al., 2020) and *Aspergillus niger* (Lima et al., 2019) considering the fact that it itself is from a fungal source and no reports are available of its expression in *B. subtilis* or *Y. lipolytica* till now. Despite the fact that many different systems have been successfully used to express lipases, the *E. coli* expression system still dominates the bacterial expression systems and is still the preferred system for laboratory research, initial development in commercial activities, or as a useful benchmark for comparison between various expression platforms with its many available host strains. In our study, the expression of TLIP using *E. coli* as heterologous host (Table 3) was achieved using the vector carrying a weak inducible T5 promoter such as pQE30. The protein was not expressed in pET23a vector carrying a strong T7 inducible promoter (Figure 2). We might consider promoter occlusion to be the reason for the incapability of the T7 promoter to inhibit its function and not express the protein (Adhya & Gottesman, 1982; Joseph et al., 2015). This problem could be resolved by repositioning or engineering of the T7 promoter (Adhya & Gottesman, 1982). This needs to be optimized further.

Amongst the different strains of *E. coli* screened, TLIP was successfully expressed in XL1 Blue and BL21 Shuffle. However, the enzymes expressed in *E. coli* are often produced with low-level enzymatic activity and mainly in the form of inclusion bodies. Several studies have been reported where a signal peptide sequence has been fused to the N-terminus of the recombinant proteins for its secretory production (Cabrita, Dai, & Bottomley, 2006; Zhang et al., 2018). To avoid the production of inclusion bodies and achieve a secretory expression of enzyme, TLIP was successfully cloned and expressed in*B. subtilis* with a secretory expression system.

***Table 3**: Expression profile in different hosts*

| **Expression system** | E. coli | E. coli | E. coli | B. subtilis | Y. lipolytica | Y. lipolytica |
|---|---|---|---|---|---|---|
| **Expression type** | Intra-cellular | Intra-cellular | Intra-cellular | Extra-cellular | Intra-cellular | Extra-cellular |
| **Cloning vector** | pET23a | pQE30 | pQE30 | pBES | pYLEX | pYLSC |
| **Expression host** | XL1 Blue BL21 DE3 BL21 codon plus | XL1 Blue | BL21 Shuffle | *B. subtilis* RIK128 | *Y. lipolytica* | *Y. lipolytica* |
| **Protein Expression** | No | Yes | Yes | Yes | Yes | Yes |

Since they grow quickly in inexpensive media, properly carry out post-translational modifications, and don't produce endotoxins, *Y.lipolytica* is also used as an expression host in the industry for the production of enzymes with significant economic value (lipases, proteases, amylases, mannanases, and laccases) (Madzak, 2015; Madzak & Beckerich, 2013). Therefore, considering it as a cheaper large scale protein production

system, it was considered as a host for gene expression in our study. TLIP was successfully cloned and expressed in *Y. lipolytica* (intracellular as well as secretory as shown in table 3) expression systems.

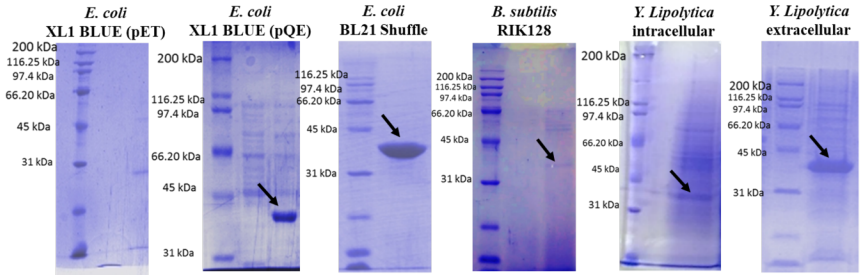


***Figure 2:*** *SDS PAGE profile of TLIP in different hosts*

Total protein in all the samples were visualized using a 12% SDS–PAGE gel electrophoresis. TLIP expressed in intracellular expression hosts was purified by IMAC whereas TLIP expressed in extracellular hosts was concentrated and run on SDS PAGE as shown in Figure 2. Based on the molar extinction coefficient, the concentration of the protein was calculated for all the samples as listed in Table 4. Among the prokaryotic expression systems screened, *E. coli* BL21 Shuffle had the highest protein yield of 2.5- 3.0 mg/ gm of cells followed by*B. subtilis* with a protein yeild of 1.0-1.5 g/L broth. Whereas, for *Y. lipolytica* , the protein yield was higher for secretory expression (5.5- 6.5 g/L broth) followed by intracellular expression (1.5-2.0 mg/ gm cells).

Enhancement in solubility by addition of NaCl did not result in an increased yield of active TLIP, suggesting that folding was not the only problem when the protein was expressed in *E. coli* XL1 Blue. Expression in BL21 Shuffle improved the activity significantly, implying that disulfide bond formation was a limiting factor (Xu et al., 2008) or insufficient translation. Also, different media compositions can affect the cell growth and hence the protein expression. Therefore, rich media source might generally result in a higher cell density, which might result in a higher yield of expressed protein. In the case of *B. subtilis* , considering it to be a secretory expression system, the protein yield was less. Although it can be cultivated to very high cell densities in simple medium but the poor protein yield might be correlated to the fact that *B. subtilis* secretes high amounts of proteases, can occasionally show plasmid instability and might have reduced/ absent expression of the protein of interest (Yin, Li, Ren, & Herrler, 2007). In contrast, *Y. lipolytica* , a unicellular eukaryote, has the capacity to create soluble, properly folded recombinant proteins that have undergone all the post-translational modifications required for their roles. It features a powerful hp4d promoter to drive the expression of the TLIP gene, making it easier and more affordable than most other eukaryotic systems to produce large quantities of lipase (Lobstein et al., 2012).

*Activity profile:*

pNP-dodecanoate was used as a substrate to determine the specific activity of all the proteins involved in this study. It was interesting to find that *E. coli* BL21 Shuffle showed the highest specific activity (Table 4) among the prokaryotic strains screened as it is capable of correctly folding disulfide bonded proteins in its cytoplasm (Lobstein et al., 2012; Abdollahi et al., 2022; McElwain et al., 2022). The activity of protein seemed clearly influenced by the protein yield achieved using each strain. Thus, stating the correct and efficient folding of protein using Shuffle strain. Although we are aware of the low yields of TLIP produced in *B. subtilis* (Table 4), it is interesting to remark that the success in achieving the functional expression of TLIP in this prokaryote. This opens new strategies to produce more robust tailor-made enzymes. Further experiments would be required to optimize TLIP expression in this host.

***Table 4****: Activity profile in different hosts*

| **Expression host** | XL1 Blue BL21 DE3 BL21 codon plus (pET23a) | XL1 Blue (pQE30) | BL21 Shuffle (pQE30) | *B. subtilis* RIK128 | *Y. lipolytica* (pYLEX) | *Y. lipolytica* (pYLSC) |
|---|---|---|---|---|---|---|
| **Protein yield** | - | 1.0-1.5 mg/ gm cells | 2.5-3.0 mg/ gm cells | 1.0-1.5 g/L broth | 1.5-2.0 mg/ gm cells | 5.5-6.5 g/L broth |
| **Activity (U/mg)** | - | 4.21 ± 0.98 | 13.9 ± 0.99 | 2.2 ± 0.07 | 5.69 ± 0.09 | 27 ± 1.02 |

Although, properties like post-translational modifications, protein folding, protein processing, disulfide bond formation, glycosylation and the use of signal sequences for heterologous protein expression and targeted secretion are enough to justify the high protein yield and activity profile of TLIP in *Y. lipolytica.* It is able to grow on a range of carbon sources such as glucose, alcohols, acetates, alkanes, fatty acids, and oils (Barth & Gaillardin, 1997;Park & Ledesma-Amaro, 2022; Zieniuk & Fabiszewska, 2019). Proteases, lipases, and phosphatases are just a few of the proteins that *Y. lipolytica* is renowned for being able to release into the environment (Park & Ledesma-Amaro, 2022). In general, *Y. lipolytica* is a good host for the production of recombinant proteins. It must be optimised and expressed in less expensive growth medium and carbon sources.

**Conclusion:**

In conclusion, we investigated the expression of a triacylglycerol lipase, a possible biotechnologically relevant enzyme, in prokaryotic and eukaryotic hosts. The TLIP Lipase gene was effectively expressed in three novel hosts, the bacteria *E. coli* and *B. subtilis*and the GRAS yeast *Y. lipolytica.* The yeast secretory expression system produced the most TLIP Lipase. Low-cost, uncomplicated cultivation, and little to no downstream processing are necessary for large-scale protein synthesis. With a manufacturing method that successfully secretes the enzyme into the growth medium, these objectives can be met. In this context, *Y. lipolytica* offers industrial bacterial systems a desirable alternative. Also, *E. coli* BL21 shuffle strain greatly expands the toolkit for cell biologists by enabling the use of bacterial production in place of more cumbersome eukaryotic expression systems (Abdollahi *et al* ., 2022).

A significant problem for biotechnology, and indeed any laboratory that studies proteins, is the development of quick expression and purification methods for recombinant proteins. A significant portion of the development of high-throughput cloning, expression, and purification technologies has been driven by the genomic and structural genomic groups.

**Author Contributions** : Surabhi, Arvind and Annamma conceptualized and designed the experiments; Surabhi collected and analysed the data; and Surabhi, Arvind and Annamma wrote the paper. All authors read and approved the final manuscript.

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**Conflicts of Interest** : The authors have no conflicts of interest to disclose.

**Data availability statement:** Data available upon reasonable request from the authors.

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