RESEARCH ARTICLE
Bull Biotechnol (2020) 1(1):23-29
Journal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Investigation of cloning strategies for the recombinant expression of a putative immune modulator TIR domain protein from probiotic *Lactobacillus casei* 21/1

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Received : 10/05/2020
Accepted : 17/06/2020

Abstract: TIR domain proteins have a key role in Toll-Like Receptor (TLR) signalling pathway in innate immunity. Bacteria can produce TIR domain proteins and those from pathogens were shown to manipulate TLR signaling via mimicking host proteins. Probiotics can also affect TLR signaling, but the molecular details have not been yet elucidated. In this study, a putative protein from probiotic *Lactobacillus casei* was identified as a TIR domain protein (LcTIR) based on sequence conservation. Multiple sequence alignments showed that LcTIR has high similarity to known TIR domains and the structural model of LcTIR verified the presence of the TIR domain fold. Following this, the gene encoding LcTIR was cloned in several *Escherichia coli* plasmids in order to obtain pure protein for structural and biochemical studies. Several fusion partners, promoter systems, different *E.coli* host strains and induction conditions were investigated to achieve recombinant protein production. In all conditions, recombinant LcTIR was produced at low amounts. The highest amount of protein obtained was GST-LcTIR fusion; in Rosetta(DE3)pLysS cells at 37°C with 0.5mM IPTG induction, where nearly all the protein was found in inclusion bodies. Furthermore, for all the constructs and strains tested, the low amount of LcTIR production suppressed cell growth and this might indicate its potential as an antimicrobial agent which opens a new era on bacterial TIR domains. This study is one of the first studies investigating the presence of probiotic TIR domain proteins, and future studies are needed to obtain soluble protein to assay their effect on TLR signalling mechanisms.

Keywords:TIR domain proteins; molecular cloning; probiotic; structural bioinformatics; toxic recombinant protein

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1 Introduction

In recent years, studies on intestinal microbiome have increased. Interaction mechanisms of both pathogens and probiotics with intestine attract many researchers. Innate immunity plays a pivotal role when the system encounters microorganisms. Pattern recognition receptors (PRR) recognize microbial molecular patterns (MAMP) and initiate innate immune responses. Toll-like receptors (TLR) are a group of PRRs and function in recognition of several microorganisms and controlling the first immune response. Organization of TIR (Toll/interleukin-1 receptor) domain proteins in this pathway are very important in the transmission of the signal (O'Neill and Bowie 2007).

The TIR domain has a conserved fold, consisting of five parallel beta sheets in the center surrounded by alpha helices and loop regions. TIR domain is found in the structure of both TLR receptors (TLR1-10) and adaptor proteins (MyD88, TRIF, TIRAP (MAL), SARM and TRAM) in the cytoplasm. TIR domain proteins show high amino acid sequence similarity at especially conserved regions, and TIR domain proteins can be identified by sequence comparison (Ve et al. 2015). Such bioinformatics analyses showed that all kingdoms of life possess TIR domain proteins; including bacteria (Turner 2003). Studies were mostly focused on TIR domains from pathogenic organisms. It was shown that TIR domain proteins from several pathogens were able to interact with human TIR domain proteins (MyD88 and TIRAP) and this resulted in suppression of TLR signalling (Chaudhary et al. 2012; Cirl et al. 2008; Kaplan-Türköz 2017; Newman et al. 2006; Radhakrishnan et al. 2009; Salcedo et al. 2008; Snyder et al. 2013). These proteins have the conserved TIR domain fold (Kaplan-Türköz et al. 2013) and with this structural mimicry, they can interact with human TIR domain proteins, resulting in manipulation of the signaling pathway.

Recent research points out evidence that probiotics can also manipulate TLR signaling, but the mechanism has not been completely elucidated. Studies have shown that some probiotic strains can regulate mostly TLR2, TLR4, TLR9 and TLR5 signaling pathways. *Bifidobacterium breve* C50, *B.breve* Yakult and their cell free components were shown to induce production of IL-10 (anti-inflammatory cytokine) through TLR2 (Hoarau et al. 2006; Jeon et al. 2012). *Lactobacllus casei* CRL 431 were shown to induce IL-6 production through TLR2 signaling (Galdeano et al. 2007). *L.casei* Zhang was shown to increase the expression of TLR2 and TLR9 and reduce production of proinflammatory cytokines in a rat model (Wang et al. 2016). Similarly, an

increased expression of TLR2, TLR4, TLR5 and TLR9 in intestinal epithelial cells were detected in the presence of *L.casei* (Castillo et al. 2011).

Probiotic DNA was shown to be responsible for the modulation of TLR9 signaling pathways (Kitazawa et al. 2003). However, the molecular mechanism of probiotic action on other TLR pathways has not been illuminated. According to our genome analysis, we found gene regions encoding TIR domains in probiotic genomes and our hypothesis is that probiotics can also produce TIR domain proteins which can interact with human partners to regulate TLR signaling pathways.

2 Materials and Method

2.1 Materials

E. coli strains TOP10, BL21(DE3), BL21Star(DE3), BL21(DE3)pLysS, Rosetta2(DE3), Rosetta(DE3)pLysS cells were used in the study. LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl) was used for cell growth. Antibiotics were added when appropriate; ampicillin (100 µg/ml), kanamycin (50 µg/ml), chloramphenicol (34 µg/ml). All chemicals were obtained from Merck or Sigma. All molecular biology reagents and kits were obtained from Thermo Scientific.

2.2 Bioinformatics methods

BLAST was used to identify probiotic origin TIR domain proteins (Madden 2002). Brucella TIR domain protein BtpA (pdb id: 4lzp) was used as query sequence for BLAST (blastp suite) and the search was restricted with *Lactobacillales* (taxid: 186826) taxonomic group to reveal probiotic origin TIR proteins.

Multiple sequence alignments between bacterial and human TIR domains were done using T-COFFEE (Expresso) (Notredame et al. 2000). The structural homology model of *Lactobacillus casei* 21/1 TIR domain (LcTIR) was built by RaptorX using *Paracoccus denitrificans* TIR protein (PdTIR) (PDB id: 3h16A) as the template protein (Kallberg et al. 2016). Structural models were displayed using graphics software PyMOL.

2.3 Cloning and protein expression

The genomic DNA of *L.casei* 21/1 was a gift from Prof. Dr. James L. Steel (Wisconsin University). Putative TIR domain gene region from *L.casei* 21/1 (507 bp) was obtained by PCR and the PCR fragment was cloned into several different plasmids (Table 1) and *E. coli* TOP10 cells were transformed with the recombinant plasmids.

Table 1 Properties of used plasmids

Plasmid	Antibiotic resistance	Promoter	Copy number
pET151/D-TOPO	Ampicillin	T7	Low
pQE80L	Ampicillin	T5	High
pETM11-SUMO3GFP	Kanamycin	T7	Low
pGEX-4T-2	Ampicillin	tac	Low

In order to express LcTIR, three different tag and/or fusion partners were tested; 6-Histidine tag (6His), small ubiquitin-related modifier (SUMO) and glutathione S-transferase (GST) fusion proteins. Some properties of the expected proteins are summarized in Table 2.

Table 2 Properties of expressed proteins

Plasmid	Expressed protein	Length (amino acid)	MW (kDa)	pI
pET151/D-TOPO	6His-LcTIR	201	23	5.05
pQE80L	6His-LcTIR-2	180	20	5.30
pETM11-SUMO3GFP	6His-SUMO-LcTIR	276	32	5.15
pGEX-4T-2	GST-LcTIR	399	46	5.24

Single colonies carrying the recombinant plasmids were grown in 5 ml culture medium overnight at 37°C and 150 rpm shaking. The next day 2% of culture was inoculated to fresh medium and were incubated at 37°C until an OD_{600} of 0.7- 0.9 and induction was done by adding 0.5-1 mM IPTG. The cultures were grown at 37°C for 6 hours or 25°C or 20°C overnight after induction and were monitored for protein expression using SDS-PAGE and Western Blot. After induction, cells were pelleted and equal number of cells were resuspended in loading dye, boiled and applied to 12% polyacrylamide gels. Gels were stained with either Coomassie brilliant blue R250 and/or blotted to PVDF membranes for 6His tagged proteins. Monoclonal anti-polyHistidine antibody (mouse) and anti-mouse IgG-alkaline phosphatase antibody and BCIP/NBT substrate were used for detection.

2.4 Protein solubility test and small scale protein purification

Solubility test and affinity purification were done for GST-LcTIR protein. Briefly, culture was centrifuged at 6500 rpm for 10 minutes at 4°C and the cell pellet was stored at -86°C. Pellets were resuspended in lysis buffer (50 mM Tris pH 8.0, 300 mM NaCl, 10 mM $MgCl_2$. 10% Glycerol, 1 mg/ml Lysozyme, 10 µg/ml DNase, 1 mM DTT, 1X Protease inhibitor) and incubated for 20 minutes on ice. Equal volume glass beads were added and vortexed for lysis. 1% Triton X-100 was added on the lysate and was centrifuged at high speed (16000 g for 20 minutes at 4°C) to separate soluble and insoluble fractions. The insoluble pellet was resuspended with lysis buffer (insoluble fraction) and the cleared supernatant (soluble fraction) was diluted with dilution buffer (50mM Tris pH 8.0, 5% Glycerol). For affinity purification, soluble fraction were mixed with glutathione resin which was previously equilibrated with Buffer A (50mM Tris pH 8.0, 150mM NaCl, 5% Glycerol) and allowed binding for 1 hour on ice with gentle rocking. After incubation the suspension was loaded on a disposable

column and flow through was collected. The column was washed with Buffer A and 10mM reduced glutathione pH 8.0 in Buffer A was used for elution.

3 Results and Discussion

3.1 Bioinformatics analysis and structural comparison

Our bioinformatics analyses have shown the presence of a hypothetical protein (LCA211_1668) in the genome of *Lactobacillus casei* 21/1 and this protein was named as LcTIR. The protein sequence was aligned with those of several different TIR domain proteins (Fig 1) and was found very similar especially in the conserved regions. Considering the sequence similarities given in Table 3, the high similarity of LcTIR sequence to bacterial TIR sequences is remarkable. In order to compare the similarities at the structural level, homology model of LcTIR was built using Raptor X (Fig 2a). The model contains five parallel beta sheets in the core and they are surrounded by alpha helices and loop regions, which is the conserved TIR domain fold (Ve et al. 2015). The LcTIR model is considered of high quality according to the criteria of the modeling software with P-value of 1.22e-04 and uGDT of 105.

Conserved regions (CR) for TIR domains were shown on LcTIR model (Fig 2b). Buried beta sheets on CR1 which are responsible of structural stability in mammalian TIR proteins (Ve et al. 2015), are similarly located in buried center at LcTIR model. Two loop regions; BB-loop and DD-loop, were shown to significantly contribute to TIR:TIR interactions which is necessary for TLR signaling (Zhang et al. 2012) These loops are located on the surface of LcTIR model and therefore has potential to contribute to protein interactions (Fig 2b).

LcTIR model was aligned structurally to other human and bacterial TIR domain proteins (Table 3) and the fit to bacterial origin TIR domains highlight the high degree of structural similarity (Fig 2c).

Table 3 Structural and sequence similarity between LcTIR and some other TIR domains

	Structral alignment with LcTIR model RMSD (A°)	Sequence identity with LcTIR sequence (%)
Bacterial TIR domains		
PdTIR (3h16)	0.580	35.56
BtpA (4lzp)	0.394	40.14
Human TIR domains		
MyD88 (2z5v)	1.405	26.67
TIRAP (2y92)	3.140	19.23
TLR1 (1fyv)	3.981	35.56
TLR2 (1fyw)	3.659	23.08
TLR6 (4om7)	2.859	25.23
TLR10 (2j67)	2.872	25.22

Protein production optimization

The gene encoding LcTIR was cloned into several different expression plasmids (Table 2) in order to produce enough protein for purification and characterization studies. The gene contains six rare codons, thus 6His-LcTIR protein expression studies were initially done using Rosetta2 (DE3) cells (Fig 3a and 3b).

6His-LcTIR protein was induced from pET151D-TOPO at three different temperatures (20°C, 25°C and 37°C) with 1mM IPTG and western blot showed that the amount of produced protein was very low, with no protein at 20°C (Fig 3a). Interestingly, the amount of protein did not increase with time and the biomass did not increase after 1.5 hours of induction (Fig 3b). These results suggested that LcTIR might be toxic to *E.coli* cells. In order to solve the toxicity problem, BL21(DE3)pLysS cells were used for expression and the amount of protein produced at 37°C were higher (Fig 3c). In order to increase the amount of protein, different IPTG concentrations were tested to control the toxicity and protein amount relatively increased with 0.2 and 0.5mM IPTG (Fig 3d). Still, the protein produced was not enough for further studies.

Next, a plasmid with a weak promoter was tested in order to slow down toxic LcTIR production and therefore the cells could accumulate protein over time. 6His-LcTIR-2 protein was expressed from pQE80L at 37°C with 0.5mM IPTG induction using different expression cells. In all the conditions tested, protein amount did not increase over time and therefore, toxicity problem was not solved (Fig 3e).

Following these, different fusion partners were tested in order to decrease the toxicity of LcTIR. 6His-SUMO-LcTIR was expressed from pETM11SUMO3GFP vector at 20°C with 1mM IPTG induction (Marblestone et al. 2006) (Fig 3f), or at 37°C with 0.5mM IPTG induction (Fig 3g). Relatively moderate amount of protein was obtained from Rosetta (DE3) pLysS cells, however, the problem of toxicity still persisted and protein sufficient for purification was not obtained.

Another recommended fusion partner for solving the toxicity problem is GST (Saluta 1998). *E.coli* cells carrying pGEX4T2-LcTIR plasmid were not affected by protein expression, as cell growth continued to increase at different temperature and inducer concentrations (data not shown). Furthermore, the amount of protein was shown to increase after induction. Therefore GST fusion seemed to eliminate the toxic effect of LcTIR. Although the molecular weight of the expected protein is 46kDa, the observed protein in the polyacrylamide gel appeared just below 40kDa (Fig 3h).

Although the reasons for this cannot be fully explained in the literature, it has been reported that GST fusion protein might denaturate under reducing conditions in SDS-PAGE and it can position below expected in the gel (ThermoFisher 2019). The highest amount of protein was obtained at 37°C, with 0.5mM IPTG, from Rosetta (DE3) pLysS cells (Fig 3h).

Fig. 1 Multiple alignment of human TIR domains, probiotic TIR domain LcTIR and bacterial TIR domains using T-COFFEE. NCBI or PDB id of the sequences used in the alignment: human origin; MyD88 (2z5v), TIRAP (2y92), TRIF (2m1x), TRAM (2m1w), SIGIRR (CAG33619.1), TLR1 (1fyv), TLR2 (1fyw), TLR3 (ABC86908.1), TLR4 (AAF89753.1), TLR5 (AAI09119.1), TLR6 (4om7), TLR7 (AAZ99026.1), TLR8 (AAZ95441.1), TLR9 (ACQ41824.2), probiotic origin; LcTIR (EKQ02965.1), pathogenic bacterial origin; BtpA (4lzp), PdTIR (3h16), TlpA (WP_000028416), TcpC (ADO30448), YpTIR (KNX91904.1). Conserved regions are shown with boxes.

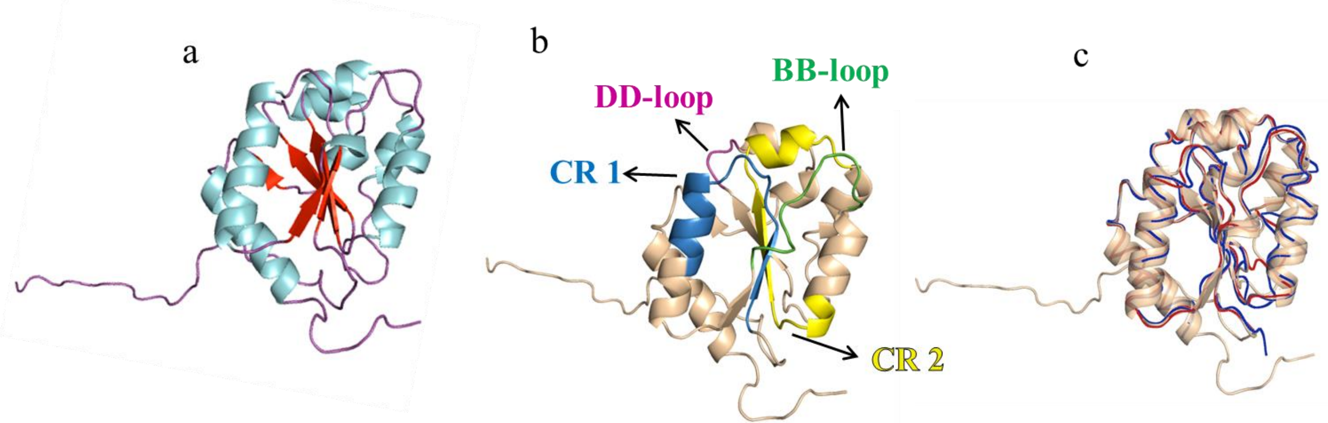

Fig. 2. LcTIR homology model. a. Ribbon display, colored for secondary structures; beta sheets:red, helices:blue, loop regions:purple. b. Conserved regions (CR) displayed on LcTIR model; CR 1 : blue, CR 2: yellow. BB-loop: green, DD-loop:pink. c. Structural alignment LcTIR and bacterial TIR domains; LcTIR shown as cartoon (wheat), PdTIR (3h16) blue ribbon and BtpA (4lzp) red ribbon.

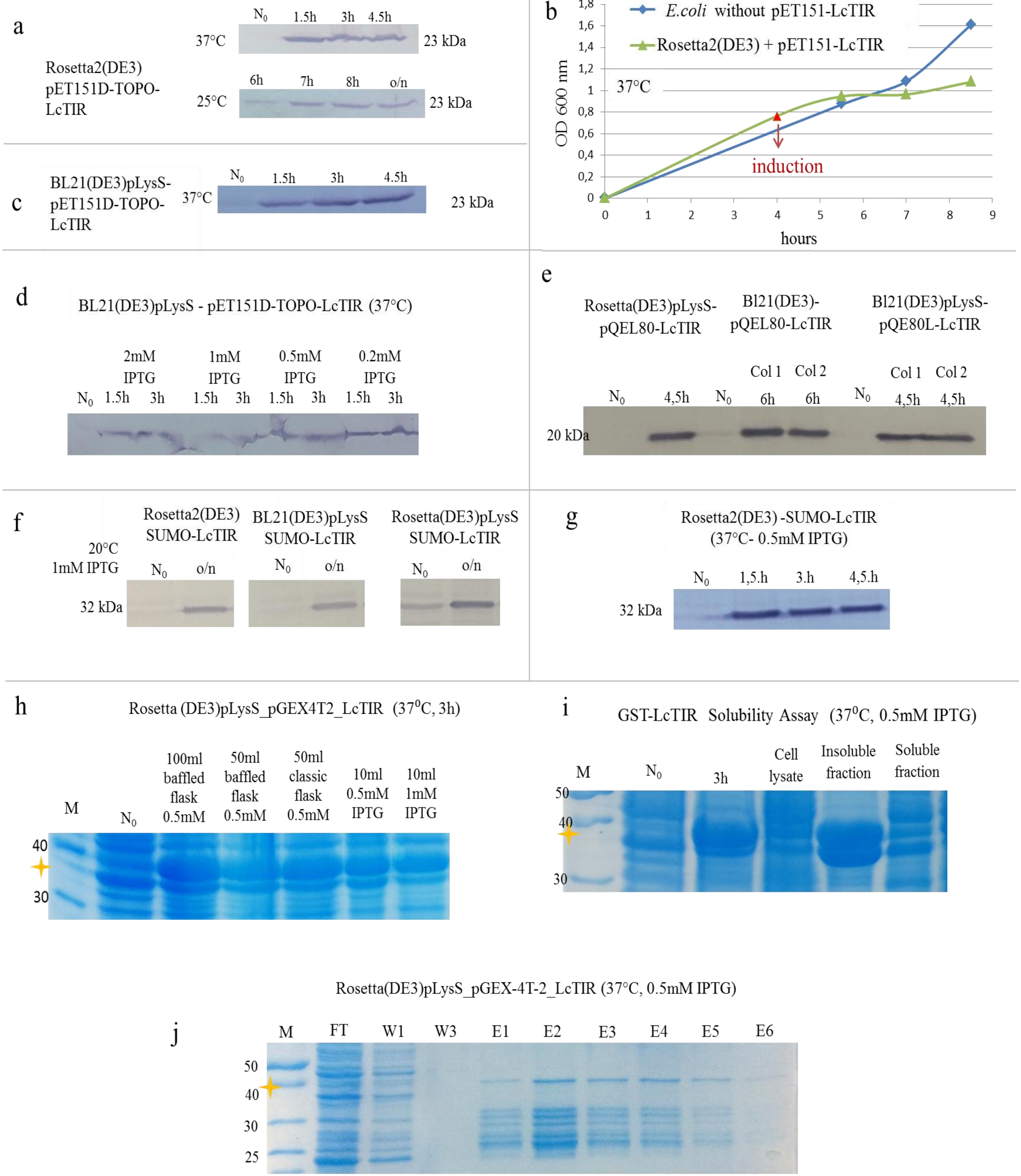

Fig. 3. Recombinant LcTIR production from different plasmids and expression hosts. a,c,d,e,f,g: Western blot detection of different His-tagged LcTIR fusion. b: Growth curve of cells expressing 6his-LcTIR (green) and cells without recombinant LcTIR plasmid (blue). h,i: Coomassie blue staining of GST-LcTIR SDS-PAGE GST-LcTIR bands are shown with a yellow star.. M: marker,N_0: before induction , o/n:overnight, Col: colony, baffled: baffled flask, classic:classical flask. j: Affinity purification fractions of GST-LcTIR analyzed on SDS-PAGE. FT: flowthrough, W:wash, E:elution fractions

Large scale protein production was done for the purpose of purification. However, most of the protein was found in the insoluble fraction; inclusion bodies; as shown by solubility test (Fig 3i). Thus, GST-LcTIR was not toxic to the cells as the protein was trapped in the inclusion bodies. Purification was performed with the small amount of soluble protein. Elution fractions contained very low amount of GST-LcTIR, not enough for further studies (Fig 3j).

For all the above mentioned recombinant plasmid-expression cell combinations, several colonies were screened but no difference was observed among them. In addition, other suggestions in order to overcome the toxicity problem; plating method (Suter-Crazzolara and Unsicker 1995), expression in terrific broth or LB broth containing 3% ethanol (Chhetri et al. 2015) were tested, with no success (data not shown).

Toxic protein is a common challenge in recombinant expression systems and various ways to solve this have been proposed (Ahmad et al. 2018; EMBL 2019; Kaur et al. 2018). In this study several methods such as, using pLysS cells, expression from weak promoter and fusing with partners were tested. There are other suggested methods such as; using pBAD promoter which with protein production is more tightly controlled (Rosano and Ceccarelli 2014), expression with other fusion partners like NusA, Trx, MBP, Fh8 (Costa et al. 2013; Rosano and Ceccarelli 2014), periplasmic expression (Bloois 2012) and using C41 (DE3) or C43 (DE3) cells for toxic protein production (Wagner et al. 2008). Also different expression systems; lactic acid bacteria, yeast or insect; can be evaluated (Song et al. 2017; Tripathi and Shrivastava 2019). Another suggestion is purification from inclusion bodies and refolding (Rosano and Ceccarelli 2014). However, it was not preferred for LcTIR protein since there is no verified method to test functional properties after refolding.

4 Conclusion

In this study, a putative TIR domain protein from a probiotic strain of *L.casei* was identified using bioinformatics methods. Sequence and structural alignments of the protein showed its similarity to other TIR domain proteins Therefore, LcTIR can be considered to have potential to mimick human TIR domains and regulate TLR signaling similar to other bacterial TIR domain proteins.

In order to investigate this potential, attempts were made to produce recombinant LcTIR from *E. coli* cells. After several different conditions, it was shown that protein has a toxic effect on cells and therefore could not be produced at high quantities. The highest amount of protein obtained was GST-LcTIR fusion; from Rosetta(DE3)pLysS cells, where nearly all the protein was found in inclusion bodies.

This study is one of the first studies on probiotic origin TIR domains, and future work will contribute to our understanding of probiotic effect on TLR signaling.

Studies are underway to investigate other cloning methods including expression as MBP fusion and recombinant expression from *Lactococcus lactis* to produce and purify LcTIR for further characterization studies. These characterization studies will focus on solving the solution structure using small angle X-ray scattering and crystal structure via X-ray crystallography. Furthermore, it will be necessary to investigate the *in vitro* interaction of LcTIR with human TIR domains using pull down assays and gel filtration chromatography.

These studies will show the functionality of the probiotic TIR domain and will pave the way for the production of targeted probiotic molecules for food and medicine applications.

Acknowledgements

This research was supported by the Scientific and Technical Research Council of Turkey, TUBITAK (Project No: 116Z299). Authors would like to thank to EMBL for pETM11-SUMO3GFP plasmid and M.Sc.Dicle Dilara Akpınar for technical assistance.

References

Ahmad I, Nawaz N, Darwesh NM, ur Rahman S, Mustafa MZ, Khan SB, Patching SG (2018) Overcoming challenges for amplified expression of recombinant proteins using *Escherichia coli*. Protein Expression Purif 144:12–18. doi:10.1016/j.pep.2017.11.005

Bloois EV (2012) Biotechnological applications of periplasmic expression in *E. coli*. Enz Eng 1:2. doi:10.4172/eeg.1000e104

Castillo NA, Perdigán G, De Moreno De Leblanc A (2011) Oral administration of a probiotic *Lactobacillus* modulates cytokine production and TLR expression improving the immune response against *Salmonella enterica* serovar Typhimurium infection in mice. BMC Microbiol 11(1):177. doi:10.1186/1471-2180-11-177

Chaudhary A, Ganguly K, Cabantous S, Waldo GS, Micheva-Viteva SN, Nag K., Hlavacek WS, Tung CS (2012) The Brucella TIR-like protein TcpB interacts with the death domain of MyD88. Biochem Biophys Res Commun 417(1):299–304. doi:10.1016/j.bbrc.2011.11.104

Chhetri G, Kalita P, Tripathi T (2015) An efficient protocol to enhance recombinant protein expression using ethanol in *Escherichia coli*. MethodsX 2:385–391. doi:10.1016/j.mex.2015.09.005

Cirl C, Wieser A, Yadav M, Duerr S, Schubert S, Fischer H, Stappert D, Wantia N, Rodriguez N, Wagner H, Svanborg C, Miethke T (2008) Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. Nat Med 14(4):399–406. doi:10.1038/nm1734

EMBL (2019) Decreasing Protein Toxicity. Available via Dialog. https://www.embl.de/pepcore/pepcore_services/index.html of subordinate document. Accesed 10 June 2019

Galdeano CM, LeBlanc ADM, Vinderola G, Bonet MEB, Perdigon G (2007) Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. Clin Vaccine Immunol 14(5):485–492. doi:10.1128/CVI.00406-06

Hoarau C, Lagaraine C, Martin, L (2006) Supernatant of *Bifidobacterium breve* induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. J Allergy Clin Immunol 50:696–702. doi:10.1016/j.jaci.2005.10.043

Jeon SG, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, Tsuji NM, Kiyono H, Ma JS, Kusu T, Okumura R, Hara H, Yoshida H, Yamamoto M, Nomoto K, Takeda K (2012) Probiotic *Bifidobacterium breve* induces IL-10-producing Tr1 cells in the colon. PLoS Pathog 8(5):1–15. doi:10.1371/journal.ppat.1002714

Kallberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, Xu J (2016) Template-based protein structure modeling using the RaptorX web server. Nat Protoc 7(8):1511–1522. doi:10.1038/nprot.2012.085

Kaplan-Türköz B (2017) A putative Toll/interleukin-1 receptor domain protein from Helicobacter pylori is dimeric in solution and interacts with human Toll-like receptor adaptor myeloid differentiation primary response 88. Microbiol Immunol 61(2):85–91. doi:10.1016/j.febslet.2013.09.007

Kaplan-Türköz B, Koelblen T, Felix C, Candusso MP, O'Callaghan D, Vergunst AC, Terradot L (2013) Structure of the Toll/interleukin 1 receptor (TIR) domain of the immunosuppressive *Brucella* effector BtpA/Btp1/TcpB. FEBS Lett 587(21):3412–3416. doi: 10.1016/j.febslet.2013.09.007

Kaur J, Kumar A, Kaur, J (2018) International Journal of Biological Macromolecules Strategies for optimization of heterologous protein expression in *E . coli* : Roadblocks and reinforcements. Int J Biol Macromol 106:803–822. doi:10.1016/j.ijbiomac.2017.08.080

Kitazawa H, Watanabe H, Shimosato T, Kawai Y, Itoh T, Saito, T (2003) Immunostimulatory oligonucleotide, CpG-like motif exists in *Lactobacillus delbrueckii* ssp. bulgaricus NIAI B6. Int J Food Microbiol 85(1):11–21. doi:10.1016/S0168-1605(02)00477-4

Marblestone JG, Edavettal SC, Lim Y, Lim P, Zuo X, Butt TR (2006) Comparison of SUMO fusion technology with traditional gene fusion systems : Enhanced expression and solubility with SUMO. Protein Sci 15:182–189. doi:10.1110/ps.051812706.for

Newman RM, Salunkhe P, Godzik A, Reed JC (2006) Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. Infection Immun 74(1):594–601. doi:10.1128/IAI.74.1.594-601.2006

Notredame C, Higgins DG, Heringa J (2000) T-coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol 302(1):205–217. doi:10.1006/jmbi.2000.4042

O'Neill LAJ, Bowie AG (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 7(5):353–364. doi:10.1038/nri2079

Radhakrishnan GK, Yu Q, Harms JS, Splitter GA (2009) Brucella TIR domain-containing protein mimics properties of the toll-like receptor adaptor protein TIRAP. J Biol Chem 284(15): 9892–9898. doi:10.1074/jbc.M805458200

Rosano GL, Ceccarelli EA (2014) Recombinant protein expression in *Escherichia coli* : advances and challenges. Front Microbiol 5:1–17. doi:10.3389/fmicb.2014.00172

Salcedo SP, Marchesini MI, Lelouard H, Fugier E, Jolly G, Balor S, Muller A, Lapaque N, Demaria O, Alexopoulou L, Comerci DJ, Ugalde RA, Pierre P, Gorvel JP (2008) *Brucella* control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. PLoS Pathog 4(2):e21. doi:10.1371/journal.ppat.0040021

Saluta M, Bell PA (1998) Troubleshooting GST fusion protein expression in *E.coli*. Life Sci News 1:1–3.

Snyder GA, Cirl C, Jiang J, Chen K, Waldhuber A, Smith PT, Durr S, Cirl C, Jiang J, Jennings W, Luchetti T, Snyder N, Sundberg EJ, Wintrode P, Miethke T, Xiao TS (2013) Molecular mechanisms for the subversion of MyD88 signaling by TcpC from virulent uropathogenic *Escherichia coli.* Proc Natl Acad Sci U.S.A. 110(17):6985–6990. doi:10.1073/pnas.1215770110

Song AA, In LL, Lim SHE, Rahim RA (2017) A review on *Lactococcus lactis*: from food to factory. Microb cell fact 16(1), 55. doi:10.1186/s12934-017-0669-x

Suter-Crazzolara C, Unsicker K (1995) Improved expression of toxic proteins in *E. coli*. BioTechniques 19(2), 202—204.

ThermoFisher (2019) GST-tagged Proteins – Production and Purification. Available via Dialog. https://www.thermofisher.com/tr/en/home/life-science/protein-biology.html of subordinate document. Accessed 15 March 2019

Tripathi NK, Shrivastava A (2019) Recent Developments in Bioprocessing of Recombinant Proteins : Expression Hosts and Process Development. Front Bioengi Biotech 7:420. doi:10.3389/fbioe.2019.00420

Turner JD (2003) A bioinformatic approach to the identification of bacterial proteins interacting with Toll-interleukin 1 receptor-resistance (TIR) homology domains. FEMS Immunol Med Mic 37(1):13–21. doi:10.1016/S0928-8244(03)00095-6

Ve T, Williams SJ, Kobe B (2015) Structure and function of Toll/interleukin-1 receptor/resistance protein (TIR) domains. Apoptosis 20(2):250–261. doi:10.1007/s10495-014-1064-2

Wagner S, Klepsch MM, Schlegel S, Appel A, Draheim R, Tarry M, Wijk KJV, Slotboom DJ, Persson JO, Gier JD (2008) Tuning *E. coli* for Membrane Protein Overexpression. Proc Natl Acad Sci U.S.A. 105(38): 14371–76. doi:10.1073/pnas.0804090105

Wang Y, Xie J, Li Y, Dong S, Liu H, Chen J, Wang Y, Zhao S, Zhang Y, Zhang H (2016) Probiotic *Lactobacillus casei* Zhang reduces pro-inflammatory cytokine production and hepatic inflammation in a rat model of acute liver failure. Eur J Nutr 55(2):821–831. doi:10.1007/s00394-015-0904-3

Zhang Q, Zmasek CM, Cai X, Godzik A (2012) TIR domain-containing adaptor SARM is a late addition to the ongoing microbe–host dialog. Dev Comp Immunol 35(4):461–468. doi:10.1016/j.dci.2010.11.013