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Cloning and cDNA Synthesis of PE & PPE Gene of Mycobacterium Strain H37rv5

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Abstract— *Pathogenic, slow-growing Mycobacterium tuberculosis and other Mycobacterium tuberculosis complex (MTBC) species include the pe/ppe genes. In the pathogen-host connection, these genes are crucial. Despite the fact that the activities of most PE/PPE family proteins are unknown, mounting evidence shows they play a role in M. tuberculosis infection. The role of PE/PPE proteins, which are thought to be involved in the ESX system's action, is investigated. During complicated host-pathogen processes, we also discuss how PE/ PPE proteins are involved in host-pathogen interactions, immune response regulation, and cell fate determination. Finally, we discuss future PE/PPE protein research priorities as well as how present information might be used to develop more accurate diagnostics and vaccines for worldwide TB control. The pe and ppe genes, which are thought to be critical in TB pathogenesis, are only found in mycobacteria. However, nothing is known about how these genes' expression is regulated. Understanding the regulatory control of genes present only in mycobacteria, such as the pe and ppe gene families, might help researchers figure out why the illness is so effective. A transposon mutagenesis method was employed to better understand pe and ppe control. Rv1403c, a previously unknown transcriptional regulator, was discovered as a consequence of this study.*

Keywords— ***Cloning, Mycobacterium tuberculosis, H37Rv.***

I. INTRODUCTION

Mycobacterium tuberculosis (M. tb) is a TB-causing Mycobacteriaceae pathogenic bacteria. Due to the presence of mycolic acid, which was first identified by Robert Koch in 1882, M. tuberculosis has a distinctive waxy coating on its cell surface. M. tuberculosis may look Gram-negative or Gram-positive due to this coating, which makes the cells resistant to Gram staining. Instead, M. tuberculosis is detected using a microscope using acid-fast stains like Ziehl–Neelsen or fluorescent stains like auramine. To live, M. tuberculosis has an aerobic physiology that needs a lot of oxygen. It is mostly a mammalian respiratory disease affecting the lungs. The most common tuberculin tests include the tuberculin skin test, acid-fast stain, culture, and polymerase chain reaction. M. tuberculosis causes tuberculosis, which is an airborne infection. One-third of the world's population is thought to be latently infected with M. tuberculosis, with three million people dying from the illness each year. The growth of drug-resistant strains is exacerbating the problem. Despite substantial research, the pathophysiology, virulence, and durability of M. tuberculosis infection remain unclear. A significant area in TB pathogenesis is the discovery of M. tuberculosis virulence factors that are related to human sickness. Weakening mycobacteria strains might be utilized to uncover genes that are critical for pathogenesis. The most well-studied virulent laboratory strain of M. tuberculosis is H37Rv. It is infectious, but it does not replicate in macrophages, and it is similar to M. tuberculosis in that it does not replicate in macrophages during latent infection. The causes for the reduced pathogenicity are unclear at this time. The genetic and behavioral differences between these strains have been widely researched in the hopes of discovering virulence factors. M. tuberculosis H37Rv has a

faster in vivo growth rate than M. tuberculosis H37Ra, and the better intracellular survival gene and exported repetitive protein genes help M. tuberculosis survive in macrophages. Genomic alterations such as insertions, deletions, and single nucleotide polymorphisms have been identified in both virulent and attenuated Mycobacteria, in addition to recognized virulence factors. Other studies looked at the phenotypic consequences and changes in gene expression independent of the genetic differences between H37Ra and H37Rv. In M. tuberculosis H37Ra, several genes were identified to be repressed. As a consequence, although finding genes connected to M. tuberculosis virulence is critical, the gene products at the protein level that are responsible for virulence should also be prioritized. For identifying whether genes are genuinely expressed, proteomics characterization is a significant adjunct to genomics. Thanks to enhanced label-free processes, proteomic technologies have recently taken on a new dimension. Proteins that are differentially expressed, such as proteins that are up-regulated or down-regulated, as well as proteins that are impacted by shaking culture conditions, may be found in the BCG proteome. This is something that no amount of genetic study will be able to uncover. Furthermore, proteomics of M. tuberculosis H37Rv revealed six open reading frames that had not been predicted by genomics. Differences in protein composition between attenuated and virulent M. tuberculosis strains may help researchers design novel vaccines and therapies. M. tuberculosis is an intracellular pathogen found in the host's macrophages. When M. tuberculosis infects host cells, it uses membrane and surface proteins to communicate with them. These proteins are hypothesized to be involved in intracellular proliferation and the bacterial response to microbicidal actions in the host. M. tuberculosis possesses a real outer membrane, which adds to the complexity of bacterial-host interactions and gives important information on anti-mycobacterial therapeutic sensitivity.

II. REVIEW OF LITERATURE

GENERAL FEATURES OF PP AND PPE GENES

It's vital to understand the features that determine a protein's function before looking at it. Separate the protein of interest into the conserved PE or PPE domains and the highly variable C-terminal domains as a first step in investigating PE/PPE proteins. Despite the fact that their function is mostly unclear, molecular and biochemical investigations have substantially enhanced our knowledge of the PE/PPE domains. All PE proteins have a highly conserved N-terminus, which consists of roughly 100 amino acids organized in a helix-turn-helix sequence. The ten N-terminal amino acids include the pro-glu residues that give the protein its name, whereas the PE-C-terminus domain contains the conserved YxxxD/E TypeVII secretion signal. This secretion signal is necessary for the secretion of these proteins and may have a role in TypeVII secretion systems' substrate identification. PPE proteins share a lot of characteristics. The pro-pro-glu motif may be found towards the N-terminus of the PPE domain, which is generally roughly 180 amino acids long. Because PE and PPE proteins' amino acid sequences are not always similar, this should not be used as the only criteria for identifying PPE proteins. All PPE proteins contain the WxG motif between the second and third alpha helix, but they do not have the YxxxD/E secretion. PE and PPE proteins were shown to be secreted as heterodimers in all studies with sufficient biochemical characterisation. The three N-terminal alpha-helices of the PPE protein form hydrophobic contacts with the homologous PE protein. The conserved WxG of the PPE-protein and the YxxxD/E of the PE protein are near together in this heterodimeric structure, and these sequences are expected to create a composite recognition structure for TypeVII secretion. This PE/PPE complex resembles Esx protein heterodimers, such as EsxA-EsxB (ESAT-6/CFP10), or even Esx protein homodimers in Staphylococcus aureus. PE/PPE proteins have a structure that is very similar to the ESX-1 secretion-associated proteins (esp) family's EspA/C and EspE/F heterodimers, as well as EspB. The helix-bundle structure and composite secretion signal are unique features of PE/PPE heterodimers and other TypeVII-associated substrates, according to these studies.

SUBCLASSIFICATION OF PE/PPE PROTEINS

The C-terminal domains of PE/PPE proteins contain the bulk of the protein's variation and, potentially, the most fascinating properties. By splitting PE/PPE proteins into subgroups, these C-terminal domains may also assist researchers build viable study hypotheses and give functional or evolutionary insight. The polymorphic gc-rich sequences (PGRS) are the most common kind of PE protein, accounting for 65 of the 99 found in the M. tuberculosis reference genome H37Rv. The PE PGRS subgroup is the most challenging to analyze, with gccontents over 80% in certain instances, hydrophobic glycine-rich repetitions, and enormous sizes (up to 1400 amino acids). Based on C-terminal sequences, the majority of the PE-proteins cannot be clearly subgrouped into separate functional groups. The PE-domain sequences, on the other hand, and their genetic background provide further information. Gey van Pittius et al. classified PE-proteins into five sublineages based on their sequence phylogeny. Sublineage I and II relate to the ancestral pe-genes encoded by ESX-1 (PE35) and ESX-3 (PE5), respectively. Sublineage III, which comprises the ESX-5 substrate PE25 as well as the esx2-localized PE36,

is the most loosely defined sublineage. Sublineage IV refers to a subset of secreted PE proteins from ESX-5, whose genes are frequently bicistronic transcripts with PPE-SVP genes. All PE PGRS proteins are found in Sublineage V, as well as a variety of proteins with various C-terminal domains, including putative hydrolases, lipases, and cutinases, the most well-known of which is LipY. Phyre2 was used to identify a variety of additional PE proteins (PE1, PE3, PE4, and PE16) as potential hydrolases (three closest hits with 99 percent confidence: PDB 3AJA, 1QOZ, and 3HC7).

The PF08237 domain, dubbed the 'PE/PPE domain' by Adindla and Guruprasad, is a hydrolase-like fold. The PFAM08237 domain has also been discovered in the PPE proteins PPE28, PPE42, and PPE63, as well as non-TypeVII substrate proteins, suggesting that these putative enzymatic domains may have evolved into TypeVII substrates via genetic recombination with the pe and ppe genes. PE2, which also contains PFAM08237, is unlikely to be a true PE protein since it lacks any of the PE, YxxxD/E, or helix-turn-helix domains. In M. tuberculosis, LipY is a PE protein, but in M. marinum, it is a PPE protein. According to the finding of these ESX-5-secreted lipases, putative hydrolases, and cutinases, TypeVII-secreted PE and PPE proteins may be involved in cell envelope production and homeostasis, as well as lipid-based nutrition absorption.

Within the PPE protein, there are even more subclasses. The PPE-PPW proteins are the first well-identified subclass, aside from the ESX-1-specific (PPE68) and ESX-2-specific (PPE69) PPEs. Several proteins have a PxxPxxW amino acid motif between 10 and 30 amino acids from the C-terminus. M. tuberculosis H37Rv has ten PPW proteins, despite the fact that PPE48 and PPE67 are truncated proteins that may be detrimental. With 26 members, the PPE-SVP proteins are the largest subgroup of PPE proteins, named after the conserved SVP (Serine-Valine-Proline) amino acid sequence present in their C-terminal domain. PPE50 is most likely a truncated protein, and PPE9 is not a true PPE-SVP because it only includes a PPE domain of 180 amino acids, although it was identified as such by phylogenetic analysis. The size of the remaining 24 PPEs varies from 350 to 468 amino acids.

Finally, PPE-MPTR proteins that include the Major Polymorphic Tandem Repeat (MPTR) are the most recently produced subclass of PPE proteins. The MPTR-repeat is made up of NxGxGNxG motif repeats that vary in size and form. While some PPE-MPTR proteins are small, others in the family have released proteins that may be as large as 3716 amino acids.

SECRETIONS OF PP AND PPE GENES

To begin with, although all pe/ppe genes are often omitted from bioinformatic databases, in the great majority of situations, this is an unnecessarily strict approach. Even short-read sequencing techniques can successfully map practically all pe/ppe genes if only pe pgrs and ppe-mptr genes/transcripts are excluded, thanks to paired-end technology and longer read lengths. Understanding a PE/subgroup PPE's may also be a great place to start when guessing on the most probable route of secretion, and may even indicate roles or redundancy. Individual proteins, even within the groupings indicated below, may behave quite differently from one another.

As a consequence, subgrouping may aid in the generation of testable concepts, however these should always be experimentally confirmed before drawing firm conclusions. PPE4, which is genetically encoded inside the ESX-3 chromosomal region and is likely secreted through this secretion route with its secretion partner PE5, is the best-studied member of the PPE-PPW proteins. Mycobactin-mediated iron absorption requires these proteins. In one experiment, the PPE-PPW PPE20 and its cognate partner PE15 were discovered to be ESX-3 substrates. PPE36 and PPE37, two more PPW proteins, are known to have a role in iron homeostasis through heme-iron acquisition activities. These results, together with a sequence study of the EspG-binding region, indicate that PPE-PPW proteins use ESX-3 rather than ESX-5 for translocation.

Experimental investigations show that ESX-5 secretes all PPE-SVP proteins. PPE-SVP proteins are often transcribed as bicistronic transcripts including a sublineage IV PE-protein and two Esx-proteins. These operonic clusters are most likely the first pe/ppe genes transferred from the ESX genetic locus when the ESX-5 system was introduced into the slow-growing mycobacteria's most recent common ancestor. PE/PPE proteins are encoded by these operons. These operons generate PE/PPE proteins that are exclusively secreted as PE/PPE heterodimers and are not promiscuous with other secretion partners. The PE8-PPE15-EsxI-EsxJ operon, which has been discovered to increase protein secretion, is an outstanding example of such an operon structure.

H37R5 STRAIN

Mycobacterium tuberculosis strain H37Rv is the most studied TB strain in research facilities. In 1905, Dr. Edward R. Baldwin became the first to isolate it. The strain was discovered in a 19-year-old patient with chronic pulmonary tuberculosis at the Trudeau Sanatorium in Saranac Lake, New York. It was initially identified as strain H37, and it was maintained alive at the Trudeau Sanatorium for many years using serial culture transfer. It was observed that the virulence of it in animal models varied according on the

substrate it was cultivated on throughout time. After that, strains with different levels of virulence were produced on purpose, with H37R being less virulent after growing in acidic media and H37S being more virulent after growing in alkaline media in guinea pigs (with R standing for resistant to environment, and S for sensitive to environment). The more virulent strain was subsequently given the name H37Rv, with R designating rough morphology and v denoting virulent. The strain was used in a number of laboratory studies before finally becoming the TB standard. Koch first identified Mycobacterium TB as the cause of tuberculosis in 1892, but the strains he studied were not preserved, and it is unknown what happened to them. Koch discovered Mycobacterium tuberculosis as the cause of tuberculosis in 1892, but the strains he studied were not preserved, so it's unclear how closely H37Rv is related to those strains. H37Rv has remained the most widely used tuberculosis strain in laboratories, and its whole genome was revealed for the first time in 1998. It does not, however, have some characteristics found in recent clinical isolates, such as the capacity to produce caseous necrosis in rabbits. It does not, however, have some characteristics found in recent clinical isolates, such as the capacity to produce caseous necrosis in rabbits. Strains produced from H37Rv in different labs have also been found to evolve over time, with one study of six strains revealing between five and 10 polymorphisms per strain. These comprised IS6110 transposable element insertions and deletions that changed the spoligotype of the strain. The authors of the research cautioned against using all H37Rv strains as a reference since there might be significant differences depending on the laboratory where it is housed.

ROLE OF PP AND PPE PROTEINS IN HOST PATHOGEN INTERACTION

Table 1 summarises the function and location of PE/PPE proteins based on the distinct stages of contact with the host, and Figure 1A highlights the fascinating roles: I surviving intracellular stress, phagocytosis, and phagolysosome maturation; (ii) cell fate determination

ROLE OF PPE PROEINS IN INTERACTION WITH HOST CELLS AND IMMUNE REGULATION

PE/PPE proteins can interact directly with their host targets after being exposed to the surface or secreted into the extracellular environment. Some proteins are said to bind with receptors on macrophages' surfaces, such as TLR2/4, triggering downstream signalling cascades. TLR4 interacts with PE9-PE10 and PE PGRS5 to trigger downstream signalling and alter cytokine production. PPE26, PPE32, PPE57, PPE65, PE PGRS33, and PE PGRS11 are only a few of the PE/PPE proteins that can interact with TLR2. PPE18 has been shown to promote IL-10 release, which may generate a Th2 response when it interacts with TLR2, as well as limit the generation of NF-kB/rel-mediated pro-inflammatory cytokines by upregulating suppressor of cytokine signalling 3 protein (SOCS3). PE PGRS17 was also discovered to mature DCs via TLR2 and trigger host cell death and cytokine production via Erk kinase, resulting in improved intracellular survival. PE/PPE protein binding to cell surface receptors triggers downstream signalling pathways such as NF-kB and MAPK (p38, JNK, and ERK), which influence cytokine production and result in a pro-inflammatory or anti-inflammatory response. th was overexpressed by PPE27. The PPE27 overexpressed strain had a stronger capacity to produce NO and reduce IL-6 production, which was blocked by inhibitors of NF-kB, p38, and ERK. PPE39, a PE/PPE protein identified in the hypervirulent strain Beijing/K, was found to mature DCs and stimulate the Th1 immune response via NFkB and MAPK, a TLR4 agonist. PE13, PE27, PPE26, PPE32, PPE44, PPE57, PE PGRS11, and PE PGRS17 are among the proteins that modulate the cytokine profile via NF-kB and MAPK signalling. It's also been proposed that PE/PPE proteins have an influence on mycobacterial invasion and macrophage phagocytosis. The invasion efficiency of the PPE38- mutant of Mycobacterium marinum was much greater, but the phagocytosis ratio of PPE29 mutants was lowered as predicted. Another need for bacterial invasion is adhesion to the cell surface. PE11 knockdown strains have been shown to increase the synthesis of fibronectin attachment protein, which aids in the attachment to the host extracellular matrix. PE PGRS60 has the ability to bind to fibronectin, resulting in increased adhesion and invasion.

ROLE OF PP/PPE PROTEINS IN INTRACELLULAR SURVIVAL

Pathogens adapt to the intracellular environment, such as low pH, reactive oxygen, and nitrogen species, as they enter macrophages, and so create their own niche. PPE60 and PE13 can also boost intracellular survival by increasing cell resilience to low pH, surface stressors, and antibiotic exposure. PPE11 also maintains a high bacterial load in mouse tissue, exacerbating organ disease, and increases early bacterial survival rate under circumstances comparable to the internal macrophage environment, such as the presence of lysozymes, acidity, and active nitrogen intermediates (RNI).

Mtb lives in macrophages by blocking phagosomal acidification and phagosome-lysosome fusion after it has acclimated to the harsh circumstances. The knockout strains PE PGRS30 and PE PGRS47 lost their capacity to suppress phagosome fusion. Similarly, overexpression of PE PGRS62 prevents phagosome maturation. PPE25 transcription is increased during phagocytosis, and the

PPE25 mutant strain loses its capacity to proliferate within macrophages and prevents phagosome-lysosome union. Furthermore, a PPE60-overexpressing strain has been discovered to boost intracellular survival and change cell destiny to pyroptosis, a newly described form of programmed cell death that is linked to intracellular growth limitation, heightened host immunological response, and IL-1b and IL-18 maturation.

- Pathogenic, slow-growing Mycobacterium tuberculosis and other M. tuberculosis complex (MTBC) species include the pe/ppe genes. These genes are important in the interactions between the host and the pathogen. Despite the fact that the function of most PE/PPE family proteins is unknown, mounting evidence shows that they are involved in M. tuberculosis infection.
- PPE22 At 30 days, mass spectrometry identified it in M. tuberculosis H37Rv-infected guinea pig lungs, but not at 90 days. M. tuberculosis H37Rv whole cell lysates, but not the culture filtrate or membrane proteinfraction, were identified by mass spectrometry.
- Rv1403c Intermediary respiration and metabolism Mutant In vitro development of H37Rv in a MtbYM rich media requires this non-essential gene. H37Rv tuberculosis Rv1403c-Rv1404-Rv1405cm utant develops at a slower rate than wild-type

1. **PPE 22**

Protein Sequence

MDFGALPPEVNSGRMYCGPGSAPMVAAASAW NGLAAELSVAAVGYERVITTLQTEEWLGPASTL MVEAV

APYVAWMRATAIQAEQAASQARAAAAAYETA FAAIVPPPLIANRARLTSLVTHNVFGQNTASIAA TEAQYA EMWAQDAMA MYGYAGSSATATKVTPFAPPPNTTSPSAAATQL SAVAKAAGTSAGAAQSAIAELIAHLPN

TLLGLTSPLSSALTAAATPGWLEWFINWYLPISQ LFYNTVGLPYFAIGIGNSLITSWRALGWIGPEAA EAAAA APAAVGA

AVGGTGPVSAGLGNAATIGKLSLPPNWAGASPS LAPTVGSASAPLVSDIVEQPEAGAAGNLLGGMP LAGS

GTGTGGAGPR YGFRVTVMSR PPFAG

2. **Rv1403c**

Protein sequence:

MTVYTPTSERQAPATTHRQMWALGDYAAIAEE LLAPLGPILVSTSGIRRGDRVLDVAAGSGNVSIP AAMAG

AHVTASDLTPELLRRAQARAAAAGLELGWREA NAEALPFSAGEFDAVLSTIGVMFAPRHQRTADE LARVC

RRGGKISTLNWTPEGFYGKLLSTIRPYRPTLPAG APHEVWWGSEDYVSGLFRDHVSDIRTRRGSLT VDRFG

CPDECRDYFKNFYGPAINAYRSIADSPECVATLD AEITELCREYLCDGVMQWEYLIFTARKC

III. METHODOLOGY

1. Selection of M.tb strain, Antigens and retrieval of protein sequences
2. To check the sub localization of protein
3. To design Primer for Gene PPE 22 and Rv1403c
4. PCR Reaction of Genes
5. PCR Product Separation by Gel Electrophoresis
6. Extraction OF Amplified Products
7. Preparation of Competent cells
8. Insert of Gene of Interest in competent cells
9. Blue and white selection of Transformants and non-transformants

Selection of M.tb strain, Antigens and retrieval of protein sequences.

M.tb strain is selected on the basis of previous work related to selection of vaccine candidate that is lab strain H37Rv .Antigen can be selected on the basis of which protein show up regulation. Antigen choosed from the H37Rv and its basic information like Gene Sequence , protein Sequence, molecular weight , promoter etc. were find through mycobrowser application.(..\mycobrowser.html).

To check the sub localization of protein

To determine whether or not proteins are present within the cell. psortb (..psort b.html) is a programme that evaluates the amino acid composition of a query protein sequence and compares it to proteins of known localization, the presence of a signal peptide, transmembrane alpha-helices, and motifs related to specific localizations.

To design Primer for Gene PPE 22 and Rv1403c

Primer is required for the amplification of Rv2628 in PCR. Primer is designed on the basis of some properties like length (17-30 bp), GC content (more than 50%), melting temperature difference between forward and reverse primer is less than 5°C, hair loop formation, self dimmers should be checked. Different software are used to make primers like Primer 3, oligoanalyzer(IDT) (..\oligoanalyzerIDT.html) to check whether designed primers at different parameters. After primer designing add the restriction endonuclease which is non cutter for Rv2628 (..\NEB.html) in the primers for further cloning process.

Designing primers PPE 22

- **Forward Primer sequence (5' – 3')**

  ATG GAT TTT GGG GCG TTG CCA CCG

  Length: 24

  GC content: 58.3%

  Melting temperature: 64.7 ºC

  Melting temperature of hairpin loop structure: 47.9ºC

- **Reverse Primer sequence (5' – 3')**

  TTA TCC GGC AAA CGG CGG CCG

  Length: 21

  GC content: 66.7%

  Melting temperature: 65.4 ºC

  Melting temperature of hairpin loop structure: 57.1 ºC

Primer Designing Rv1403c

Forward primer: 5' ATGACTGTCTACACACCCACC 3'

GC content: 52.4 %

Melting temperature: 56.5 ºC

Hairpin: Tm = 20.4◦C

Self-dimer: no significant self-dimers

Reverse primer: 5' TCAACACTTCCGGGCGGT 3'

GC content: 61.1 %

Melting temperature: 59.7 ºC

Hairpin: Tm = 31.6◦C

Self-dimer: Only 1 significant self-dimers

PCR Reaction of Genes

The polymerase chain reaction (PCR) is a technique for producing vast amounts of DNA.

• A DNA target (100-35,000 bp in length).

• Two complementary primers (synthetic oligonucleotides of 17-30 nucleotides length) surrounding the target DNA.

• dATP, dCTP, dGTP, and dTTP are the four deoxyribonucleotides.

• A DNA polymerase that can endure temperatures of up to 95 degrees Celsius

• Denaturation: When the temperature is raised to around 95°C for 1 minute, the DNA denatures and the two strands split.

• Renaturation or annealing: The primers base pair with complementary areas flanking target DNA strands when the temperature of the mixture is gently lowered to around 55°C.

Synthesis

The 3'-hydroxyl end of each primer is where DNA synthesis begins.

- The primers are lengthened by connecting complimentary bases on DNA strands.

- The synthetic process is similar to leading strand DNA replication.

- DNA polymerases need that the temperature be kept at a certain level.

-Taq DNA polymerase has an optimal temperature of 75°C, while E.coli DNA polymerase has an optimum temperature of 37°C.

-Raise the temperature to to 95°C to cease the process. -Each PCR cycle lasts roughly 3-5 minutes.

Reaction Mixture Preparation

NFW- 26.7 µl

Phusion Buffer- 10µl

10mM DNTP- 1µl

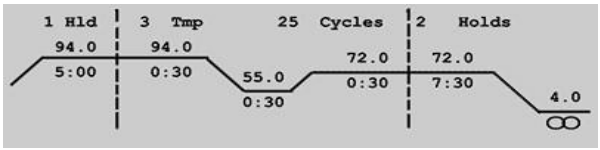
10µM FP – 2.5µl

10µM RP- 1.5µl

DMSO- 1.5µl

Phusion Polymerase- 0.8µl

Template – 5µl



PCR Product Separation by Gel Electrophoresis

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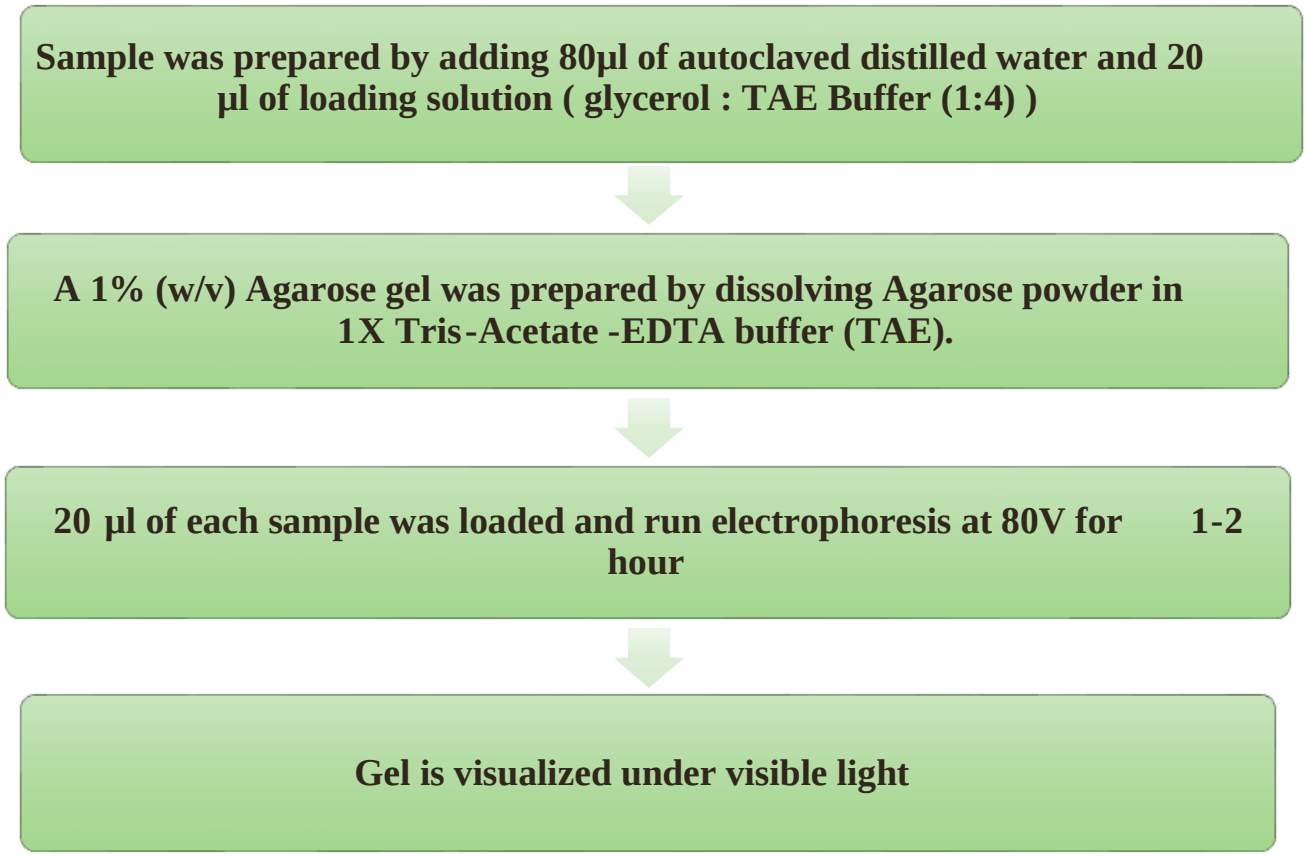

Extraction OF Amplified Products

- Using a clean scalpel or razor blade, remove the gel slice containing the DNA fragment. To reduce the gel volume, cut as near to the DNA as feasible. Place the gel slice in an eppendrof and that has been pre-weighed, and then weigh it.
- To the gel slice, add a 1:1 volume of binding buffer (volume: weight).
- Incubate the gel mixture for 10 minutes at 50-60°C, or until the gel slice is fully dissolved. To speed up the melting process, invert the tube every few minutes. Check to see if the gel has totally dissolved. Before placing the gel mixture onto the column, vortex it briefly.
- Fill the Gene Jet Purification column with up to 800 l of the solubilized gel solution. For 1 minute, centrifuge at 10,000 rpm. Remove the flow-through and re-insert the column into the collecting tube.
- Quantify the sample with Nanodrop before moving on to the next step.

Preparation of Competent cells

- Isolate E.coli DH5 colonies on LBM Plates (without ampicillin) and incubate at 37°C overnight (16-20hours).
- Collect cells from a single colony with a sterile inolculating loop and inoculate 50 ml sterile 1X LBM Broth to grow at 37°C overnight in an incubator shaker. To equilibrate the temperature of the medium, place 2 flasks of 250 ml of 1X LBM in the incubator.
- Fill each 250 mL flask with 25 mL of the overnight culture. To equilibrate the temperature of the medium, place another flask of 150 ml 1X LBM in the incubator. The cultures should be grown to an OD650 of 0.2. In each flask, add 75 mL of equilibrated 1X LBM and incubate for 30 minutes.

- Pellet the cells in a cold autoclaved eppendroff and centrifuge for 10 minutes at 5000 rpm. Resuscitations can then be done in the same bottle.
- Finally, place the tubes holding the cells on ice in the cold chamber.
- Decant the supernatant and resuspend the cells in 100mM Mgcl2 at freezing temperature for 5 minutes. Transfer the cells to sterile centrifuge tubes that have been pre-chilled, then centrifuge at 4000rpm for 10 minutes at 4°C.
- Drain the supernatant and resuspend the cells in ice cold 100mM Cacl2, then incubate for 20 minutes on ice. Pellet the cells by centrifuging them for 10 minutes at 4°C at 4000rpm.
- Drain the supernatant and resuspend the cells in a mixture of 85 percent v/v Cacl2 and 15% v/v glycerol. And I kept it in an eppendorf freezer at -80°C for later use.

Insert of Gene of Interest in competent cells

- Put the competent cells on ice to thaw.
- In a 1.5 ml microcentrifuge tube, chill approximately 5ng (2l) of the ligation mixture.
- Toss in 50 litres of competent cells with the DNA. Pipette up and down 4-5 times to gently mix the cells; do not vortex.
- Freeze the mixture for 30 minutes. Do not combine.
- Heat shock for 30 seconds at 42°C. Do not combine.
- Fill the tube with 950 l of room temperature medium.
- Preheat the oven to 37°C and bake the tube for 60 minutes. Rotate or shake vigorously.
- Preheat the selection plates to 37°C.
- Cover the plates with 50-100 l of the cells and ligation mixture.
- Incubate at 37°C overnight.

Blue and white selection of Transformants and non-transformants

- Prepare and autoclve LB Agar. Reduce the temperature of the autoclaved growth medium agar to 50°C.
- Make an IPTG solution of 100mM and an X-Gal solution of 20mg/ml.
- To attain a final concentration of 1mM, add 10l X-Gal solution per 1 ml medium and 10l per ml media.
- Add ampicillin as a screening antibiotic.
- Pour the mixture onto the dishes and let aside to cool to room temperature.
- As needed, disperse the changed competent cells.

IV. FUTURE APPLICATIONS OF PE/PPE FAMILY PROTEINS IN TB VACCINE DESIGN AND DIAGNOSTIC TOOL DEVELOPMENT

Although serological antibody tests are commonly used, there is no gold standard for TB diagnosis. PE35, an RD1-encoded antigen, can tell the difference between pulmonary and extrapulmonary tuberculosis patients and healthy BCG-vaccinated people. Another noteworthy example is PPE17, whose N-terminal elicits a significant immunogenic response and has more potential to be a sero-diagnostic marker than full-length PPE17, which can screen latently infected individuals. PPE2 might be used as a serodiagnostic marker to identify extrapulmonary and smear-negative pulmonary patients. The examination of IFN-g T cell responses elicited after infection indicated the highly immunogenic features of PE/PPE proteins. PE18, PE19, PPE25, PPE26, and PPE27, which are CD4+-specific epitope-rich PE/PPE proteins, are strong inducers of cell-mediated immune responses. During human and bovine infection, Vordermeier et al. looked at cellular immune responses to a panel of 36 PE/PPE proteins and found that several of them were key targets of the cellular immune response to TB. PPE68 epitopes that are HLAA*0201-restricted also induce a strong cellular response. In addition, during intradermal testing, the PE5 protein and EsxI have been shown to be a diagnostic antigen for bovine TB. In the IFN-g releasing test for identifying active TB, a combination of PPE57 can improve the sensitivity of ESAT-6 or CFP-10. PE/PPE proteins may be superior diagnostic and vaccine options for intracellular survival of bacteria due to the more cellular immune response. PE/PPE proteins are also polymorphic among clinical isolates and can be resistant to degradation, which limits MHC processing. Surprisingly, researchers discovered that the PPE18 protein, which increased IL-10 production while inhibiting the inflammatory response, may be investigated as a treatment for sepsis caused by excessive inflammatory reactions. The utilisation of immunodominant epitopes of PE/PPE proteins or thorough characterization of candidates may make vaccine production easier.

V. DISCUSSION

PE/PPE family has been regarded as unique to mycobacteria, especially pathogenic species, since its discovery over 20 years ago. PE/PPE protein expression is now well-established as being connected to ESX gene clusters, according to several studies. Our understanding of the biological role of individual PE/PPE proteins has greatly improved as our understanding of the ESX system has improved. Furthermore, structural biology research has begun to unravel and explain the functions of protein complexes involved in PEPPE and ESX secretion. PE/PPE proteins' function and structure, on the other hand, are significantly less well studied than other mycobacterial proteins. The structure of PE/PPE proteins and their interactions with ESX systems will be crucial in gaining a better understanding of how the PE/PPE protein family, in conjunction with the ESX secretion system, contributes to Mtb pathogenicity. This is critical for learning more about mycobacteria's virulence methods, and it might lead to the discovery of new antimycobacterial targets.

Another characteristic of PE/PPE proteins is that they are frequently discovered as co-operonic pairs comprising primarily one PE- and one PPEcoding gene, whose products interact and are thought to assemble as heterodimers. As in the cases of PPE41 and PE25, PE35 and PPE68, and PE19 and PPE51, such interactions have been predicted using bioinformatic methods and validated by experimental data. PPE51 deletion caused Mtb cells unable to proliferate in propionamide, glucose, or glycerol, according to Korycka-Machaa et al. Mtb also requires several PE/PPE proteins during Mg2+ and PO32 restriction, such as PE20/PPE31 and PE32/PPE65. PPE36/PPE62 and PPE37 are required for Mtb growth and heme iron accumulation. In addition, mutant PPE51 and PE19 bacteria showed resistance to 3bMP1, a tuberculosis-fighting chemical. These findings show that at least some PE/PPE proteins operate as solute-selective pores, enabling exogenous chemicals or nutrients necessary for proliferation to pass through. Thus, concentrating on pe/ppe family genetic alterations, which are frequently overlooked when evaluating next-generation sequencing data of clinically drug-resistant populations, may aid in the discovery of antituberculosis drug resistance mechanisms. In conclusion, we anticipate that research into the PE/PPE family will continue to be quite busy, with many fascinating aspects still to be found.

VI. CONCLUSION

Mycobacteria have a strong protective outer coating that protects them from the elements both outside and within the host. However, the bacteria must produce proteins over this outer barrier in order to cause illness. Mycobacteria have type VII secretion systems that help them do this. The ESX-5 secretion system, for example, is found only in the group of slow-growing mycobacteria, which includes the majority of harmful species. The ESX-5 system is required for mycobacteria development, according to this research. The ESX-5 system is required for mycobacteria development, according to this research. The ESX-5 system was no longer required for development when we created a 'leaky' outer membrane by interfering with the outer membrane's formation or adding an outer membrane porin. We also discovered that ESX-5 promotes fatty acid absorption, implying that ESX-5 substrates can build particular transport networks or holes in the outer membrane essential for nutrient uptake. Understanding the involvement of ESX-5 in outer membrane permeability sheds light on a key distinction between fast- and slow-growing mycobacteria. Because most pathogenic mycobacteria are slow-growing, we can better grasp what mycobacteria need to cause disease.

REFERENCES

[1] Ates LS, Ummels R, Commandeur S, van de Weerd R, Sparrius M, Weerdenburg E, Alber M, Kalscheuer R, Piersma SR, Abdallah AM, Abd El Ghany M, Abdel-Haleem AM, Pain A, Jiménez CR, Bitter W, Houben EN. Essential Role of the ESX-5 Secretion System in Outer Membrane Permeability of Pathogenic Mycobacteria. PLoS Genet. 2015 May 4;11(5):e1005190. doi: 10.1371/journal.pgen.1005190. Erratum in: PLoS Genet. 2015 May;11(5):e1005270. van der Weerd, Robert [corrected to van de Weerd, Robert]. PMID: 25938982; PMCID: PMC4418733.

[2] Samantaray, M. U. S., & Santra, M. P. (2021). Human Adenovirus Serotypes Efficiently Transducing HEK293 Cells: An In Vitro Propagation of HAdv. International Journal for Research in Applied Sciences and Biotechnology, 8(5), 17-21. https://doi.org/10.31033/ijrasb.8.5.3

[3] Mr. Utkalendu Suvendusekhar Samantaray, & Ms. Swapnarani Sethi. (2021). Isolation and Biochemical Characterization of Indole-3-Acetic Acid (IAA) Produced in Pseudomonas Sp. Isolated from Rhizospheric Soil. International Journal for Research in Applied Sciences and Biotechnology, 8(4), 1-14. https://doi.org/10.31033/ijrasb.8.4.1

[4] Mr. Utkalendu Suvendusekhar Samantaray, & Ms. Tanima Singh. (2021). Optimization of Modified Murashige and Skoog (MS) Medium Promoting Efficient Callus Induction in Zinnia elegans. International Journal for Research in Applied Sciences and Biotechnology, 8(3), 202-211. https://doi.org/10.31033/ijrasb.8.3.25

[5] Samantaray, M. U. suvendusekhar, & Tripathy, M. S. (2021). Quantitative Analysis of Nutrients and Biochemical Evaluation in Pomfret Fish (Pampus argenteus) During Frozen Storage. International Journal for Research in Applied

Sciences and Biotechnology, 8(3), 180-195. https://doi.org/10.31033/ijrasb.8.3.23

[6] Samantaray, M. U. suvendusekhar, & Patro, M. A. (2021). Comparative Study of Antioxidant Activity and ROS Production Profile among Clarias batrachus, Cirrhinus reba, Channa punctatus (Freshwater Species). International Journal for Research in Applied Sciences and Biotechnology, 8(3), 160-179. https://doi.org/10.31033/ijrasb.8.3.22

[7] Samantaray, M. U. S., & Sahu, S. (2021). Characterization and Cellulase Production Activity of different Aspergillus Sp. Isolated from "Puri, Odisha" Marine Fungal Strains. International Journal for Research in Applied Sciences and Biotechnology, 8(3), 71-87. https://doi.org/10.31033/ijrasb.8.3.11

[8] Mr. Utkalendu Suvendusekhar Samantaray, Ms. Ankita Mishra, & Yengkhom D. Singh. (2020). Biosynthesis and Antimicrobial activities of Silver Nanoparticles (AgNPs) by using Leaf Extracts of Tagetes erecta (Marigold) and Tridax procumbens (Tridax). International Journal for Research in Applied Sciences and Biotechnology, 7(6), 209-226. https://doi.org/10.31033/ijrasb.7.6.31

[9] SamantarayUS, SahuS, PatroA,TripathyS, SethiS, Phytochemicals: A novelantiviraltherapeutic approach for Prevention of lung injury and respiratory infection during COVID 19,SPR, Volume 2, issue 1, Page No.: 528-540. DOI: https://doi.org/10.52152/spr/2021.177