

1 **Characterization of the pscC (*Type III secretion*) gene of *Pseudomonas aeruginosa***
2 **(PA01) and assessment of immunogenicity of pscC protein in rats**

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4 Bhuiyan SA¹, Vanitha DJ¹, Sultana H², Opook, F¹ Rodrigues KF¹

5 ¹ Biotechnology Research Institute, University Malaysia Sabah, 88400 Kota Kinabalu, Sabah,
6 Malaysia.

7 ² Rangpur Medical college, Rangpur, Bangladesh

8 Corresponding author: kennethr@ums.edu.my

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10 **1. Md Safiul Alam Bhuiyan**

11 Biotechnology Research institute
12 University Malaysia Sabah, Malaysia
13 Email: dr.s_alambh@yahoo.com

14 **3. Daisy Vanitha John**

15 Associate Professor
16 Biotechnology Research Institute
17 University Malaysia Sabah, Malaysia
18 Email: daisyv@ums.edu.my

19 **5. Fernandes Opook**

20 Biotechnology Research Institute
21 University Malaysia Sabah, Malaysia
22 Email: fernandes_opk@yahoo.com

22

23

24

25

2. **Kenneth Francis Rodrigues**

Associate Professor
Biotechnology Research Institute
University Malaysia Sabah, Malaysia
Email: kennethr@ums.edu.my

4. **Hafiza Sultana**

Assistant professor,
Department of Microbiology and Immunology
Rangpur Medical College, Rangpur, Bangladesh
Email: dr.hafizasultana@yahoo.com

26 **ABSTRACT**

27 **Proteins associated with the bacterial membrane can be recruited for application as**
28 **antigens for the development of vaccines. This preliminary study was directed towards**
29 **evaluating the antigenic properties of the *Pseudomonas aeruginosa* (PA01) pscC protein**
30 **which is a component of the Type III secretion system. Gene specific primers were**
31 **designed to isolate the *pscC* gene which was isolated, ligated onto the multiple cloning**
32 **site of vector pGS21(a), cloned and expressed in *Escherichia coli* (BL21). The molecular**
33 **weight of the expressed pscC protein was determined by SDS-PAGE (10% sodium**
34 **dodecyl sulphate-polyacrylamide gel electrophoresis) and was found to be around 57**
35 **KDa and purified by the size exclusion chromatography. Finally, the purified pscC**
36 **protein was injected subcutaneously into adult Sprague Dawley® rats with a range of**
37 **concentrations (50, 100 and 150 µg per rat) respectively. Recombinant pscC antigen**
38 **induced a specific humoral immune response against the antigen, which was validated**
39 **by Enzyme-linked immunosorbent assay (ELISA). The results concluded that anti-pscC**
40 **antibody was elicited in the animal model.**

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43 ***Key words:***

44 *Pseudomonas aeruginosa*, pscC gene, type III secretion system.

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48 1. INTRODUCTION

49 *Pseudomonas aeruginosa* is a versatile Gram negative aerobic bacillus, found in a wide
50 range of environmental habitats. This opportunistic pathogen causes both acute and chronic
51 infections in patients with hospital-acquired pneumonia [1]. It has been classified as the
52 fourth leading cause of nosocomial infection and is associated with cystic fibrosis; burn
53 wound infection, and pneumonic septicaemia [2] [3]. Due to recurrent causes of nosocomial
54 infections *Pseudomonas* infections have become more complex and life-threatening
55 infection, as standard treatments are becoming ultimately ineffective. This organism exhibits
56 intense signs of antibiotic resistance to a wide variety of antimicrobial treatments, including
57 Beta-lactams, Chloramphenicol and Fluoroquinolone [4]. Therefore, specific immune therapy
58 is more desirable than conventional antibiotic therapy [5].

59 It is an urgent demand of time to implement therapeutic vaccination schemes against
60 *Pseudomonas* infections. *P. aeruginosa* have its own type III secretion system (T3SS), with a
61 protein translocation apparatus and the effector proteins which can be injected into host cells.
62 The secretion and translocation stages comprise over 20 proteins gathered into a needle-like
63 structure termed as injectisome [6]. The T3SS is the part of important virulence determinants
64 of *P. aeruginosa*, in which the pscC protein is a fundamental part of the needle tip for *P.*
65 *aeruginosa* (Fig 1). The pscC protein has been established to be an important protective
66 antigen of the bacterium and regulate the secretion of translocator proteins to attach with host
67 cell membranes. Once the bacterium interacts with host cell membranes, the T3SS system is
68 activated and this in turn inhibits signal transduction resulting in cellular cytotoxicity or
69 changes in the host immune responses. The T3SS antigen may react with the host cell, whose
70 maximum component should play a significant role in the host immunity. Therefore,
71 immunization against T3SS antigen can prevent translocon association. However, the

72 functional and regulatory information about this multi-mechanism of pscC antigen will
73 facilitated for the design of novel recombinant vaccines as well as therapeutics.

74 Effective vaccines are designed to stimulate the innate immune response, as well as
75 carry antigens to specific sub-cellular sites for the elicitation of antigen-specific cytotoxic T
76 cells. Physical delivery to specific locations within a cell is one of the major challenges when
77 developing a suitable candidate for T3SS antigen as a vaccine. Secretory needle antigens of
78 T3SS are easily processed through T3SS pathways. These complexes form a host pathogen
79 interaction is important to identify the molecular pathogenesis for developing an effective
80 vaccine. As a result, the secretory T3SS antigen directly stimulates antigen-specific cytotoxic
81 T cells through the delivery of antigens to the antigen presenting cells (APC), causing a
82 humoral immune response [7] [8]. The proteins for transportation of T3SS pathway have
83 distinct signals that direct them to the secretion machine to stimulate T-helper cells,
84 conferring protection to a diversity of infectious diseases [9].

85 The preliminary aim of this study was to determine the immune response of the T3SS
86 pscC antigen elicited in animal model. It has been suggested that recombinant or purified
87 protein immunization may allow long-term persistence of immunogenic action in host cells
88 without any risk of infection [10] [11]. Th1 and Th2 cells collectively responses on either Ag
89 are in the secretory product (Th2) or an intracellular or membrane-anchored molecule [12].
90 These approaches are mostly reliant on the antigen presentation which has been capable to
91 improve immune stimulation to recombinant vaccines. Clinical trials have attested to the
92 safety, efficiency and efficacy, as well as wide application of this immunization technique
93 [13]. The cell surface display of a heterologous antigenic determinant is advantageous for the
94 induction of an antibody against a specific antigen. The orientation of target antigens has
95 been used to develop recombinant vaccines for immunization on rats for the capture and
96 detection of an antibody from serum [14]. The recombinant version of the pscC needle

97 protein of *P. aeruginosa* can be used as a specific antigen for indirect ELISA to screen the
98 antibodies generated against specific protein in response to immunization of the rats.

99

100 **2. MATERIALS AND METHODS**

101 **2.1 Growth of *Pseudomonas aeruginosa* (PA01) and Isolation of Bacterial DNA**

102 The *Pseudomonas aeruginosa* PA01 culture was obtained from the ATCC. Cultures of *P.*
103 *aeruginosa* were grown in autoclaved LB (Luria-Bertani) broth and agar containing peptone
104 (10g/L), Yeast extract (5g/L), NaCl (10g/L) and agar (15g/L). Bacterial DNA isolation was
105 performed via following Aljanabi and Martinez method which was used for rapid salt
106 extraction of high quality genomic DNA for PCR base technique [15]. The plasmid vectors
107 pGS-21a (Gene script, New Jersey, USA) was used as vector for cloning and expression. The
108 complete Freund's adjuvant (Sigma, USA) was used as an adjuvant for delivery of protein in
109 rats. LB media containing 100µg/ml of ampicillin were used to grow *E.coli* clones at 37°C.

110

111 **2.2 Development of gene constructs for expression of recombinant pscC gene.**

112 PCR amplification of pscC gene (1.46kb) of *P. Aeruginosa* was performed using specific
113 primers. Primers were designed according to the pscC gene sequence which was retrieved
114 from the NCBI GenBank (NP_250407). Then nucleotides were selected from both 5' and 3'
115 ends of the pscC gene and restriction sites for *EcoRI* and *HindIII* were introduced at the 5'
116 and 3' ends respectively in order to facilitate cloning. The forward primer:
117 TCAGaattcCCAGCCTGCCTTACGACTAT, with the restriction site for *EcoRI*, and Reverse
118 primer: CGC-ccatgg-CAACTCGTCGATTCAAGCA, with the restriction site for *HindIII*.
119 Amplification of the gene was performed 20 µl of total volume containing 2 µl DNA as a
120 template, 4 µl of 5×PCR buffer (Promega), 1.2 µl 1.5Mm Mgcl₂ (Promega), 0.2 µl of 10 Mm
121 dNTPs (Promega), 0.2 µl of tag polymerase (Fermentas, USA), 2 µl of primer (1st BASE

122 Laboratories, Malaysia) oligonucleotides (Forward & Reverse), and 10.4 µl sterilizes distilled
123 water.

124 The amplification was performed in a thermal cycle program for initial denaturation at 96°C
125 for 3 min, 30 cycles of 20 Sec at 94°C, 40 Sec at 56°C and 2 min at 72°C. After final
126 extension of 10 min at 72°C, the sample was cool to 4°C. The presence and yield of specific
127 PCR products of approximately 1,500 base-pair was confirmed by gel electrophoresis in 1%
128 agarose gel. DNA samples were loaded in wells and the electrophoresis was carried out at
129 100 volts. Then the DNA bands were visualized under ultraviolet (UV) trans-illuminator.

130

131 **2.3 Cloning and expression of *pscC* gene**

132 The PCR products were purified using the gel purification kit according to the manufacturer's
133 instructions (Fermentas, USA). The purified PCR product was cloned into the pGS-21a
134 vector containing T7 promoter to induce the expression of the cloned gene and selected
135 *EcoRI* and *HindIII* were the appropriate restriction sites on pGS-21a vector. Plasmid pGS-
136 21a and *pscC* gene (PCR products) were further double digested with *EcoRI* and *HindIII*
137 (New England Biolabs, Inc, Beverly, MA). The PCR product of the *pscC* gene was ligated
138 into the vector using T4 DNA ligase (Fermentas, USA) according to the manufacturer's
139 instructions. The ligation product was used to transform into *E. coli* of Top10 competent cells
140 by CaCl₂ method [16]. The insert plasmids were identified by colony PCR. The inserted
141 sequence and its reading frame were confirmed by *EcoRI* and *HindIII* digestion and DNA
142 sequence analysis. The generated pGS-*pscC* inserted plasmid was again sub-cloned into
143 competent expression cells of *E. coli* BL21 (DE3) following same method described as
144 cloned of Top10 competent cells.

145 The transformant cells were inoculated into the LB containing ampicillin in test tubes
146 and incubated at 37°C for 16 hours with continuous stirring (200 xg) until the optical cell

147 density reached 0.4-0.6 at OD₆₀₀. Subsequently, the culture was induced with 0.5mM IPTG
148 and maintained at 37°C on a rotary shaker set at 200xg. The un-induced sample (1 ml) was
149 withdrawn prior to induction for used as a control. Afterwards, induced sample was taken
150 hourly and last sample was picked after 16 hours of induction to observe the time course
151 study of the expression. The soluble and insoluble fractions were obtained by treating the
152 pellet with 1ml of PBS (Phosphate Buffer Saline) and lysed using ultra sonication on ice. The
153 insoluble fractions of the protein were run in the 10% SDS-PAGE gel using coomassie
154 Brilliant blue with protein marker (Bio-5150, 1st BASE). After extraction of the intense
155 protein gel, the identification of protein by mass spectrometry was performed. Peptides were
156 extracted according to the Brangan's methods followed by digestion with trypsin which was
157 analyzed using MALDITOF-TOF mass spectrometer with proteomics Analyzer (48000) [17].
158 Spectra were analyzed to identify the protein of interest by Mascot sequence matching
159 software with Ludwig NR Database.

160

161 **2.4 Purification and Immunization of pscC protein in rats**

162 The soluble fraction of pscC protein was purified by using by Size exclusion chromatography
163 (SEC). The SEC system (Akta Prime) was calibrated according to the supplier's instruction.
164 S-100HR column (GE Healthcare) attached to the Akta Prime System was equilibrated using
165 0.05M Sodium Phosphate, 0.15M NaCl, pH 7.2 at a flow rate of 2.6ml/min. The possible
166 fractions were generated through the significant peaks and collected the specific column for
167 desired protein by chloroform/methanol precipitation method. The fractions were further
168 analysed using 10% SDS-PAGE with Pre-staining Protein Marker.

169 The male adult Sprague-Dawley Rats (4-8 weeks old) weighing 150-200gms were
170 obtained from Tes Jaya laboratory services, Pulau Pinang, Malaysia. All these animals were
171 acclimatized and quarantined before commencement of the experiment. This study had

172 approval from the Animal Care and Use Committee and Institutional Biosafety Committee
173 (IACUC), Malaysia prior setup the experimental design. Animals were reared in plastic cages
174 using paddy husk bedding at room temperature ($25 \pm 1^\circ\text{C}$) and humidity ($50 \pm 5\%$) in BSL-1.
175 For evaluation of the immunogenicity of pscC protein, soluble protein was administrated in
176 rats with various doses [Low (50 μg), medium (100 μg) and high (150 μg)]. Rats were
177 randomly assigned to four groups composed of 6 animals in each. Standard restraints were
178 used during the injection. Group 1 received 100 μl 0.1 M phosphate buffered saline (PBS)
179 with equal of complete Freund's adjuvant (Sigma, USA) via the subcutaneous route as a
180 control. The Group 2 immunized with 50 μg of total soluble protein mixed with the same
181 volume of complete Freund's adjuvant. The group 3 & 4 were injected following the same
182 technique with the equivalent of same adjuvant which was treated with 100 μg (medium) and
183 150 μg (High) in every 5 weeks. Serum antibody was measured by indirect ELISA and
184 antibody level responds of immunized rat sera which were collected on 7, 14, 21, 28 and 35
185 days after a single dose of administration. Retro-orbital bleeding was done for blood
186 collection and blood samples were collected under general anaesthesia (95% Diethyl ether
187 with enclosed induction chamber). Serum samples from immunized and control rats were
188 centrifuged at 11,600xg, and stored at -80°C . The collected sera were subjected to enzyme-
189 linked immunosorbent assay (ELISA) assay for the determination of the level of antibodies in
190 sera.

191

192 **2.5 Enzyme-linked immune-sorbent assay (ELISA)**

193 Each sample was screened by indirect ELISA for specific immunoglobulin IgG+IgA (H+L
194 chain specific) using goat anti rat-antibody (Southern Biotech, USA). The recombinant
195 antigen was mixed and poured into the 96-well Polystyrene micro-titre (Membrane solution,
196 USA) plates. After coating the antigenic samples, the micro plates were covered and

217 incubated on a shaker at 4°C for 16hours which was washed three times with 100µl 0.05%
218 (V/V) Tween 20 in PBS, blocked by adding 150 µl of PBS -3%(W/V) BSA followed by
219 incubation for 90min at room temperature. Control and test sera were diluted 1:100 in the
220 blocking agent of PBS-A-0.5% to which 0.05% of Tween 20 was added. Further, after the
221 serial dilution of serum samples at 1:300, 1:600 & 1:900 they were loaded to the micro plate
222 separately and incubated. The plates were washed and applied on goat anti-rat IgM+ IgG
223 antibodies followed by incubation and another wash step. 100 µl of conjugated alkaline
224 phosphatase secondary Abs in PBS-1% and BSA (1/1000) was added to the wells and
225 incubated at room temperature followed by the wash step. P-Nitro phenyl phosphate
226 (Southern Biotech, Birmingham) substrate tablet (5mg/tablet) was dissolved in DSB
227 (1tablet/15ml of DSB) buffer. Subsequently, 100µl of substrate solution was mixed to all
228 tests well and kept at room temperature for 15min until a yellow colour developed. The
229 optical density (OD) was measured using an ELISA plate reader (Infinite ® M-200 PRO,
230 TECAN). The OD results of all samples were calculated as percentage of positive control by
231 using the OD value of the positive control serum by deducting the average OD value of blank
232 serum.

233 **3. RESULTS AND DISCUSSION**

234 **3.1 Plasmid DNA constructions**

235 PCR amplified product was run on a 1% agarose gel and desired band for the respective
236 target pscC gene was found without any primer and dimer. The PCR fragments were found to
237 be approximately 1.46 kb (Figure 2A) amplified from the genomic DNA.

238 After confirmation, the PCR products were excised out of the agarose gel and purified
239 using a gel extraction kit and digested with restricted enzyme for conformation which was
240 represented in Figure 2B. The synthesized PCR product was then ready for cloning into pGS-
241 21a plasmid vector. The pGS-21a plasmid was constructed by PCR amplification of the

222 coding region of pscC with *EcoRI* and *HindIII* sites and ligating the pGS-21a vector cut with
223 the same enzymes. To determine whether the pscC fragment was successfully ligated in the
224 pGS-21a plasmid, which were then digested with the *EcoRI* and *HindIII* to release the
225 expected size of pscC gene. After 1% agarose gel examination, the RE mixture exposed the
226 presence of two bands. The upper band was consistent to linear according to plasmid size
227 (Approximately 6.2 kb) and another band was corresponding to the expected molecular size
228 of pscC gene around 1.46kb.

229 **3.2 Cloning and expression of pscC gene in *E. coli***

230 The pGS-21a plasmid was transferred to *E. coli* TOP10 and BL21 (D3) and confirmed
231 respectively by restriction digestion, colony PCR and sequencing. The clones were subjected
232 to colony PCR and an amplified 1.46 kb band was obtained as that of positive control. The
233 plasmid showed the presence of 1kb insert and clones were confirmed through PCR and
234 restriction analysis in Figure 3. The PCR positive recombinant clones were also subjected to
235 restriction digestion using *EcoRI* and *HindIII*. The presence of about 1.46kb insert was
236 confirmed by electrophoresis and the positive clone was sequenced with T7 promoter primer
237 (Forward and Reverse). Later than confirmation, clones were further subjected to plasmid
238 extraction, followed by RE and plasmid sequence analysis.

239 The sequences were then analyzed via BLAST, compared with the reference
240 sequences formerly deposited in NCBI. The sequences was compared to the NCBI database
241 which was revealed about 97% similarity compared the sequences of pscC in BLASTn
242 algorithm. As of the colony PCR the pGS-21a with inserted pscC gene was found
243 approximately 1.46kb size and control showed the 1.0kb. Therefore, it can be concluded that
244 the recombinant plasmid carried the targeted pscC gene.

245 The protein expression was observed in host strains of *E.coli* BL21 (D3) cells and the
246 most prominent band was appeared at ~84 kDa in 4 samples which was illustrated in Figure

247 4. The soluble and insoluble fractions were obtained by re-suspending the cell pellet in buffer
248 and sonicated on ice. The slightly visible soluble band for scarce was detected in supernatant
249 phase after visualized under SDS-PAGE (Figure 5). Consequently, a prominent band of the
250 target protein pscC appeared which was visually distinguishable from other proteins based
251 the un-induced controls. Recombinant *E. coli* also showed a distinct band with an
252 approximate cumulative size of ~57 kDa with His tag (6XHis) and GST Tag at the size of
253 1kDa & 26kDa respectively. For further substantiation, the protein samples were digested by
254 trypsin and peptides were extracted by MALDITOF-TOF mass spectrometer. Spectra were
255 analyzed to identify the protein of interest using Mascot sequence matching software. The
256 result indicated that the amino sequence of pscC protein specified a high ionic scoring match
257 and similar with two peptide sequences appeared in the same protein. The protein identity
258 and mass match were statistically more significant. Protein was analysed and noticed the one
259 matched peptide to the same pscC protein, which was higher confidence for correct protein
260 identified in Figure 6.

261 **3.3 Purification and Immunization of pscC protein in rats**

262 The crude fraction was further purified and subjected to gel filtration on a Superdex followed
263 by purification analysis using SDS-PAGE. The chromatogram showed a mixture of monomer
264 and dimer state. The distinct peak was observed in 40 to 47 fractions and the small picks were
265 noticed in 22 to 41 fractions. Additional stage was precipitating the fractions followed by the
266 protocol of Wessel and Flugge [18]. The precipitated proteins were run once again in SDS-
267 PAGE and protein in the lane of fraction 27, 28, 29 columns presented the correct band as
268 their predicted molecular weight of approximately ~84kDa in Figure 7.

269 After immunization, the antibody levels were measured by indirect ELISA. The result
270 showed that the rat antibody demonstrated a positive value against the immunized pscC
271 protein. As shown in the graphical representation in figure 8, the level of immunoglobulin

272 measured over the 0 to 5th week for all the immunized rats. The OD₄₀₅ Values for different
273 weeks of serum from the post immunized blood were represented in Table 1. The 4th and 5th
274 weeks of all groups of immunized sera were shown in positive value. Four weeks after
275 immunization, The OD₄₀₅ values at 1:300 dilutions were 0.56, 0.64 and 0.63 for the low,
276 medium and high dose respectively. Similarly, at the 5 weeks the antibody level was further
277 increased and the OD₄₀₅ value at 1:300 dilutions to 0.80, 1.04 and 1.22 for the low, medium
278 and high dose respectively. Though for the titer measurement, a standard was needed since
279 we do not have. The OD value of unimmunized and immunized serum was compared in
280 rodent model. It was found that immunized OD value was 2 to 3 folds higher than the
281 control OD value after 3 weeks. The result illustrated that the rat antibody demonstrated a
282 positive value against the immunized serum based on OD value. The levels of the humoral
283 immune responses showed higher antibody of Ag-specific serum immunoglobulin compared
284 to with the controls group. The control groups were always (Group-1) negative to the pscC
285 antigen throughout the study. The humoral immune responses were detected in all plasmid
286 immunized groups and continued increasing after 4th weeks of immunization as indicated by
287 the antibody level. The increased antibody was dose dependant until the 5th weeks. It was
288 noted that the medium dose (100 µg) and higher dose (150 µg) of protein antigen elicited a
289 stronger immune response as compared to the lower dose (50µg).

290 *Pseudomonas aeruginosa* is a major cause of most opportunistic and nosocomial
291 infections. Although conventional antibiotics are more resistance to *Pseudomonas* organism,
292 now it is challenging to find an alternative immunize therapy. In these circumstances, a
293 monoclonal antibody-based approach is still exploration for the inhibition of *Pseudomonas*
294 infection [19]. Monoclonal antibodies have been established to improve of bacterial
295 permission, stop the colonization and invasion, and avoid the destruction which is triggered
296 by cytotoxic influences [20]. During the past few years, recombinant protein vaccines have

297 shown the highest innovation in modern medical technology. Immunization with microbial
298 antigen has delivered the protective immunity in animal models and considered a potential
299 useful vaccine strategy [21]. Based on previous findings, there are various kinds of
300 immunization have been observed against *Pseudomonas* infection such as killed vaccine [22],
301 purified outer membrane subunit vaccine [23], plasmid immunization [24] and synthetic
302 peptide immunization [25]. The immune response in a mouse animal model to consecutive
303 recombinant protein base vaccine could able to neutralizing the bacterial virulent toxin [26].
304 It is supported that the use of genetic immunization technique in which a random assortment
305 of genes from a bacteria's genome to practice for immunization purposes [27]. So, it is
306 supposed to be clear that immunization is the best way of protection against outer membrane
307 secretin antigen of T3SS.

308 A previous study described about the immunogenicity of *Pseudomonas* cap segment
309 (PcrV) of T3SS needle protein. The anti-PcrV monoclonal antibodies were detected after
310 recombinant PcrV protein was used to immunize in a mouse model. The subgroup of anti-
311 PcrV antibody can repressed T3SS function which was observed in cell lysis assay and
312 designed for assessment of protective activity in a *P. aeruginosa* mouse acute pneumonia
313 [28]. Protection or defence in animal models gives only the recommendation of the potential
314 efficiency of that vaccine in humans [29]. Recently, Mingzi *et al.*, 2014 have published the
315 immunogenicity of pcrV gene (T3SS) encoding needle protein was immunized on mice and
316 evaluated the efficiency of vaccines in *Pseudomonas* encoding single antigen [30]. The
317 results revealed that mammalian expression vector with the pcrV gene could elicit a sufficient
318 level of specific antigen to induce the humoral antibody. Based on Brain *et al.*, 2001 study,
319 outer membrane of oprF gene of *P. aeruginosa* was immunized by plasmid that indicated that
320 the efficacy of this vaccine can be able to elicited anti-oprF that confers protection of rodents
321 against chronic pulmonary infection. Moreover, the results are similar to previous published

322 results producing defence mechanism in rodent models by immunization with purified outer
323 membrane protein from *P. aeruginosa* [31].

324 The fact is, without testing its effectiveness in animal models, the recombinant
325 antigen has not yet been applied to induce in the human body for assessment of their efficacy
326 and level of immune responses. It is necessary to conduct a challenging experiment in which
327 live *Pseudomonas* can be injected into immune animals to examine the viability of rat and
328 colony contents of *Pseudomonas* spp. Our further expectation, we tried to determine an
329 applicable vaccine against *Pseudomonas* for clinical trial. Clinical challenge is essential for
330 recombinant antigen efficacy in animal models for assessing their efficacy and level of
331 immune responses. It was also not clear yet, what about the optimal vaccine modalities to
332 induce potent neutralizing antibody responses or to strong humoral immune respond ensures
333 capable to destroy against the infected cell. It is factual that, the recombinant protein vaccine,
334 which is able to induce protection from *Pseudomonas* infection, might be more realistic for
335 ending global.

336 In précis, it is an appropriate challenging strategy for exploration of surface T3SS
337 pscC antigen, which has been characterized from *P. aeruginosa* PAO1 genome as a vaccine
338 candidate. The pscC gene (1.46 kb) encodes a 57-kDa immunogenic outer membrane
339 associated protein of *P. aeruginosa* PAO1 strain. The protein was characterized in SDS-
340 PAGE and purified from *E. coli* as a fusion protein to identify anti-pscC specific antibodies in
341 the serum of Sparagury rat. Therefore, it is posited that recombinant pscC protein is capable
342 of becoming a strong carrier and processor for the presentation of target foreign peptides in
343 MHC II to stimulate the T helper cell based humoral immune system in a host cell. The
344 expectations for treatment and prevention of human disease by immunization are changing,
345 and the recurrent refinement of these abilities signify a fit for the current as well as the future
346 manufacture of a vaccine platform against *Pseudomonas* infection.

348 **4. CONCLUSION**

349 This study provides preliminary experimental evidence indicating that the outer membrane
350 protein pscC can serve as an elicitor of the humoral immune response in rats. We recommend
351 that future studies focus on a larger sample size and explore a range of open reading frames
352 to determine the precise location of the antigenic component of the pscC protein.

353

354

355 **BIOSAFETY AND ANIMAL ETHICS**

356 The study was approved by the Biotechnology Research Institute Institutional Biosafety
357 Committee (BRIBC). The animal study was carried out in the Level 3 Biological
358 Containment Facility (ABSL-3) at the Biotechnology Research Institute, Universiti Malaysia
359 Sabah.

360 Recombinant protein expression procedures were carried out using *Escherichia coli* (BL21)
361 which is classified as a (B) strain that is exempted under the Malaysian Biosafety Law.

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