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CLONING AND EXPRESSION OF SEC2 GENE OF STAPHYLOCOCCUS AUREUS FOR ANTIBODY PRODUCTION.

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ABSTRACT

Staphylococcus aureus is an opportunistic pathogen causing a wide range of diseases and intoxications in warm blooded animals. Methods for fast and reliable detection and identification of food borne pathogens and toxins are highly desired by food industries to provide early information's about the sample being tested. The aim of the present investigation was to produce pure SEC2 (A type of enterotoxin produced by *S. aureus*) by heterologous expression and study its utility in raising anti-SEC2 antibodies which can be further used for the development of a suitable detection kit against SEC2 toxin, based on immunological detection system. But the success of immunoassay depends on purity of SEC2 used for raising anti-SEC2 antibodies. Therefore, in the present study amplified SEC2 toxin gene (*sec*2) *S. aureus* isolate 36 was cloned and co-expressed with a fusion protein (6x His tag) for ease of purification. Recombinant protein was purified under native condition using Ni-NTA resin. The purified; dialyzed recombinant SEC2 protein was then used for immunization. Recombinant SEC2 fusion protein was further used for raising antibodies in rabbit and mice to generate humoral immune response to study its suitability for detection of SEC2. These antibodies were further tested for their sensitivity and specificity for their later use in the development of a suitable detection test like ELISA. Results obtained in the present study with respect to purity of r-SEC2 and titer of anti SEC2 antibodies indicate that the heterologous expression of SEC2 is a suitable alternative for producing high tittered and specific antibodies .

Keywords: *Staphylococcus aureus*, SEC2, Cloning, Antibody Production

No of Figures: 8 **No of References:31**

INTRODUCTION

Staphylococcus was first observed in human pyogenic lesions by Recklinghausen in 1871. Pasteur (1880) obtained liquid cultures of the cocci from pus and produced abscesses by inoculating them into rabbits. It was Sir Alexander Ogston (1880) a Scottish surgeon, who established conclusively the causative role of the cocci in abscesses and other suppurative lesions. The name Staphylococcus derived from the Greek name Staphyle meaning a bunch of grapes and coccus meaning a grain or berry; was first used by Sir Alexander Ogston (1882).

Rosenbach (1884) was the first to grow Staphylococci in pure culture and studied their characteristics in laboratory. He described that most staphylococcal strains from pyogenic lesions produce golden yellow colonies, named them *Staphylococcus pyogenesaureus* and the strains from normal skin produce white colonies on solid media named them *Staphylococcus pyogenesalbus*. *Staphylococcus pyogenes aureus* was later renamed as *Staphylococcus aureus*. *Staphylococcus aureus* is an opportunistic pathogen causing a wide range of diseases and intoxications in warm blooded animals. These include skin infections such as ulcers, carbuncles, impetigo and epidermitis necrosis; infections of the internal organs such as bacteremia, endocarditis, pericarditis, osteomyelitis, meningitis, and intoxications such as toxic shock syndrome and food poisoning (Bergdoll, 1983).

Main cause of food poisoning are inadequate refrigeration, inadequate cooking or heat processing and holding in warming devices congenial for bacterial growth (Jay, 1992).

Staphylococcal food poisoning is an acute gastroenteritis due to the ingestion of enterotoxin produced in food by certain strains of *Staphylococcas aureus*. The most common symptoms which include nausea, vomiting, abdominal pain, diarrhea, sweating, headache and sometimes fall in body temperature appear 2-6 hours after eating the contaminated food (Jay, 1992). In more severe cases prostration and dehydration occur. The illness is relatively mild, normally lasting only a few hours to a day, with a high morbidity but a low mortality rate. More than 20 serologically distinct enterotoxins A-E and G-U have been implicated in food poisoning outbreaks (Lim *et al*., 1985).

Staphylococci produce a wide range of toxins and exoenzymes including membrane damaging toxins (alpha, beta, gamma, and delta toxin and leucocidin), epidermolytic toxin, and enterotoxin. Staphylococcal enterotoxins (SEs) are low molecular weight (26 to 30 kDa), single chain proteins with isoelectric points between 5.7 and 8.6. Their molecular composition is characterized by the presence of only two residues of half cysteine and one or two residues of tryptophan; they contain relatively large amounts of lysine, tyrosine, aspartic and glutamic acid. They are resistant to degradation by proteolytic enzymes and heat.Staphylococcal enterotoxins are superantigens and are presented to T cells on antigen presenting cells without being internalized and processed by these cells. Enterotoxins bind directly to invariant regions of MHC class II (Dinges *et al*., 2000) and are most potent activators of T

lymphocytes coupled with release of cytokines. Cytokines have been reported to result in stimulation of neuro-receptors in the intestinal track and trigger the vomiting center in brain (Komisar *et al*., 1994). Currently more than 20 different types of enterotoxins are recognized, but the most important ones implicated in SFP are SEA, SEB, SEC1, SEC2, SEC3, SED and SEE. The genes responsible for the production of SEA, SEB and SEC are on the chromosome, where as those of SED are on a plasmid. SECs are typical secondary metabolites and are produced by the cell at the end of the growth phase and the beginning of the stationary phase.

SEC is subdivided into SEC1, SEC2, SEC3 based on their minor epitopes. Among the staphylococcal enterotoxins, SEC shows the highest heat stability followed by SEB and SEA (Tibana *et al*., 1987). Even heat treatment at 60^{o}C for 30 minutes could not inactivate SEC (Borja and Bergdoll, 1967).

It is essential to devlop immunological detection system for SEC2. Success of immune based detection system based on purity of SEC2 is used for raising anti-SEC2 antibody. SEC2 was purified initially by the method of ion-exchange chromatography, chromate focusing and gel filtration (Borja *et al*., 1972). Use of these techniques in tandem is tedious, time consuming, expensive and sometimes does not result in purification of staphylococcal enterotoxin as well as methods for fast and reliable detection and identification of food borne pathogens and toxins are highly desired by food industries to provide early informations about the sample being tested. Therefore, SEC2 toxin gene (sec2) was cloned and co-expressed with a fusion protein (6x His tag) for ease of purification. The aim of the present investigation was to produce pure SEC2 by heterologous expression and study its utility in raising anti-SEC2 antibodies which can be further used for the development of a suitable detection kit against SEC2 toxin.

MATERIALS AND METHODS

All chemicals until specified were purchased from Sigma -Aldrich (USA). Glasswares were purchased from Borosil (India) and Schott-Duran (Germany). Plasticwares were procured from Greiner Bio-One (Austria), Tarsons products Pvt. Ltd. (India), Axygen (USA)and Nunc (Denmark). Animal experimentation was carried out as per the guidelines of Institutional Animal Ethics Committee. Experiments and assays were performed in different instruments for example Thermal Cycler (iCycler and Gene cycler, Bio-Rad, USA), Gel Electrophoresis system (Bio-Rad,USA), Gel documentation system (Fluorchem SP, USA),Incubator shaker (Kühner, Switzerland),Deep Freezer (-85º C, -35º C,New Brunswick Scientific, USA),Refrigerated centrifuge (Sigma Instruments Germany), pHmeter (Metler Toledo, Germany), Autoclave (Harayama, Japan), Water purification system (Millipore, USA), Bio safety cabinet type II (Kalenzaids Bioclean Device, India), ELISA plate washer & plate reader (Bio-TekInstrumentsInc., USA).

Sec 2 f- primer:
5’GAGAGTCAACCAGACCCTACG3’&
Secrprimer5’TTATCCATTCTTTGTTGTAAG GTGG3’ was used for Amplification of sec 2 gene.

1) **Isolation of total DNA**

SEC2 producing strain *S. aureus* was streak plated on BHI agar medium for purification. Total DNA was isolated using GenEluteTMBaterial Genomic DNA Kit (Sigma, USA). DNA yield was calculated using λ260/280¬(Sambrook and Russel, 2001) and the integrity of DNA was checked on 0.8% agarose by gel electrophoresis.

To enrich DNA of SEC2 gene a PCR was performed. A 25 µl PCR mix was prepared in a thin walled PCR tube:DNA Template (5ng), Sec 2f Primer (10µM), Sec r Primer (10µM), Pfu DNA Polymerase 0.25µl (2.5unit/µl, Fermentas), 10X PCR Buffer 2.5µl (Fermentas), $MgSO_4$ (20mM), dNTP (10mM, Fermentas). Thirty rounds of Amplification were performed at 94^{o}C/1min Denaturation, 53^{o}C-58^{o}C/1.5min Primer Annealing, 72^{o}C/2min Extension in a Thermocycler; a final extension step was included at 72^{o}C/10min and soaking at 4^{o}C. The amplified PCR product of sec2 gene was purified by using QIAGel Extraction Kit (Qiagen, USA). Purified sec2 product was quantified by gel quantitation method on 1.5% agarose gel using fermentas Fast Ruler TM DNA ladder.

2) **Cloning of sec2 gene in E. coli**

pQE30Xa encodes a Factor Xa Protease recognition site which is bracketed by 6x His tag coding region on 5' side and multiple cloning site on 3' side. The 5' end cloning using blunt end *Stu*I restriction site allows insertion of the gene of interest directly behind the factor Xa protease recognition site without any intervening amino acid codons.

To ligate *sec*2 gene in pQE30-Xa vector a ligation reaction was performed. A 10µl ligation mix was prepared: Linearized pQE30-Xa vector (10ng), gene insert (28.8ng), *Stu* I enzyme (2.5units), T4 DNA Ligase (1µl, Fermentas), Ligation buffer 10x (1µl, Fermentas), ATP 10mM. The ligation mix was spinned and kept at 25^{o}C for 2.5 hrs, then incubated at 65^{o}C for 10 minutes and cooled.

Electrocompetent Cells were prepared by modifying the method of Tung and Chow (1995).Electrocompetent E. coli SG13009 (kanamycin resistant) cells were transformed with recombinant vector containing sec2 insert by electroporation using Gene Pulser.Random 25 clones were patched on LB (ampicillin 100µg/ml + kanamycin 25 µg/ml) agar plates for pure colony isolation. Ampicillin and Kanamycin resistant colonies were screened for the presence of sec2 gene by whole cell PCR. Out of 25 clones, three clones were found to contain the sec2 gene in proper orientation as revealed by PCR followed by agarose gel electrophoresis. The expected size of the amplicon is ~845bp if the gene is inserted into the vector in proper orientation. The PCR Mix was prepared by using pQE-r primer (10µM), *sec* 2-f primer (µM), *Taq* DNA Polymerase (Fermenta, 0.1µl), dNTP (10mM), 10X buffer 1µl (without $MgCl_2$), $MgCl_2$ (25mM). Thirty rounds of Amplification were performed at 94^{o}C/1min Denaturation,54^{o}C/1.5min Primer Annealing, 72^{o}C/2min Extension in a Thermo cycler; a final extension step was included at 72^{o}C/10min and soaking at 4^{o}C. The PCR positive clones of sec2 were streaked on LB plates to obtain pure colonies. The vector plasmid containing sec2 gene was isolated from recombinant

clones for DNA sequencing using QIAprep spin mini prep kit (QIAGEN, Germany).

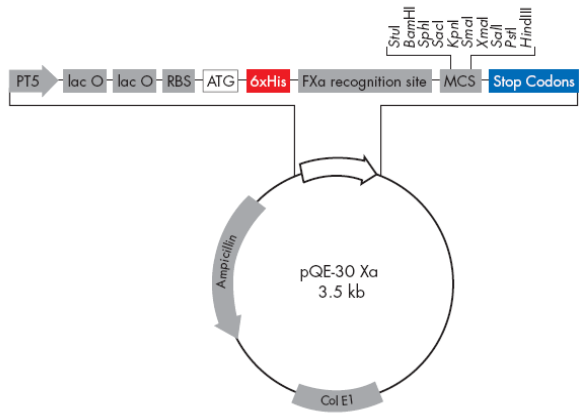


Fig1:*Vector pQE30Xa*

3) **Purification of Recombinant SEC2**

Harvested cells were lysed with lysis buffer under native conditions for cell disruption and release of cellular protein. The presence of 6xHis tag in pQE30Xa vector facilitated purification of recombinant SEC2 by its binding with Ni-NTA matrix. Proteins obtained in soluble fraction were purified under native lysis condition. Crude protein was purified by Ni-NTA affinity super flow slurry by batch process and all fractions were analyzed on SDS-PAGE. After purification, recombinant proteins were dialyzed in cellulose membrane with a cut of size of 12.4 kDa against water for the removal of salts and protein concentration was estimated using BCA protein estimation kit (Sigma, USA).

4) **Antibody Production**

In the present work, polyclonal antibodies were raised in mice and rabbit immunized with rSEC2. The immunization was done at an interval of seven days, i.e., 0, 7, 14, 21, 28, 35, 42nd day, and the dose given to rabbit was 500ng, 2µg, 10µg, 100µg, 500µg, 500µg, 500µg respectively and dose given for mice was 100ng, 500ng, 1µg, 10µg, 50µg, 50µg, 50µg respectively. The first titre was checked after 6th dose i.e. after 35 days by Western blot for both rabbit and mice. The titre was 1:64000 for both mice and rabbit. The 7th dose was given on 42nd day and once again titer was estimated by western blot and ELISA and the titer was 1:128000 by both western blotting and ELISA.

5) **Cross reactivity/Specificity analysis of mice and rabbit anti SEC2 antiserum**

ELISA was done with mice and rabbit anti SEC2 antisera for analyzing the cross reactivity against SEA, SEB, SED, SEH and BSA. Rabbit anti-SEC2 serum (1:1000) and Mice anti-SEC2 serum (1:1000) were used for the analysis.

6) **Sensitivity analysis of anti SEC2 antiserum**

Checker board ELISA results revealed that 1:400 dilution of rabbit antiserum is suitable for capturing of antigen and 1:800 dilution of mice antiserum is suitable for revealing of antigen. In Sandwich ELISA, for sensitivity evaluation 1:400 dilution of rabbit anti-SEC2 serum was used as capture and mice anti-SEC2 serum (1:800) was used as revealing antibody.

RESULT AND DISCUSSION

In the present study, sec2 gene of S. aureus isolate was amplified by PCR that resulted in 720 bpamplicon using Pfu DNA polymerase to generate blunt ends for cloning into the compatible pQE30Xa vector. The PCR amplified sec2 gene of 720 bp was ligated to pQE30Xa vector having Histidine tag as fusion partner.

The recombinant vector containing the sec2 gene was transformed into E. coli SG13009. Three of the seven transformants were found to contain the sec2 insert in proper orientation as revealed by PCR analysis (Fig 2). The vector plasmid containing sec2 gene was isolated from recombinant clones for DNA sequencing using QIAprep spin mini prep kit (QIAGEN, Germany). The gene sequence was found identical with that of sec2 gene of S. aureus available in the gene bank (Accession No- AY450554) (Fig.3a & 3b).

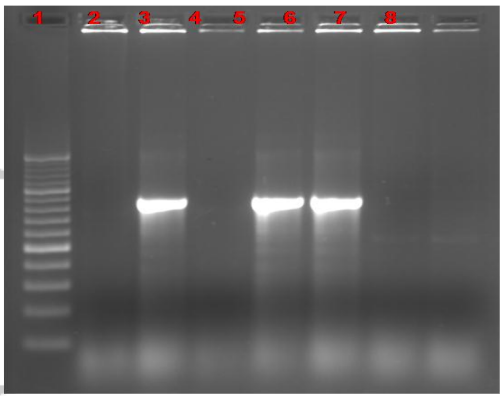
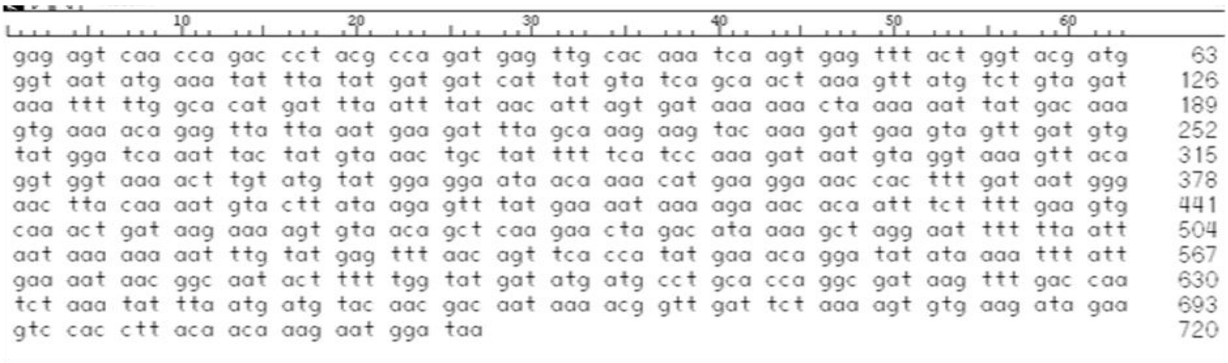


Fig 2: Screening of recombinant clones for *sec2*
Lane 1: 100 bp Ladder
Lane 2: sec2 clone 1
Lane 3: sec2 clone 2 showing amplicon at 845bp
Lane 4: sec2 clone 3
Lane 5: sec2 clone 4
Lane 6: sec2 clone 5 clone 2 showing amplicon at 845 bp
Lane 7: sec2 clone 6 clone 2 showing amplicon at 845 bp
Lane 8: sec2 clone 7

Fig 2: *Screening of Recombinant Clones for sec2*



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gag agt caa cca gac cct acg cca gat gag ttg cac aaa tca agt gag ttt act ggt acg atg    63
ggt aat atg aaa tat tta tat gat gat cat tat gta tca gca act aaa gtt atg tct gta gat   126
aaa ttt ttg gca cat gat tta att tat aac att agt gat aaa aaa cta aaa aat tat gac aaa   189
gtg aaa aca gag tta tta aat gaa gat tta gca aag aag tac aaa gat gaa gta gtt gat gtg   252
tat gga tca aat tac tat gta aac tgc tat ttt tca tcc aaa gat aat gta ggt aaa gtt aca   315
ggt ggt aaa act tgt atg tat gga gga ata aca aaa cat gaa gga aac cac ttt gat aat ggg   378
aac tta caa aat gta ctt ata aga gtt tat gaa aat aaa aga aac aca att tct ttt gaa gtg   441
caa act gat aag aaa agt gta aca gct caa gaa cta gac ata aaa gct agg aat ttt tta att   504
aat aaa aaa aat ttg tat gag ttt aac agt tca cca tat gaa aca gga tat ata aaa ttt att   567
gaa aat aac ggc aat act ttt tgg tat gat atg atg cct gca cca ggc gat aag ttt gac caa   630
tct aaa tat tta atg atg tac aac gac aat aaa acg gtt gat tct aaa agt gtg aag ata gaa   693
gtc cac ctt aca aca aag aat gga taa                                                  720
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Figure 3(a): DNA sequence of *sec2* gene

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ESQ PDP TPD ELH KSS EFT GTM GNM KYL YDD HYV SAT KVM SVD KFL AHD LIY NIS DKK LKN YDK    63
VKT ELL NED LAK KYK DEV VDV YGS NYY VNC YFS SKD NVG KVT GGK TCM YGG ITK HEG NHF DNG   126
NLQ NVL IRV YEN KRN TIS FEV QTD KKS VTA QEL DIK ARN FLI NKK NLY EFN SSP YET GYI KFI   189
ENN GNT FWY DMM PAP GDK FDQ SKY LMM YND NKT VDS KSV KIE VHL TTK NG.                   240
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Figure 3(b): Deduced amino acid sequence of *sec2* gene

Fig 3: *a) DNA Sequence of sec2 gene and b) Deduced amino acid sequence of sec2 gene*

Recombinant clone 2 which showed inducible expression of SEC2 fusion protein in E. coli SG13009 was selected for further study. A thick band of SEC2 was observed at 29.7kDa position upon induction of recombinant clones by IPTG.Position of SEC2 was localized and it was found to be present in the soluble form in the periplasmic region. Further conditions for its optimum expression were standardized like inducer concentration and induction duration. The yield of r-SEC2 obtained from clone 2 was found to be 12.975mg/L of the culture broth under optimized conditions of induction duration (4h) and IPTG concentration (0.75mM) (Fig 4).

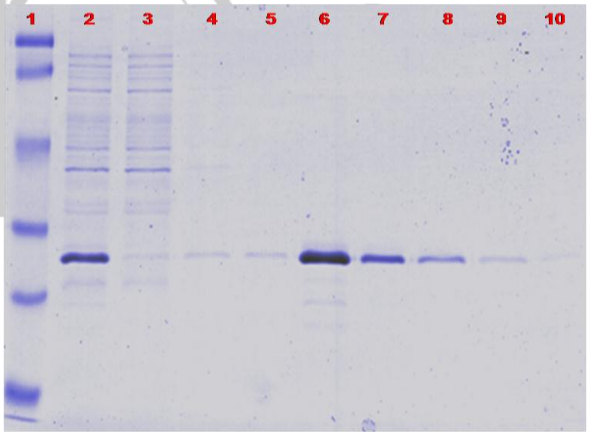

Fig 4: Affinity purification of r-SEC2 protein
Lane 1: MW marker (97.4, 66.2, 45.0, 31.0, 26.6,14.4 kDa)
Lane 2: Cell lysate
Lane 3: Flow through
Lane 4: wash 1
Lane 5: wash 2
Lane 6: elute 1 showing pure r-SEC2 protein at 29.7kDa
Lane 7: elute 2
Lane 8: elute 3
Lane 9: elute 4
Lane 10: elute 5

Fig 4: *Affinity purification of r-SEC2 protein*

Expression of recombinant SEC2 encoded by pQE30Xa is rapidly induced by the addition of isopropyl-β-D-thiogalactoside (IPTG) which binds to the lac repressor protein and inactivates it. Once the lac repressor protein is inactivated, the host cells RNA polymerase can transcribe the sequences downstream from the promoter. The transcripts produced are then translated into recombinant SEC2.In this study, recombinant protein was purified under native condition using Ni-NTA resin. Nitrilotriacetic acid (NTA) is a tetradentate chelating adsorbent which occupies four of the six ligand binding sites in the coordination spheres of the nickel ion, leaving two sites free to interact with 6xHis tag. The 6xHis tag facilitated binding of His-tagged protein with Ni-NTA for ease of purification. Further, 6xHis tag is poorly immunogenic, so its removal is not required for antibody generation against the antigen of interest. Moreover, the 6xHis tag does not interfere with the structure and function of the proteins as demonstrated for a wide variety of proteins, including enzymes (Liu *et al*., 2005), transcription factors and vaccines (Knap *et al*., 1999; Uhl *et al*., 2004; Hu *et al*., 2005; Pier *et al*., 2008). NTA binds metal ions far more stable than other available chelating resins like IDA (iminodiacetic acid) (Hochuli, 1989) and retains the ions under stringent wash conditions. The recombinant protein, SEC2, was eluted from Ni-NTA resin by decreasing the pH of the elution buffer upto 4.5 because at the lower pH (acidic) 6xHis-tag dissociates from Ni-NTA resin and can no longer compete for binding sites on the Ni-NTA resin. Purification was carried out using repeated washings to remove nonspecifically bound protein on Ni-NTA and elution steps were repeated five times so that all 6xHis recombinant protein could be retrieved. The purified recombinant protein was pure as assessed on SDS-PAGE. The whole purification process was carried out at 4^{o}C to prevent protein degradation. Purified recombinant protein was dialyzed with a gradual exchange of buffer to completely remove the salts present in elution buffer and to retain the biologically active form. The final exchange of buffer was done with deionized water. The purified, dialyzed recombinant SEC2 protein was then used for immunization.

In the present work, rSEC2 fusion protein was further used for raising antibodies in rabbit and mice to generate humoral immune response to study its suitability for detection of SEC2. Mice were immunized through intraperitonial route because it is easy and appropriate for small rodents as antigen injected into the peritoneum is drained directly into the thoracic lymphatics and then to vena cava (Harlow and Lane, 1988). On the other hand rabbit was immunized through subcutaneous route. Antigen could be given with alum adjuvant safely by this route. Alum adjuvant is the only adjuvant used widely in routine human and veterinary vaccines (Gupta, 1998).

The immunization schedule followed for rabbit was of 0, 7, 14, 21, 28, 35, 42, 49 days giving the dose of 500ng, 2µg, 10µg, 100µg, 500µg, 500µg, 500µg, 500µg. The immunization schedule followed for mice was of 0, 7, 14, 21, 28, 35, 42, 49 days giving the dose of 100ng, 500ng, 1µg, 10µg, 50µg, 50µg, 50µg, 50µg. The titer anti r-

SEC2 serum obtained was 1:128000 by both western blotting and ELISA, which is a suitably high titer for any antibody to be used for the detection system (Fig5a, Fig5b, Fig6a, and Fig6b).

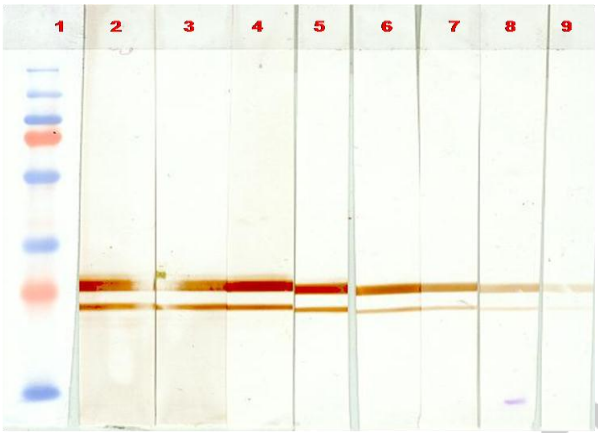

Fig 5(a): Titer of final bleed of rabbit anti r-SEC2 antiserum

Lane 1: MW marker (250, 130, 100, 70, 55, 35, 27, 15, 10kDa)

Lane 2-9: serial dilution of antiserum 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000, 1:64000, 1:128000 respectively showing band at 29.7kDa

Fig 5(a): *Titer of final bleed of rabbit anti r-SEC2 antiserum*

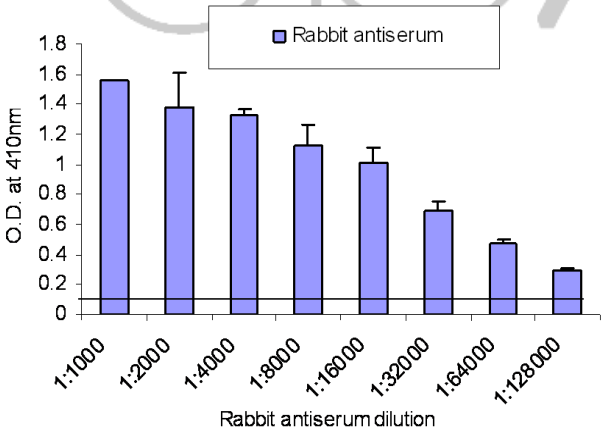

Fig 5(b): *Titer of final bleed of rabbit antiserum raised against r-SEC2. The data represent mean of triplicate ±SD. OD of negative control is 0.0685 at 1:1000 dilution.*

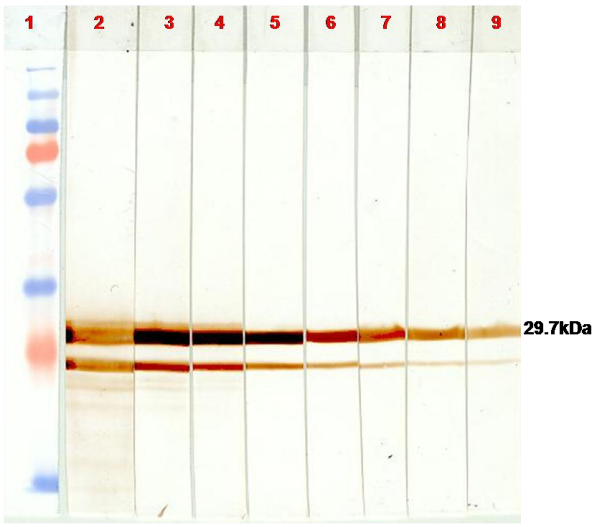

Fig 6(a): Titer of final bleed of mice anti r-SEC2 antiserum

Lane 1: MW marker (250, 130, 100, 70, 55, 35, 27, 15, 10kDa)

Lane 2-9: serial dilution of antiserum 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000, 1:64000, 1:128000 respectively showing rSEC2 band at 29.7kDa

Fig 6(a): *Titer of the final bleed of mice anti r-SEC2 antiserum*

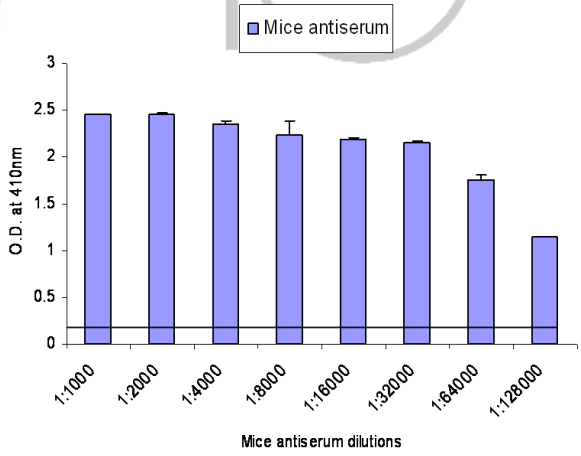

Fig 6(b): *Titer of final bleed of mice antiserum raised against r-SEC2. The ELISA titre data represent mean of triplicate ± SD. OD of negative control is 0.105 at 1:1000 dilution*

These antibodies were further tested for their sensitivity and specificity for their later use in the development of a suitable detection test like ELISA. ELISA is a useful and powerful method in estimating nanogram to picogram materials in the solution, such as serum, urine and culture supernatant (Freed *et al*., 1982; Fey *et al*., 1984; Schotte*et al*., 2000; Cook *et al*., 2007). Antisera raised in mice and rabbit against SEC2 were found to detect SEC2 with sensitivity of 0.244ng/ml through sandwich ELISA.The data suggested that antibodies raised against SEC2 proteins are suitable for use as diagnostic reagents. Sensitivity was statistically determined by calculating the cut-off level for negative control as described by several workers (Snyder *et al*., 1983) (Fig 7).

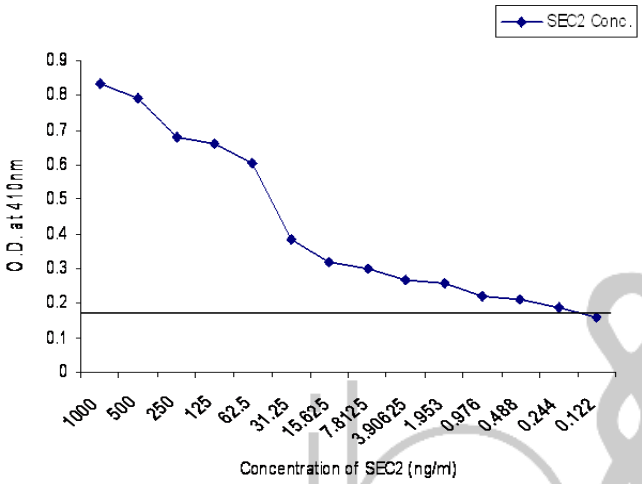

Fig 7: *Sensitivity measurement of anti r-SEC2 antiserum by sandwich ELISA. Rabbit antiserum capture at 1:400 dilution and mice antiserum revealed at 1:800 dilution. The data represents mean of duplicate ± SD. Cut off value is 0.16048.*

Antisera raised in mice and rabbit were analyzed for their cross reactivity with other closely related enterotoxins using ELISA. The cross reactivity of antisera with various toxins, i.e., SEA, SEB, SED, SEH and BSA (1µg/well) was analyzed.Results showed that the anti SEC2 antiserum was highly specific for SEC2 and it has no cross reactivity with any of the toxin type used in the present study. For sensitivity and cross reactivity analysis in ELISA, 1:1000 dilution of mice antisera and rabbit antisera were used(Fig 8a, Fig 8b).

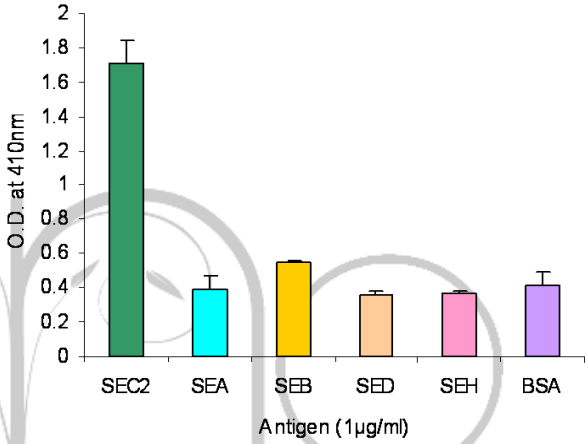

Fig 8a: *Cross reactivity analysis of anti r-SEC2 antiserum generated in rabbit model. The data represent mean of duplicate ± SD.*

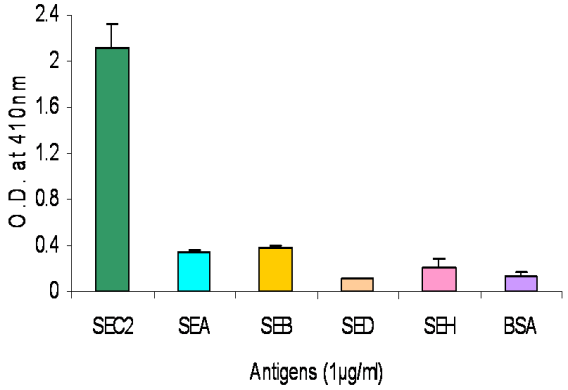

Fig 8b: *Cross reactivity analysis of anti r-SEC2 antiserum generated in mice model. The data represent mean of duplicate ± SD.*

Results obtained in the present study with respect to purity of r-SEC2 and titer of anti SEC2 antibodies indicate that the heterologous expression of SEC2 is a suitable alternative for producing high tittered and specific antibodies.

SEC2 is also one of the common causes of food poisoning. Therefore, it is essential to develop immunological detection system for SEC2. But the success of immunoassay depends on purity of SEC2 used for raising anti-SEC2 antibodies. A slight contamination of SEC2 with other bacterial proteins results in antibodies that are less specific to SEC2. Therefore it is desirable to express the protein with a suitable fusion protein to facilitate its detection and purification (Nilsson *et al*., 1997; Constans, 2002; Mukherjee *et al*., 2003, Kamboj*et al*., 2006).

Staphylococcus aureus and *Streptococcus pyogenes* produce a family of related pyrogenic toxins (PTs) with similar biological and biochemical properties. Of the seven SEs (types A, B, C1, C2, C3, D, and E), the three type C Streptococcus Enterotoxins (SEC1, SEC2, and SEC3) are immunologically most closely related (Avena and Bergdoll, 1967; Borja and Bergdol, 1967; Reiser*et al*., 1984). They possess cross-reactive and unique antigenic epitopes and react with heterologous SE type C antisera in immunodiffusion assays (Reiser*et al*., 1984). The 5 major serological groups of enterotoxins fall in 2 sub groups: SEB, SEC1, SEC2 & SEC3 which have 66-98% amino acid sequence identity and SEA, SED and SEE, which have 53-81% amino acid sequence identity (Anvari*et al*., 2008).Cross reaction between SEB and SECs and between SEA and SEE have been reported (Sergeev *et al*., 2004). The SEC subtypes (C1, C2, and C3) possess subtype-specific and type C-common epitopes that cross-react with less-related PTs (Bohach*et al*., 1988; Reiser *et al*., 1984; Thompson *et al*., 1984). They also elicit biological properties shared by all PTs, in addition to emetic capability.

In the present study the system could detect 0.244ng/100µl SEC2 with an OD value of 0.188. This value is higher than that of the cut off value which is 0.16048.The mean OD value for the various toxins in the cross reactivity study was in the range of 0.36-0.55 for rabbit anti-SEC2 serum which was significantly lower than the r-SEC2 value (1.7130). In case of mice anti-SEC2 OD values for the various toxins were in the range of 0.11-0.37, which were again lower than the r-SEC2 value (2.1220). Therefore results showed that the anti SEC2 antiserum was highly specific for SEC2 and it has no cross reactivity with any of the toxin type used in the present study. So conclusion of the whole study is that the antibody raised against recombinant SEC2 can be used as a diagnostic reagent for sensitive and specific detection of staphylococcal enterotoxin C2 (SEC2) using sandwich plate ELISA system.

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