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CLONING OF *CHIA* FROM *SERRATIA MARCESCENS* AND ITS EXPRESSION IN *E. COLI*

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ABSTRACT
Six best isolates of *Serratia marcescens* were selected for studying variant of *chi*A by ARFLP method. Except AUDS170*chi*A, *chi*A from other isolates showed similar (but different from sm141*chi*A) profile with *Pst*I. Full length sequence analysis of AUDS096*chi*A and AUDS106*chi*A showed 97.40% and 97.34% similarity with sm141*chi*A. The expression profile based on SDS PAGE and hydrolysis of colloidal chitin proved *chi*A from AUDS106 is the best. In-silico analysis of sequence revealed that most of variants in the genes are at wobble position which does not alter the amino acid they code. But the variations in AUDS096*chi*A changed the GC content (54.72% to 66.03%) leading to reduced translational efficiency in *E. coli*. The variant of gene from different isolates may vary in function to a larger extent and hence classification of isolate from less to more potent.

INTRODUCTION

Around 10% to 20% loss in crop production is due to disease, pest and weed (Kumar and Gupta, 2012). Majority of plant diseases are caused by fungi. Application of agrochemicals and developments of resistant cultivars are the major approaches for the management of plant disease. An alternative approach to avoid the undesired effects of chemical control is the biological control using antifungal bacteria that exhibit a direct action against fungal pathogens (Neeraja *et al.,* 2010). Most of plant pathogenic fungal cell wall is a complex structure composed typically of chitin, 1, 3-β and 1, 6-β glucans of β 1, 4-linked N acetyleglucosamine (Adams, 2004). Chitinases (E.C 3.2.2.14) are glycosyl hydrolases which cleaves a bond between C1 and C4 of two consecutive N-acetylglucosamine of chitin (Gokul *et al.,* 2000). The Gram negative bacterium *Serratia marcescens* secretes a variety of extracellular enzymes including chitinases (Hines *et al.,* 1988). It is one of the most effective bacteria for degradation of chitin (Monreal *et al.,* 1969). When this bacterium is cultivated in the presence of chitin, a variety of chitinolytic enzymes and chitin binding proteins are produced (Fuchs *et al.,* 1986). The chitinolytic activity of *S. marcescens* is of great interest because it is one of the best characterized chitinolytic machineries known to date (Perrakis *et al.,* 1994). Detailed studies by a number of groups have showed that *S. marcescens* produces at least three chitinases (*chi*A, *chi*B, *chi*C), a chitobiose and a putative chitin binding protein (CPB21) (Vaaje-Kolstad *et al.,* 2005). However, it is not certain that these five enzymes represent the complete chitinolytic machinery of bacterium.

S marcescens isolated from peanut hull showed antagonistic activity against mycelia growth and subsequent aflatoxin production (Wang *et al.,* 2014). *chi*A isolated from soil bacteria (*S. marcescens*) and cloned in *E. coli* and tobacco showed chitinolytic activity (Malatheshaih *et al.,* 2011). *S. marcescens* ChiA was heterologously expressed in an anti-Coleopteran *Bacillus thuringiensis* at levels even higher than that produced by the source organism (Okay *et al.,* 2008). Genetic engineering of plants with the potential genes accelerates plant breeding for disease management. However, development of resistance to transgene by pest is a major problem (Frederic, 1998). A pool of genes with some variability in nucleotide is required to overcome the problem of development of resistance by pest. Isolates of a species are known for variations in their activity which can be used for specific purpose (Pawar and Ingle, 2014). *Trichoderma harzianum* and *Pseudomonas fluorescens* isolates showed varied degree of inhibition against *Exserohilum turcicum* (Singh and Singh, 2014). So far the information on the diversity and distribution of bacterial chitinases is limited. *Chi*A isolated from different sources showed variation in 7-26 amino acid residue (Wu *et al.,* 2009). In this study, *chi*A from potent isolates of *S. marcescens* was amplified, profiled for variability by restriction polymorphism. And also full length *chi*A from two isolates was cloned, sequenced, characterized and expressed in *E. coli*.

MATERIALS AND METHODS

Isolation of DNA from isolates of *S.marcescens*

The *S. marcescens* isolates used in this study were collected from Biligiriranga hills Mysore, Karnataka and earlier studied for their chitin degradation efficiency and also profiled the chitinase gene content. Six isolates which produced 5 mm clearance zone on colloidal chitin agar and showed to harbor *chi*A, *chi*B and *chi*C were selected for this study. The DNA was isolated from 10 mL of overnight culture of *S. marcescens* by chemical and enzymatic lysis, separation by phenol:chloroform:isoamyl alcohol and precipitated by isopropanol (Sambrook and Russell, 2001). Briefly, the overnight culture was treated with 25mg lysozyme, 2.5 mg proteinaseK and 250 μg of RNaseA in 50 mM Tris and 20 mM EDTA buffer. The supernatant was mixed with equal volume of phenol:chloroform: isoamyl alcohol (25:24:1) and phase separated. The DNA was precipitated by adding pre-chilled isopropanol to aqueous layer. The pellet was washed, dried and re-suspended in 10mM Tris 1Mm EDTA.

Amplification of *chi*A and Restriction Fragment Length Polymorphism (ARFLP)

The *S. marcescens* isolates (AUDS096, AUDS106, AUDS154, AUDS165, AUDS166 and AUDS170) which produced 5mm (largest) clearance zone and contain *chi*A, *chi*B and *chi*C were used for checking novelty by Amplicon Restriction Fragment Length Polymorphism (ARFLP) (Kuo and Chak 1996). During this study, chiA isolated from sm141 by Malatheshaiah *et al.,* (2011) was used as reference. The full length amplification of *chi*A was done by polymerase chain reaction using the protocol of Malatheshaiah *et al.* (2011). PCR mixture containing 100 ng of template DNA, 2.5 pmol of each primer, 250 μM of dNTP each, 1X Taq buffer and 1 unit of Taq DNA polymerase in a final volume of 20 μL nuclease free water. The primers used for amplification of full length *chi*A are; *chi*Afp 5'TCTAGAAAAGGAATCAGTTATGCGCAAAT3', *chi*Arp 5'GGATCCAACGCAC TGCAACCGATTATT3'. The template DNA was denatured at 95ºC for 5 minutes for one cycle and amplified for 32 cycles of 94ºC for 1 minute, 55ºC for 45 seconds followed by primer extension at 72ºC for 1 minute. The amplified product was purified by using MinElute gel extraction kit (Qiagen) by following manufacturer's instruction. Based on the sequence information of *Sm141chi*A, *Pst*I, *Sal*I, *Sma*I and *Xma*I restriction endonucleases (Bangalore Genei) were used. One unit of each enzyme was used for digesting 100 ng of amplified and purified DNA in 20μL reaction mixture under appropriate buffer, pH and temperature conditions. The restricted DNA was separated in 1% agarose gel, stained by EtBr and documented.

Molecular characterization of *chi*A

*chi*A from AUDS096 and AUDS106 was cloned by TA cloning in pTZ57R/T. PCR compositions and conditions (except for final extension of 45 minutes to add dATP at the 3' end) followed were same as mentioned above. The amplified product was separated in 0.8% agarose gel and purified using MinElute gel extraction kit (Qiagen) by following manufacturer's instruction. 30 μL ligation mixture containing 1 X cohesive end ligation buffer, 0.17 pmol ends of vector, 0.51 pmol ends of insert and 1 unit of T4 DNA ligase and incubated overnight at 16ºC. Competent *E. coli* DH5a (prepared by $CaCl_2$ method, Smbrook and Russell, 2001) was transformed with the above ligation mixture by heat shock at 42ºC for 2 minutes. The transformants selected on Luria Bertani agar containing ampicilin (100μg/mL), nalidixic acid (10μg/mL), X-gal (16 mM) and IPTG (16 mM) are finally confirmed by PCR and restriction digestion (*Hind*III and *Eco*RI). The putative recombinant clones were confirmed for presence of *chi*A by PCR using gene specific primer followed by sequencing. Ends of the gene were sequenced using M13 universal primer and then primer walking using internal primers. Forward and reverse sequence reads were joined by cap contig assembly using Bioedit software (Hall, 1999). Full length sequence was submitted to NCBI for similarity search using BLAST (Altschul *et al.,* 1990). Variation in AUDS096*chi*A and AUDS106*chi*A nucleotide sequence was analysed by ClustalW multiple alignment in Bioedit tool (Thompson *et al.,* 1994). The nucleotide sequence was *in silico* translated to amino acid by Bioedit software. The similarity of deduced amino acid sequence of AUS096*chi*A and AUDS106*chi*A was compared with sm141 chitinaseA protein by BLASTp (Altschul *et al.,* 1997). The effect of mutation on activity of chitinaseA at each amino acid position was predicted by PredictProtein (www.predictprotein.org) (Rost *et al.,* 2004).

Expression of *chi*A in *E. coli*

AUDS096chiA and AUDS106chiA from cloning vector was released and cloned into prokaryotic expression vector pET32^{c+} (Invitrogen) using *Hind*III and *Eco*RI restriction endonucleases in presence of appropriate temperatre, pH and ionic concentration. The transformants were selected on Luria Bertani agar containing 100μg/mL ampicilin and the recombinant clones were confirmed by PCR and restriction digestion as mentioned above. The induction of recombinant clones and isolation of crude protein from cytoplasmic fraction of cell was done by following guidelines provided by Invitrogen. Briefly, 1mM of IPTG was used to induce the log

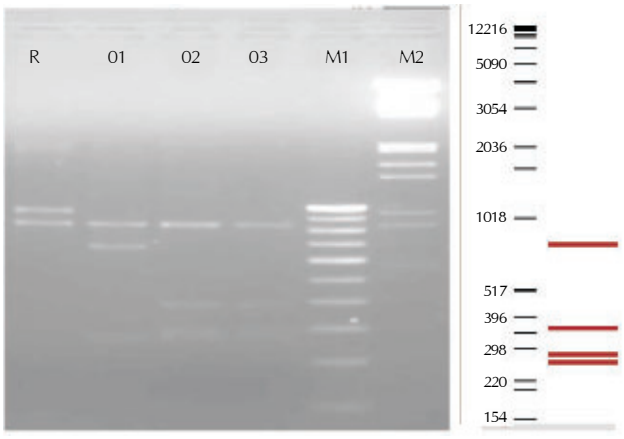

Figure 1: Amplified Restriction Fragment Length Polymorphism (ARFLP) profile of *chi*A. R is *sm*141*chi*A, 1, 2 and 3 are AUDS170*chi*A, AUDS096*chi*A and AUDS106*chi*A. M1 and M2 are 100 bp and Lambda *Hind*III single digest DNA ladder respectively. All the three isolates produce profile different from *sm*141*chi*A but the profile produced by AUDS096*chi*A and AUDS106*chi*A is same. The right panel depicts the computer simulated banding pattern of AUDS096 *chiA* and AUDS106 *chiA*

phase culture and allow it to produce protein for two hours under shaking condition at 37ºC. The protein in crude extract was quantified by Lowry's method (Lowry *et al.,* 1951). 12.5 ng of crude protein was loaded in 12% SDS-PAGE (prepared and run as mentioned by Smbrook and Russell, 2001). The gel was stained by comassie brilliant blue and then destained to get clear bands. 5 % Colloidal chitin was prepared according to Roberts and Selitrennikoff (1998). Chitin plates were prepared with chitin agar medium containing 1% colloidal chitin, 0.5% yeast extract, 0.05% $MgSO_4$, 0.2% sodium nitrate, 0.05% KCL, $FeSO_4$ pinch, 0.1% K_2HPO4 and 1.8% agar (w/v), pH adjusted to 6.0 using 1N NaOH/HCl. 40ng of total protein (cytoplasmic fraction, isolated from recombinant *E. coli* BL21DE3) was spot on 1.0% colloidal chitin agar plate. Similarly, 40 ng of uninduced and induced non-recombinant E. coli BL21DE3 cell culture was also spot on 1.0% colloidal chitin agar. The zone of clearance was measured at 5th day.

RESULTS AND DISCUSSION

Full length *chi*A was amplified from six best isolates based on colloidal chitin solubalisation assay. To find the variants of chiA, we used ARFLP method which was designed by Kuo and Chak (1996) to detect novel cry genes. This technique was also used later by Wang *et al.* (2003) to detect new cry genes. The full length *chi*A was purified and restricted with *Pst*I, *Sal*I, *Sma*I and *Xma*I restriction endonucleases. Variant form of *chi*A was observed only with *Pst*I for AUDS096, AUDS106, AUDS154, AUDS165, AUDS166 and AUDS170. Except AUDS170, the *chi*A amplified from all isolates yield similar restriction profile (but different from *sm*141*chi*A) (Fig. 1). Pooja *et al.* (2013) identify variant of *cry*1I in *Bacillus thuringiensis* using similar method. Difference in restriction profile of DNA indicates there is a difference in nucleotide sequence. But the diversity of a gene may not be detected always by restriction profiling because the variation in nucleotide might have not created site for restriction endonuclease. We carried further the cloning and expression of *chi*A from AUDS096 and AUDS106 only, henceforth they are called as AUDS096*chi*A and AUDS106*chi*A respectively.

Nucleotide sequence of AUDS096*chi*A and AUDS106*chi*A (Accession number KP728831 and HQ219075) are 97.40% and 97.34% similar with sm141chiAand 97.81% similarity between AUDS096*chi*A and AUDS106*chi*A. Similarly, amino acid sequence of AUDS096*chi*A and AUDS106*chi*A are 99.11% and 99.82% similar with sm141chiA and 99.29% similarity between AUDS096*chi*A and AUDS106*chi*A. The BLASTn and BLASTp result also showed similarity of these sequences to *chi*A of *S. marcescens* (supplementary figure). We also confirmed the isolates by 16S rDNA sequencing (results not shown). These results indicate that the cloned genes are variant of *sm*141*chi*A and are from same species (*S. marcescens*) (Tindall *et al.,* 2010). In-silico analysis of full length *chi*A showed presence of site for *Pst*I at 362, 645 and 906 bp in AUDS096chiA and AUDS106chiA. These results are in agreement with the results obtained in ARFLP where we obtained 4 bands of 786, 362, 283 and 261 bp size (Fig. 1). The variation in nucleotide and their corresponding amino acid sequence of *sm*141*chi*A and AUDS106*chi*A is shown in Fig. 2. Most of the variations in AUDS096chiA and AUDS106chiA are at wobble position and the variant genes still maintain the amino acid they codes (Fig. 2). This may be one of the reasons for low level expression of chiA in AUDS096*chi*A isolates of the same species (Liu and Xue, 2005).

The crude protein isolated from induced and uninduced culture of *E. coli* containing cloned *chi*A was separated in 12% SDS-PAGE. A presence of clear and distinct band of approximately 58.5 kDa in only the induced culture containing cloned *chi*A confirms the expression of recombinant protein (Fig. 3). Similar size of chiA band was observed by Malatheshaiah *et al.* (2011), Watanabe *et al.* (1997), and Brurberg *et al.* (1994). *chi*A of 1692bp codes for precursor protein of 563 amino acids but N terminal signal peptide is cleaved while secreting from the cell (Brurberg *et al.,* 2000) resulting an enzyme of 540 amino acids and a calculated molecular mass of 58.5 kDa (Perrakis *et al.,* 1994). The processing of N terminal signal peptide from precursor chiA of *Aeromonas caviae* and production of active enzyme in *E. coli* was observed by Sitrit *et al.* (1995). Similarly removal of N terminal signal peptide of *S marcescens chiB* was observed by Suzuki *et al.* (2002) *in E coli*. It is assumed that the N terminal signal peptide is removed during transportation of enzyme across the cell membrane. A transparent clear zone in opaque media of colloidal chitin was observed only in protein isolated from induced culture (or protein) containing *chi*A cloned from AUDS106 (fig4). Although AUDS096*chi*A produced expected size band in SDS-PAGE but it failed to show chitinolytic activity in *E. coli*. Even the band intensity of AUDS096*chi*A in SDS-PAGE is faint compare to AUDS106*chi*A. Reduced/loss of chitinolytic activity in AUDS096*chi*A cloned in *E. coli* may be due to mutation in active site or reduction in translation efficiency in heterologous system. mRNA expression among clinical isolates of *Mycobacterium tuberculosis* demonstrates that genes with important functions can vary in their expression levels between strains grown under identical conditions (Gao *et al.,* 2005).

Expression of heterologous gene in microbial expression system lead to reduced translation efficiency or errors in amino acid sequence of protein product (Smith, 2008). The GC content of wobble position is most important for gene expression (Pasha *et al.,* 2015). Most of the nucleotides are replaced by G or C at wobble position in AUDS096*chi*A leading to poor expression in *E. coli*. The overall GC content in sm141*chi*A, AUDS096*chi*A and AUDS106*chi*A and *E. coli* BL21 is 58.51%, 58.87%, 58.22% and 52.35% respectively. The GC content at wobble position in sm141*chi*A, AUDS096*chi*A and AUDS106*chi*A is 54.72%, 66.03% and 39.62% respectively. The variation in nucleotides also created variation in amino acids; six and three amino acids were changed in AUDS096*chi*A and AUDS106*chi*A respectively (Fig. 2). To check whether the changes in amino acid lead to loss of activity in AUDS096*chi*A, we fed amino acid sequence to predict protein software. Interestingly, all the altered amino acids are neutral in action except replacement of valine by glutamic acid at 130th position in AUDS096*chi*A (Fig. 5). Valine is non-polar aliphatic amino acid whereas glutamic acid is hydrophilic and negatively charged amino acid. Most of the point mutations are neutral, while some destroy protein structure and hence hamper biological functions of a cell

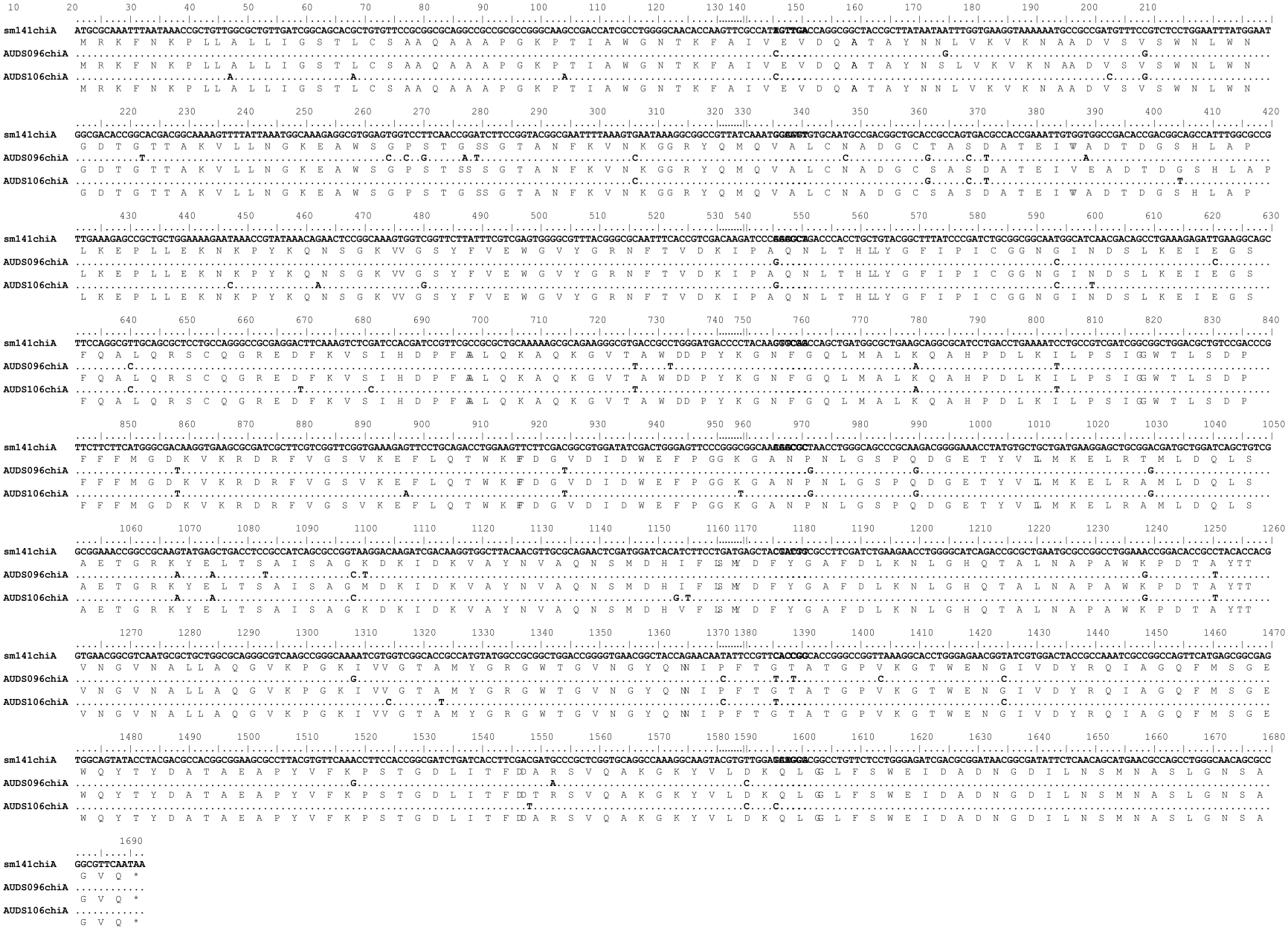

Figure 2: Comparison of nucleotide and their translated amino acid sequence of *sm*141*chi*A, AUDS096*chi*A and AUDS106*chi*A. Yellow, red and green bars indicate variation in only AUDS096chiA, only AUDS106*chi*A and both respectively. Six and three amino acids are different in AUDS096*chi*A and AUDS106*chi*A respectively compared to sm141*chi*A

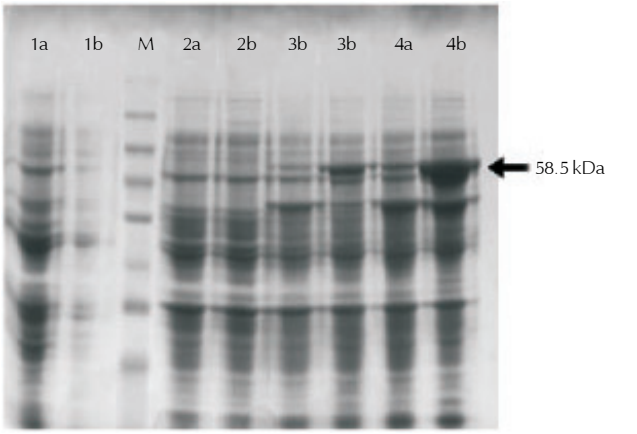

Figure 3. SDS-PAGE profile of crude protein isolated from *E.coli*. 1; *E.coli*BL21DE3, 2; *E.coli*BL21DE3 containing pET32^{C+} (prokaryotic expression vector), 3; *E.coli*BL21DE3 containing pET32^{C+} AUDS096*chi*A and 4; *E.coli*BL21DE3 containing pET32^{C+}AUDS106*chi*A. a and b are uninduced and induced cultures respectively. M: Pre-stained Protein Ladder marker SM0671. A thick band of 58.5kDa is seen only in induced cultures containing cloned *chi*A

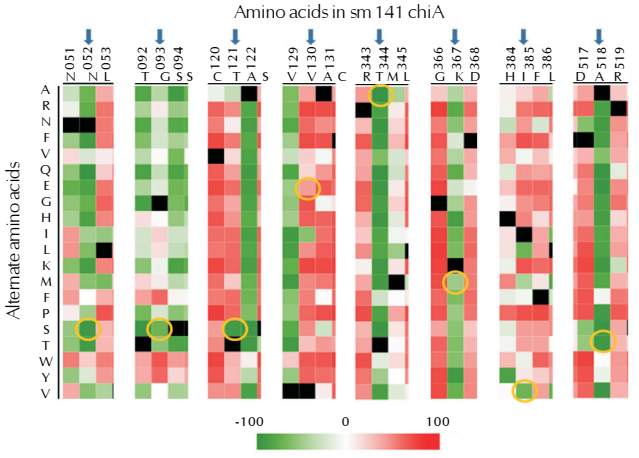

Figure 5: Snapshot of predict protein: effect of point mutation window. Amino acids in sm141chiA are in X axis (top line) and alternate are in Y axis (left vertical). Each box in different colour indicates the effect of alternate amino acid at that position. Red, white and green colour indicates strong, medium and weak/no effect of point mutation. The variation observed (yellow circle) at all positions in both genes does not have effect on activity of protein except one variation in AUDS096chiA at 130th position

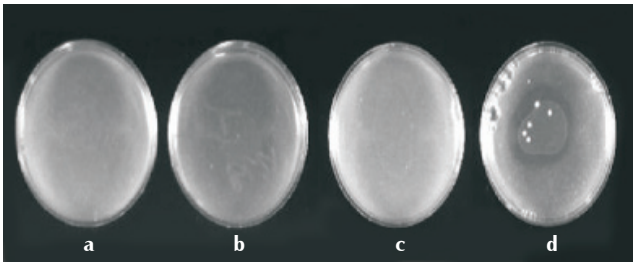

Figure 4: Colloidal chitin solubalisation assay. a and c is protein from uninduced culture of *E.coli* BL21DE3 containing pET32^{C+}AUDS096*chi*A and *E.coli*BL21DE3 containing pET32^{C+}AUDS106*chi*A respectively whereas b and d is from their induced cultures. A clear transparent zone is observed only in induced AUDS106*chi*A

Supplementary figure. BLAST analysis of AUDS096chiA and AUDS106chiA. a and b are BLASTn result of AUDS096chiA and AUDS106chiA respectively. c and d are BLASTp result of AUDS096chiA and AUDS106chiA respectively.

(Matthews, 1987). The chitinolytic activity observed in *S. marcescens* AUDS096 isolate might be due to presence of other active/functional chitinolytic enzymes (Vaaje-Kolstad *et al.*, 2013).

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REFERENCES

Adams, D. J. 2004. Fungal cell wall chitinases and glucanases. *Microbiol.* **150(7):** 2029-2035.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J. 1990. Basic Local Alignment Search Tool. *J. Mol. Biol.* **215:** 403-410.

Altschul, S. F., Madden, T. L., Schaffe,r A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25:** 3389-3402.

Brurberg, M. B., Eijsink, V. G. H. and Nes, 1. F. 1994. Chitinases ChiA and ChiB from Serratia marcescens BJLZOO. *Doctoral thesis. Agricultural University of Norway, As, Norway.*

Brurberg, M. B., Synstad, B., Klemsdal, S. S., Van Aalten, D. M. F., Sundheim, L. and Eijsink, V. G. H. 2000. Chitinases from Serratia marcescens. *Recent Res. Dev. Microbiol.* **5:** 187-204.

Frederick, R. D., Thilmony, R. L., Sessa, G. and Martin, G. B. 1998. Recognition specificity for the bacterial avirulence protein AvrPto is determined by Thr-204 in the activation loop of the tomato Pto kinase. *Mol. Cell.* **2(2):** 241-245.

Fuchs, R. L., Mcpherson, S. A. and Drahos, D. J. 1986. Cloning of a Serratia marcescens gene encoding chitinase. *Appl. Environ. Microbiol.* **51:** 504-509.

Gao, Q., Kripke, K. E., Saldanha, A. J., Yan, W., Holmes, S. and Small, P. M. 2005. Gene expression diversity among Mycobacterium tuberculosis clinical isolates. *Microbiol.* **151:** 5-14.

Gokul, B., Lee, J. H., Song, K. B., Rhee, S. K., Kim, C. H. and Panda, T. 2000. Characterization and applications of chitinases from Trichoderma harzianum- A review. *Bioprocess Engg.* **23:** 691-694.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. *Nucleic Acids Symp. Ser.* **41:** 95-98.

Hines, D. A., Saurugger, P. N., Ihler, G. M. and Benedik, M. J.1988. Genetic analysis of extracellular proteins of Serratia marcescens. *J. Bacteriol.* **170:** 41-44.

Kumar, S. and Gupta, O. 2012. Expanding dimensions of plant pathology. *JNKVV Res. J.* **46(3):** 286-293.

Kuo, W. S. and Chak, K. F. 1996. Identification of novel cry-type genes from Bacillus thuringiensis strains on the basis of restriction fragment length polymorphism of the PCR-amplified DNA. *Appl. Environ. Microbiol.* **62(4):** 1369-1377.

Liu, Q. and Xue, Q. 2005. Comparative studies on codon usage pattern of chloroplasts and their host nuclear genes in four plant species. *J. Genetics.* **84(1):** 55-62.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Malatheshaiah,T. N., Krishnaraj. P. U., Kempagangaiah, K., Swamidatta, H. S., Narasimhamurthy, K. Y. and Kuruvinashetti, M. S. 2011. Cloning,expression and development of transgenic tobacco using Chi A gene from native isolate of Serratia marcescens 141.*Trans. Plant J.* **5(1):** 72-77.

Matthews, B. W. 1987. Genetic and structural analysis of the protein stability problem. *Biochem.* **26:** 6885-6888.

Monreal, J. and Reese, E. 1969. The chitinase of Serratia marcescens. *Can. J. Microbiol.* **15:** 689-696.

Neeraja, C., Anil, K., Purushotham, P., Suma, K., Sarma, P., Moerschbacher, B. M. and Podile, A. R. 2010. Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. *Crit. Rev. Biotechnol.* **30:** 231-241.

Okay, S., Tefon, B. E., Ozkan, M. and Ozcengiz, G. 2008. Expression of chitinase A (chiA) gene from a local isolate of Serratia marcescens in Coleoptera-specific Bacillus thuringiensis. *J. Appl. Microbiol.* **104(1):** 161-70.

Pasha, M. A., Belgaumwala, A., Kumar, R., Krishnaraj, P. U. and Kuruvinashetti, M. S. 2015. Codon optimization of Serratia marcescens chiA and its expression in tobacco. *J. Anim. Plant Sci.* **25(1):** 254-260.

Pawar, N. B. and Ingle, Y. V. 2014. Protein and isozyme patterns of Rhizoctonia bataticola isolates causing chickpea root rot. *The Bioscan.* **9(3):** 1165-1170.

Perrakis, A., Tews, I., Dauter, Z., Oppenheim, A. B., Chet, I., Wilson, K. S. and Vorgias, C. E. 1994. Crystal structure of a bacterial chitinase at 2.3 a resolution. *Structure.* **15:** 1169-1180.

Pooja, A. S., Krishnaraj, P. U. and Prashanthi, S. K. 2013. Profile of cry from native Bacillus thuringiensis isolates and expression of Cry1I. *Afr. J. Biotechnol.* **12(22):** 3545-3562.

Roberts, W. K. and Selitrennikoff, C. P. 1998. Plant and Bacterial Chitinases Differ in Antifungal Activity. *J. Gen. Microbiol.* **134:** 169-176.

Rost, B., Yachdav, G. and Liu, J. 2004. The PredictProtein server. *Nucleic Acid Res.* **32:** 321-326.

Sambrook, J. and Russell, D. 2001. Molecular Cloning: a Laboratory Manual, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Singh, V. and Singh, Y. 2014. Evaluation of Trichoderma harzianum and Pseudomonas fluorescens isolates for their antagonistic potential against Exserohilum turcicum cuasing leaf blight of sorghum. *The Bioscan.* **9(3):** 1171-1175.

Sitrit, Y., Vorgias, C. E., Chet, I. and Oppenheim, A. B. 1995. Cloning and primary structure of the chiA gene from Aeromonas caviae. *J. Bacteriol.* **177:** 4187-4189.

Smith, D. W. E. 2008. Problems of Translating Heterologous Genes in Expression Systems: The Role of tRNA. *Biotechnol. Progress.* **12(4):** 417-422.

Suzuki, K., Sugawara, N., Suzuki, M., Uchiyama, T., Katouno, F., Nikaidou, N. and Watanabe, T. 2002. Chitinases A, B, and C1 of Serratia marcescens 2170 produced by recombinant Escherichia coli: Enzymatic properties and synergism on chitin degradation. *Biosci. Biotechnol. Biochem.* **66:** 1075-1083.

Thompson, J. D., Higgins, D. G., Gilson, T. J. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specifi c gap penalties and weight matrix choice. *Nucliec Acids Res.* **22:** 4673-4680.

Tindall, B. J., Rossello-Mora, R., Busse, H. J., Ludwig, W. and Kampfer, P. 2010. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int. J. Syst. Evol. Microbiol.* **60:** 249-266.

Vaaje-Kolstad, G., Horn, S. J., Sorlie, M., Eijsink, V. G. H. 2013. The chitinolytic machinery of Serratia marcescens-a model system for enzymatic degradation of recalcitrant polysaccharides *FEBS J.* **280:** 3028-3049.

Vaaje-Kolstad, G., Houston, D. R., Riemen, A. H., Eijsink V. G., van Aalten, D. M. 2005. Crystal Structure and Binding Properties of the Serratia marcescens Chitin-binding Protein CBP21. *J. Biol. Chem.* **280:** 11313-11319.

Wang, J., Boets, A., Van Rie, J. and Renc ¸, G. 2003. Characterization of genes cry1, cry2, and cry9 genes in Bacillus thuringiensis isolates from China. *J. Invertebr. Pathol.* **82:** 63-71.

Wang, K., Yan, P. and Cao, L. 2014. Chitinase from a Novel Strain of Serratia marcescens JPP1 for Biocontrol of Aflatoxin: Molecular Characterization and Production Optimization Using Response Surface Methodology. *BioMed Res. Intl.* 10.1155/2014/482623.

Watanabe, T., Kimura, K., Sumiya, T., Nikaidou, N., Suzuki, K., Suzuki, M., Taiyoji, M., Ferrer, S. and Regue, M. 1997. Genetic analysis of the chitinase system of Serratia marcescens 2170. *J. Bacteriol.* **179:** 7111-7117.

Wu, Y., Cheng, C. and Li, Y. 2009. Cloning and Expression of Chitinase A from Serratia marcescens for Large-scale Preparation of N,N-Diacetyl Chitobiose. *J. the Chinese Chemical Society.* **56:** 688-695.