Advances in Bioresearch
Adv. Biores., Vol4 (4) December 2013: 09-11
©2013 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr/abr.htm
CODEN: ABRDC3

REVIEW ARTICLE

Bacillus thuringiensis S-Layer Proteins- A Perspective for Better Crop Protection

Hanchipura Mallesh Mahadeva Swamy[1], Ramasamy Asokan[1], Riaz Mahmood[2]
[1] Division of Biotechnology, Indian Institute of Horticultural Research (IIHR), Hessaraghatta lake post, Bangalore 560089, Karnataka, INDIA
[2] Post-Graduate Department of Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Jnanasahayadri, Shankaraghatta, Shimoga 577451, Karnataka, INDIA
E-mail: clintonbio@gmail.com
E-mail: asokaniihr@gmail.com

ABSTRACT
S-layer proteins are among the most widespread in nature prokaryotic proteins (bacteria, archaea). Highly ordered protein arrays composed of single protein or glycoprotein subunits. They completely cover the cell surface during all stages of bacterial growth and division. Since their discovery S-layer proteins were found in more, than 300 different prokaryotic species, however, the number of S-layer proteins for which the sequences were identified is significantly less. S-layer proteins account for 10 to 15 % of the total cell protein production and that places them among the most abundant cellular proteins at all. This review article focused on recent innovations in B. thuringiensis S-layer proteins, and the possibility of applications of insect pest management strategies. The developments described in this review article underscore the validity of genetic manipulation to improve efficacy/ cost-effectiveness, and to expand the markets for Bt-based bioinsecticides.
Keywords: *Bacillus thuringiensis, glycoprotein subunits, S-layer proteins*

Received 09/09/2013 Accepted 28/11/2013

INTRODUCTION

The use of *Bacillus thuringiensis* as a biopesticide is a viable alternative for insect control since the insecticidal Cry proteins produced by these bacteria are highly specific; harmless to humans, vertebrates, and plants; and completely biodegradable. In addition to Cry proteins, *B. thuringiensis* produces a number of extracellular compounds, including S-layer proteins (SLP) that contribute to virulence. The S layer is an ordered structure representing a proteinaceous paracrystalline array which completely covers the surfaces of many pathogenic bacteria [1].

The S-layer is an ordered structure of proteinaceous paracrystalline arrays which completely covers the surfaces of many archaea and bacteria [2, 3] and constitutes up to 15% of the total cell protein. The function of S-layer proteins (SLP) has not been accurately defined. It was proposed that these proteins are involved in cell integrity and shape maintenance [2]. Also, it has been hypothesized that they may be involved in macromolecule exchange with the environment since they are the outermost cell envelope component [2]. In some gramnegative pathogens, they have been implicated in virulence and resistance to complement-mediated killing [2, 4]. In *Bacillus cereus*, the SLP promotes interactions with human leukocytes and with the host, contributing to pathogenicity [5]. It has been proposed that in *Bacillus anthracis*, the S-layer and the capsule might cooperate in the interaction with the host [6]. The SLP comprise a family of proteins found on the surfaces of very different bacterial species that live under different environmental conditions and have different targets. These proteins are modular in structure, consisting of two different functional domains [1].

BINDING MECHANISM OF S-LAYER SUBUNITS

In bacteria, the S-layer subunits are linked to each other and to the underlying cell envelope layer by non-covalent interactions. The N-terminal region of S-layer proteins from Gram-positive organisms recognizes a distinct type of secondary cell wall polymer as the proper binding site. Secondary cell wall polymers

(SCWPs) are covalently linked to the peptidoglycan backbone. S-layer subunits can be extracted from cell wall fragments with chaotropic agents (e. g. GHCl) and form self-assembly products after removal of the disintegrating agent.

Pena et al., [1] characterized a *B. thuringiensis* S-Layer protein which is involved in toxicity against *Epilachna varivestis* (Coleoptera: Coccinellidae). They screened two *B. thuringiensis* strain collections containing unidentified Cry proteins and also strains isolated from dead insects. Some of the *B. thuringiensis* strains assayed against *E. varivestis* showed moderate toxicity. However, a *B. thuringiensis* strain (GP1) that was isolated from a dead insect showed a remarkably high insecticidal activity. The parasporal crystal produced by the GP1 strain was purified and shown to have insecticidal activity against *E. varivestis* but not against the lepidopteran *Manduca sexta* or *Spodoptera frugiperda* or against the dipteran *Aedes aegypti*. The gene encoding this protein was cloned and sequenced. It corresponded to an S-layer protein highly similar to previously described SLP in *Bacillus anthracis* (EA1) and *Bacillus licheniformis* (OlpA). The phylogenetic relationships among SLP from different bacteria showed that these proteins from *Bacillus cereus*, *Bacillus sphaericus*, *B. anthracis*, *B. licheniformis*, and

B. thuringiensis are arranged in the same main group, suggesting similar origins. This is the first report that demonstrates that an S-layer protein is directly involved in toxicity to a coleopteran pest. This work will add a new protein to the list of insecticidal proteins produced by *B. thuringiensis*. This work also showed that classical screening strategies using *B. thuringiensis* strains isolated from soil samples could be laborious and inefficient at isolating strains highly active against a selected pest. The strategy for isolating *B. thuringiensis* strains from dead insect bodies seems to be highly effective at identifying active strains. Finally, it would be relevant to test whether other S-layer proteins produced by *B. thuringiensis* have some insecticidal activity against other insect species. This knowledge could be important for future control of insects. However, the mechanism of action of this SLP is unknown, and future work is needed to describe it.

Zhou et al., [7] screened four *B. thuringiensis* strains whose parasporal inclusions contained the S-layer protein (SLP), and cloned two *slp* genes from each strain. Phylogenetic analysis indicated these SLPs could be divided into two groups, SLP1s and SLP2s. To confirm whether SLPs were present in the S-layer or as a parasporal inclusion, strains CTC and BMB1152 were chosen for further study. Western blots with isolated S-layer proteins from strains CTC and BMB1152 in the vegetative phase showed that SLP1s and SLP2s were constituents of the S-layer. Immunofluorescence utilizing spore-inclusion mixtures of strains CTC and BMB1152 in the sporulation phase showed that SLP1s and SLP2s were also constituents of parasporal inclusions. When heterogeneously expressed in the crystal negative strain BMB171, four SLPs from strains CTC and BMB1152 could also form parasporal inclusions. This temporal and spatial expression is not an occasional phenomenon but ubiquitous in *B. thuringiensis* strains.

Finally, it would be relevant to test whether other S-layer proteins produced by *B. thuringiensis* have some insecticidal activity against other insect species. This knowledge could be important for future control of insects. However, the mechanism of action of this SLP is unknown, and future work is needed to describe it.

ACKNOWLEDGEMENTS

The authors are grateful to ICAR, New Delhi for funding this study under Network project on Application of microbes in agriculture and allied sectors (AMAAS). Infrastructure facility and encouragement by The Director, Indian Institute of Horticultural Research (IIHR) are duly acknowledged.

REFERENCES

1. Pen˜a, G., Juan Miranda-Rios., Gustavo de la Riva., Liliana Pardo-Lo´pez., Mario Sobero´n., and Alejandra Bravo. (2006) *Bacillus thuringiensis* S-Layer Protein Involved in Toxicity against *Epilachna varivestis* (Coleoptera: Coccinellidae). Applied and Environmental Microbiology., 72 (1): 353–360. 0099-2240/06/$08.00_0 doi:10.1128/AEM.72.1.353–360.2006
2. Beveridge, T. J., Pouwels, P. H., Sa´ra, M., Kotiranta, A., Lounatmaa, K., Kari, K., Kerosuo, E., Haapasalo M., Egelseer, E. M., Schocher, I., Sleytr, U. B., Morelli, L., Callegari, M.L., Nomellini, J. F., Bingle, W. H., Smit J., Leibovitz, E., Lemaire, M., Miras, I., Salamitou, S., Beguin, P., Ohayon, H., Gounon, P., Matuschek M., Sahm K., Bahl, H., Grogono-Thomas, R., Dworkin, J., Blaser, M. J., Woodland, R. M., Newell, D. G., Kessel M., and Koval, S. F. (1997). Functions of S-layers. FEMS Microbiology Reviews., 20:99–149.
3. Sa´ra, M., and Sleytr, U. B. (2000) S-layer proteins. Journal of Bacteriology., 182:859–868.
4. Pei, Z., and Blaser M. J. **(**1990) Pathogenesis of *Campylobacter fetus* infections. Role of surface array proteins in virulence in a mouse model. Journal of Clinical Investigations., 85:1036–1043.
5. Kotiranta, A., Haapasalo, M., Kari, K., Kerosuo, E., Olsen, I., Sorsa, T., Muerman, J. H., and Lounatmaa, K. (1998) Surface structure, hydrophobicity, phagocytosis and adherence to matrix protein of *Bacillus cereus* cells with and without the crystalline surface protein layer. Infections Immunology., 66:4895–4902.

6. Mignot, T., Mesnage, S., Couture-Tosi, E., Mock, M., and Fouet, A. (2002) Developmental switch of S-layer protein synthesis in *Bacillus anthracis*. Molecular Microbiology., 43:1615–1627.
7. Zhou Zhou., Donghai Peng., Jinshui Zheng., Gang Guo., Longjun Tian,. Ziniu Yu. and Ming Sun. (2011) Two groups of S-layer proteins, SLP1s and SLP2s, in *Bacillus thuringiensis* co-exist in the S-layer and in parasporal inclusions. BMB reports., 323-328.

Citation of This Article

Hanchipura Mallesh Mahadeva Swamy, Ramasamy Asokan, Riaz Mahmood. *Bacillus thuringiensis* S-Layer Proteins- A Perspective for Better Crop Protection. Adv. Biores. Vol 4[4] December 2013: 09-11