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Research Note

Burkholderia pseudomallei lectins: occurrence and expression

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Abstract. Lectins, also known as sugar binding proteins, play an essential role in the initiation of bacterial infections and biofilm production. To date, several lectins of Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Ralstonia solanacearum* and *Chromobacterium violaceum* have been identified. There are no published reports on the presence of lectins in *Burkholderia pseudomallei*, the causative agent of melioidosis. The aim of this study was to identify possible lectin genes of *B. pseudomallei* and generate recombinant proteins for assessment of hemagglutinating activity. Seven hypothetical lectins of *B. pseudomallei* were retrieved from the UniProt database. Four lectin domains, i.e., ricin B, C-type, H-type and Bulb-type lectins were identified. *In silico* analysis using a ligand binding site prediction server (3DLigandSite) predicted the presence of N-acetylglucosamine and calcium binding sites in two C-type lectins. Four recombinant proteins with the molecular weights of 11.7, 30.2, 36.2 and 46.4 kDa were expressed from the cloned genes; however none of them expressed any hemagglutinating activity. Further characterization of *B. pseudomallei* lectins may be able to provide insights into bacterial-host interaction that are required to initiate infections.

INTRODUCTION

Microbial lectins are necessary to mediate adhesion of microorganisms to host cells or tissues, and initiating the process of colonization and infection (Esko & Sharon, 2009). Lectins have been isolated from *Pseudomonas aeruginosa* (LecA and LecB) (Gilboa-Garber *et al.*, 2011), *Chromobacterium violaceum* (CV-IIL) (Zinger-Yosovich *et al.*, 2006), *Ralstonia solanacearum* (RSL) (Sudakevitz *et al.*, 2004) and *Burkholderia cenocepacia* (BC2L) (Lameignere *et al.*, 2008). *LecA* gene of *P. aeruginosa* encodes a tetrameric protein (PA-IL) consisting of four 12.75 kDa subunits which bind D-galactose and its derivatives (Diggle *et al.*, 2006). *LecB* encodes a tetrameric protein (PA-IIL) consisting of four 11.73 kDa subunits with high specificity for L-fructose but low affinity for D-mannose (Gilboa-Garber *et al.*, 2000). Lectins of *C. violaceum* (CV-IIL) (Zinger-Yosovich *et al.*, 2006) and *R. solanacearum* (RS-IIL) (Sudakevitz *et al.*, 2004) resemble the *P. aeruginosa* PA-IIL lectin. Both lectins (CV-IIL and RS-IIL) bind preferentially to L-fucose and L-galactose (Sudakevitz *et al.*, 2002; Zinger-Yosovich *et al.*, 2006) and exhibit almost similar molecular weights (~ 12 kDa). *B. cenocepacia* has been reported to harbor three soluble carbohydrate-binding proteins (BC2L-A, BC2L-B and BC2L-C) which are related to the fucose-binding lectin (PA-IIL) of *P. aeruginosa* (Lameignere *et al.*, 2010). Lectins are important factors in the

development of *P. aeruginosa* biofilms (Tielker *et al.*, 2005), and the production of high levels of lectins had increased the virulence potential of this organism (Gilboa-Garber & Garber, 1992).

Melioidosis, a life-threatening disease of man and animals, is caused by *Burkholderia pseudomallei* which originates from soil and water. The bacterium can stay dormant in the human host for many years and relapse/reoccurrence of infections is often reported (Ngauy *et al.*, 2005). The dormancy and latency of *B. pseudomallei* have been linked to its ability to form biofilms in the human host (Kamjumphol *et al.*, 2013; Koh *et al.*, 2013; Limmathurotsakul *et al.*, 2014; Ramli *et al.*, 2012; Taweechaisupapong *et al.*, 2005). However, unlike *P. aeruginosa* lectins which have been identified as important factors in the development of biofilms (Tielker *et al.*, 2005), biofilm formation in *B. pseudomallei* has not been reported to be associated with any virulence factors. The genus *Burkholderia* (originally considered as genus *Pseudomonas*) is closely related to the genus *Pseudomonas* (Yabuuchi *et al.*, 1992), and this raises the possibility that lectins may play a similar role in the pathogenicity of melioidosis.

The aim of this study was to identify, clone and express hypothetical lectin genes of *B. pseudomallei*, by using the bioinformatic approach for identification and *in silico* analysis of the lectins for ligand-binding residues for the protein sequences. Subsequently, the hypothetical genes were cloned and expressed as recombinant proteins. As most microbial lectins are detected based on their ability to agglutinate red blood cells (Esko & Sharon, 2009), hemagglutinating activity of the recombinant proteins was assessed using a conventional assay.

MATERIALS AND METHODS

Bacterial isolates

B. pseudomallei reference strain, K96243 was used as the target organism in this study. DNA was extracted from an overnight culture using a Wizard® Genomic DNA purification kit (Promega, Madison, USA, Lot #268023). Additionally, genomic DNA was extracted from ten clinical isolates of *B. pseudomallei* in our culture collection (Koh *et al.*, 2013). *P. aeruginosa* ATCC 27852 was used to generate recombinant lectin proteins (LecA and LecB) as positive controls for hemagglutination assay.

Identification of lectin genes using bioinformatic approach

Two keywords, i.e., "lectins" and "K96243" (*B. pseudomallei* K96243 reference strain) were used for searching hypothetical lectins in the UniProt database (http://www.uniprot.org/). Sequences of potential lectin genes of *B. pseudomallei* K96243 were subjected to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in search of homologous gene sequences in other *B. pseudomallei* strains and closely related *Burkholderia* species, including *B. thailandensis*, *B. mallei*, *B. oklahomensis* and *B. cenocepacia*. A web server, 3DLigandSite (Wass *et al.*, 2010), was used to predict the ligand binding residues in the hypothetical lectins.

Determination of the occurrence of lectin genes in *B. pseudomallei* using PCR assays

Based on the sequences of the seven hypothetical lectin genes (*BPSS1649*, *BPSS2022*, *BPSS0767*, *BPSL2056*, *BPSS1124*, *BPSS0713*, and *BPSS1488*), specific PCR assays were designed to determine the occurrence of lectins in *B. pseudomallei* K96243 and ten *B. pseudomallei* clinical isolates. The presence of two hypothetical lectin genes, i.e, *BPSS1649* and *BPSS2022* was investigated using a multiplex PCR assay (Koh *et al.*, 2013), while the presence of the other five hypothetical lectin genes, i.e., *BPSL2056*, *BPSS0713*, *BPSS0767*, *BPSS1124* and *BPSS1488*, were investigated by a singleplex PCR assay, using primers as shown in Table 1. All PCR assays were performed in a total volume of 15 µl containing 5 µl of purified genomic DNA, 0.2 mM of each dNTP, 5% of DMSO, 1x Pol Buffer C, 2 mM of $MgCl_2$, 0.75 unit Perpetual *Taq* DNA polymerase (EURx,

Gdansk, Poland), and 0.2 µM of each forward and reverse primers, using the thermal cycling condition as described previously (Koh *et al.*, 2013). PCR products were analyzed by electrophoresis on a 1.5% agarose gel (wt/vol) at 140 V for 20 min.

Cloning and expression of bacterial lectins

Known bacterial lectin genes, i.e., *LecA* and *LecB* genes of *P. aeruginosa* and seven *B. pseudomallei* hypothetical lectin genes (*BPSS1649*, *BPSS2022*, *BPSS0767*, *BPSL2056*, *BPSS1124*, *BPSS0713*, and *BPSS1488*) were cloned into pET-46EK/LIC vector (Novagen, USA) using ligation-independent cloning (LIC) approach. Table 1 shows the primers (indicated with asterisk) which had been designed to ensure that all expressed proteins had an additional 6x His purification tag extension in the N-terminal, to facilitate protein purification using affinity chromatography and detection using western blot. A stop codon was designed and incorporated in the reverse primer.

PCR reactions were performed in a total volume of 50 µl, consisting of 1X KOD buffer (Novagen, USA), 1.5 mM $MgSO_4$, 0.2 mM of each dNTPs, 0.3 µM of each forward and reverse primer, 1U of KOD Hot Start DNA Polymerase, and 5 µl of DNA template. The amplification was initiated with a denaturation step at 95ºC for 2 minutes, followed by 30 cycles of denaturation at 95ºC for 20 seconds, annealing at 60ºC for 10 seconds and extension at 70ºC for 1 minute and 30 seconds. Each PCR product was purified prior to cloning into a pET-46EK/LIC vector (Novagen, USA) in accordance with the manufacturer's recommendation. The ligated vector was then transformed into NovaBlue GigaSingles™ competent cells (Novagen, USA) and plated on LB agar containing 50 µg/ml carbenicillin. Selected colonies were propagated in Lysogeny Broth (LB) containing 50 µg/ml carbenicillin supplemented with 1% (w/v) glucose. Plasmids were isolated and transformed into competent cells [*E. coli* BL21 (DE3) pLysS strain or ROSETTA-GAMI 2 (DE3) pLysS strain] (Novagen, USA).

Recombinant *E. coli* strains were cultured at 30ºC under shaking condition (150 rpm) in 25 ml LB broth supplemented with 50 µg/ml carbenicillin and 1% (w/v) glucose, using IPTG (Fermentas, Lithuania # R0393) as an inducer for protein expression. The bacterial cells were harvested and sonicated, and the recombinant proteins were purified by gravity flow using a Protino® Ni-TED 1000 Packed Column (Macherey-Nagel, Germany). The eluted proteins were analysed for purity using SDS-PAGE and Western blots.

Hemagglutination assay

Hemagglutination assay was performed as described by Zinger Yosovich *et al.* (2006). Briefly, 50 µl of a 2% (v/v) rabbit erythrocyte suspension was added to an equal volume of an eluted recombinant protein sample (standardized at 15 µg/ml) in a microtiter well and incubated at 37ºC for 1 hour. The recombinant proteins of *P. aeruginosa* (LecA and LecB) were used as the positive controls, while PBS buffer was used as a negative control.

RESULTS AND DISCUSSION

Seven hypothetical lectins of *B. pseudomallei* annotated in the UniProt database were investigated in this study. Using primers designed in this study (Table 1), all the hypothetical lectin genes were amplified from *B. pseudomallei* K96243 reference strain as well as all ten clinical isolates of *B. pseudomallei*, indicating that the genes were highly conserved within *B. pseudomallei*. None of the hypothetical lectin genes exhibited sequence similarity with known lectin gene sequences of *P. aeruginosa*, *C. violaceum* nor with *B. cenocepacia*, suggesting that lectins present in *B. pseudomallei* are of different types. BLAST analysis showed that all seven hypothetical lectin genes were present (sharing 99-100% sequence similarity) in *B. pseudomallei* strain 1710b. However, only five hypothetical lectin genes (sharing 98-99% sequence similarity with those of *B.*

Table 1. Primers used in this study

Gene (Uniprot. accession no.)	Primer label	Sequence (5' – 3')	Amplicon size (bp)
BPSL2056	ADP-BPSL2056F*	gacgacgacaagatgccgacggcgccggat	2568
(Q63TB1)	ADP-BPSL2056R*	gaggagaagcccggtcaggcgccgatccggat	
	2056F_702	cgggtactggcagttcgtat	702
	2056R_702	acgagccacatgtgattgtc	
BPSS0713	ADP_BPSS0713_F*	gacgacgacaagatgacgaagaacgaagaatcg	1236
(Q63ME4)	ADP_BPSS0713_R*	gaggagaagcccggtcagagatctctcgcaagacg	
	0713F_646	ctgatcctgacggacatcct	646
	0713R_646	tgaacttgccgttgtattcg	
BPSS0767	ADPBS0767F*	gacgacgacaagatgactcaaaaattcgtcggtac	324
(Q63M93)	A2DP-BPSS0767R*	gaggagaagcccggtcacgcgggcgtctcggcga	
	0767F_226	tcaaaaattcgtcggtacgc	226
	0767R_226	gttgaccgtgaagtcggtct	
BPSS1124	ADP_BPSS1124_F*	gacgacgacaagatgacgctcaagctggcc	984
(Q63L84)	ADP_BPSSS1124_R*	gaggagaagcccggtcatcgcgactccacgag	
	1124F_378	gtcacgaacctcgaataccg	378
	1124R_378	gacgtattcctcgacgttgc	
BPSS1488	ADP_BPSS1488_F*	gacgacgacaagatgcacgcgccgtcattc	837
(Q63K77)	ADP_BPSS1488_R*	gaggagaagcccggtcatggtcgatctccagaaa	
	1488F_224	aatgggtcgacgatttcaac	224
	1488R_224	tcatggtcgatctccagaaa	
BPSS1649	ADPB1649SF*	gacgacgacaagatgcatttctttcgattcgc	2421
(Q63JR7)	A2DP-BPSS1649R*	gaggagaagcccggtcatgagatccggatcgatg	
	71F	agctcgcagatgaactggat	709
	71R	gctgatcgttgttcgtcgta	
BPSS2022	ADPB2022SF*	gacgacgacaagatgaaaaaatacgcattggcg	906
(Q63IP7)	A2DP-BPSS2022R*	gaggagaagcccggtcaacccggcgcgtagca	
	32F	tctggttcatgctggtttca	321
	32R	ggccgtaataccagttgctc	
CV-IIL	CV2L345-F*	gacgacgacaagatggctcagcaaggcgtg	342
	CV2L345-R*	gaggagaagcccggtcagcccagcggccagtt	
LecA	PA*LECA*361-F*	gacgacgacaagatggcttggaaaggtgaggt	366
	PA*LECA*361-R*	gaggagaagcccggtcaggactgatcctttccaata	
LecB	PA*LECB*348-F*	gacgacgacaagatggcaacacaaggagtgttc	345
	PA*LECB*348-R*	gaggagaagcccggttagccgagcggccagtt	

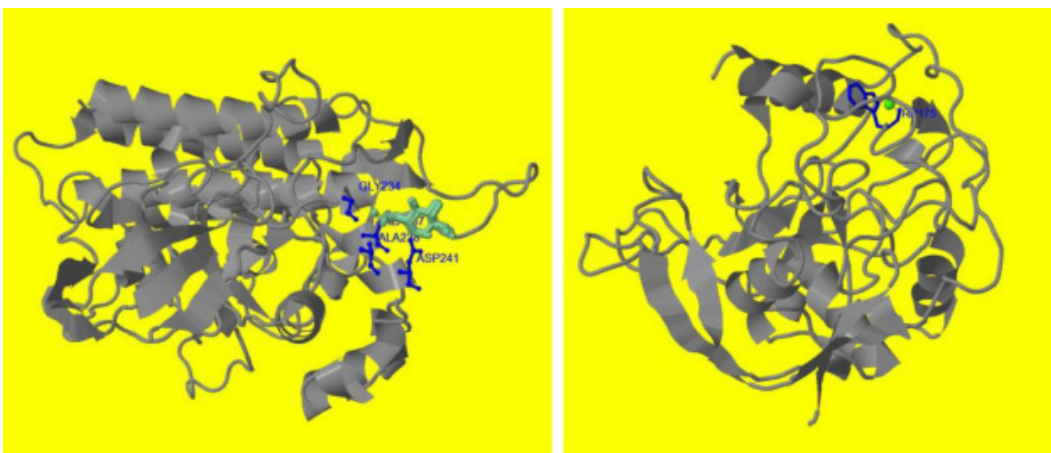
*Primers used for cloning purpose

pseudomallei) were found in *B. mallei*. All seven hypothetical lectin genes were present in *B. thailandensis* and *B. oklohomensis*, sharing 86-98% sequence similarities, except one (*BPSL2056*). Only one lectin gene (*BPSS0713*) with low sequence similarity (79-80%) was found in *B. cenocepacia*. The uniqueness in the lectin profiles of *B.*

pseudomallei has been used as a basis for development of a multiplex PCR assay for differentiation of closely related *Burkholderia* species as reported in our previous study (Koh *et al.*, 2013).

Based on the information derived from the UniProt database, ricin B lectin (Q63TB1 and Q63JR7), C-type lectin fold (Q63ME4, Q63L84 and Q63K77), H-type lectin (Q63M93) and Bulb-type lectin (Q63IP7) were identified from *B. pseudomallei* hypothetical lectins. The results obtained from 3DLigandSite predicted the presence of N-acetylglucosamine (Q63ME4) and calcium (Q63K77) binding sites in two hypothetical lectins. Figure 1 shows the structural view for the hypothetical lectins and the ligand binding sites. Ligands were not identified from other hypothetical lectins due to insufficient homologous structures.

The ricin B lectin domain has been reported in one or more copies in carbohydrate-recognition proteins of plant and bacterial AB-toxins, glycosidases or proteases (Hazes 1996; Hazes & Read, 1995; Hirabayashi *et al.*, 1998). Recently, a ricin-like lectin in *Mycobacterium tuberculosis* has been reported as an immunologically recognized molecule during active tuberculosis (Nogueira *et al.*, 2010). Calcium-dependent C-type lectin has been annotated in three hypothetical lectins in this study, of which two (Q63ME4 and Q63K77) have been successfully expressed as recombinant proteins. C-type lectin shares a common sequence motif with two disulfide



Protein	Predicted binding site		Heterogens present in predicted binding site		
	residue	Amino acid	Heterogen	count	Source structures
Q63ME4	234 237 238 241	GLY ALA ALA ASP	N-acetylglucosamine	14	2aii_X,1y1e_X,2aik_X,2aij_X, 1y1i_X,1y1h_X,1z70_X,1y1f_X, 1y1j_X,2hi8_X,1y1g_X,2afy_X, 2hib_X,2aft_X
Q63K77	175	TRP	calcium	14	1y1i_X,2aij_X,2hib_X,2aik_X, 2aft_X,2aii_X,2afy_X,1z70_X, 1y1e_X,1y1j_X,2hi8_X,1y1f_X, 1y1g_X,2q17_C

Footnote: Ala, Alanine; Asp, Asparagine; Gly, Glycine; Trp, Tryptophan

Figure 1. Structural view of two *B. pseudomallei* lectins, Q63ME4 (left) and Q63K77 (right), generated from 3DLigandSite. N-acetylglucosamine (Q63ME4) and calcium binding (Q63K77) sites were the heterogens/ligands present in the predicted binding sites of the hypothetical lectins. The heterogens/ligands are highlighted in green and the binding sites in blue.

bonds and a set of hydrophobic residues (Drickamer, 1993). The lectins demonstrated primary and secondary structural homology in their carbohydrate-recognition domains and a fold (referred as C-type lectin fold) with highly variable protein sequences which enables organisms to tolerate massive amino acid variation, as shown by the major tropism determinant (*Mtd*) of *Bordetella* bacteriophage (McMahon *et al.*, 2005). C-type lectins usually differ significantly in the types of glycans they recognize, for instance, different ligands, i.e., N-acetylglucosamine and calcium binding sites were identified in two hypothetical lectins (Q63ME4 and Q63K77) in this study (Figure 1).

The H-type lectin domain was predicted for only one of the hypothetical lectin (Q63M93) investigated in this study. The name has been proposed for *Helix pomatia* agglutinin (HPA)-like lectins in the albumen gland of the roman snail (Sanchez *et al.*, 2006). Sequence similarities have also been found in several bacterial putative proteins from the genera *Silicibacter*, *Magnetococcus*, and *Rhodobacter* (Jimbo *et al.*, 2005; Sanchez, *et al.*, 2006). This lectin is known to be involved in self/non-self recognition of cells through binding with carbohydrates (Sanchez *et al.*, 2006), hence, its importance in cell adhesion. The bulb lectin (B lectin) was annotated in one hypothetical protein (Q63IP7) in this study, which is very similar to that of *Dictyostelium discoideum* comitin, an actin binding protein, and *Curculigo latifolia* curculin, a sweet tasting and taste-modifying protein (Barre *et al.*, 1997; Hester *et al.*, 1995). Recently, bulb lectins with antibacterial activities have been reported from *Cynoglossus semilaevis* tongue sole (Sun *et al.*, 2016). Further characterization and confirmation of the lectin in *B. pseudomallei* is necessary since we believe that this is the first report of lectin in *Burkholderia* and probably the first in any bacteria.

Of the seven *B. pseudomallei* hypothetical lectin genes investigated in this study, only four (*BPSS0713*, *BPSS0767*, *BPSS1124*, and *BPSS1488*) were successfully expressed as recombinant proteins with the molecular weights of 46.4, 11.7, 36.2 and 30.2 kDa (Figure 2). On the contrary of *P. aeruginosa* (LecA and LecB), the recombinant proteins of *B. pseudomallei* did not show agglutinating activity with rabbit erythrocytes. The reason for the lack of hemagglutinating activity expressed by the recombinant proteins is not known. One of the important factors for a recombinant protein to be functional is the post-translation modification of the expression host which governs and determines the structure, localization and specific activity of the

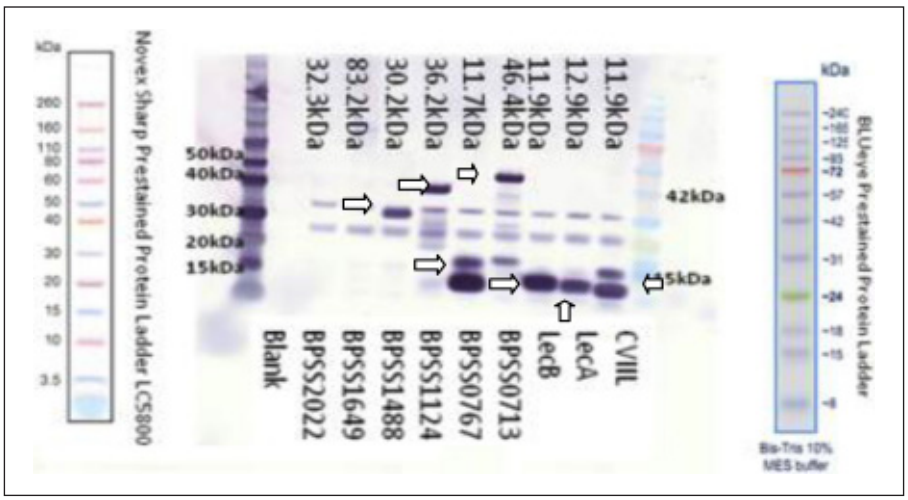


Figure 2. Western blot analysis of recombinant proteins expressed. Arrows show position of recombinant proteins.

protein (Wani *et al.*, 2014). It is speculated that the recombinant proteins expressed in this study might not have undergone appropriate post-translational modifications or conformational changes as required for hemagglutinating activity, as *E. coli* is not a native host for the proteins. Additionally, co-factors such as metal ions, or complexes with other protein monomers which are needed for hemagglutinating activity are probably absent (Etzler, 2009). Nevertheless, as hemagglutination assay could be limited in terms of sensitivity, other lectin probing assays such as glycan array, isothermal titration calarimetry or surface plasmon resonance should be used for characterization of *B. pseudomallei* lectins.

The primary function of lectins is to facilitate attachment or adherence of bacteria to host cells. Investigation of the lectin-host interaction should be able to provide insights on the establishment of bacterial infections in the human hosts. Additionally, some lectins expressed as outer membrane proteins (as annotated for Q63IP7W in this study) may induce specific immune responses in the infected individuals. In a recent study, high titers of IgG against ricin-type lectin has been reported in *M. tuberculosis* (Nogueira *et al.*, 2010), suggesting that lectin may have the potential as a diagnostic biomarker and target for immunization.

CONCLUSIONS

Lectins are a diverse group of proteins, consisting of many different families which are evolutionarily unrelated (Loris, 2002). Our present understanding of the structure and function of lectins in *B. pseudomallei* is limited. The findings from bioinformatic approach in this study showed that lectin genes are likely to be present in *B. pseudomallei.* Four recombinant proteins were expressed from the cloned lectin genes in this study, however; none demonstrated hemagglutinating activity with rabbit erythrocytes. More sensitive assays should be used for the detection of *B. pseudomallei* lectins.

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REFERENCES

Barre, A., Van Damme, E.J., Peumans, W.J. & Rougé, P. (1997). Curculin, a sweet-tasting and taste-modifying protein, is a non-functional mannose-binding lectin. *Plant Molecular Biology* **33**: 691-698.

Diggle, S.P., Stacey, R.E., Dodd, C., Cámara, M., Williams, P. & Winzer, K. (2006). The galactophilic lectin, LecA, contributes to biofilm development in *Pseudomonas aeruginosa*. *Environmental Microbiology* **8**: 1095-1104.

Drickamer, K. (1993). Ca^{2+} dependent carbohydrate-recognition domains in animal proteins. *Current Opinion in Structural Biology* **3**: 393-400.

Esko, J.D. & Sharon, N. (2009). Microbial Lectins: Hemagglutinins, Adhesins, and Toxins. In A. Varki, R.D. Cummings, J.D. Esko, H.H. Freeze, P. Stanley, C.R. Bertozzi, G.W. Hart & M.E. Etzler (Ed.), *Essentials of Glycobiology*. New York: Cold Spring Harbor Laboratory Press.

Etzler, M.E., Surolia, A. & Cummings, R.D. (2009). L-type Lectins. In A. Varki, R.D. Cummings, J.D. Esko, H.H. Freeze, P. Stanley, C.R. Bertozzi, G.W. Hart & M.E. Etzler (Ed.), *Essentials of Glycobiology (2nd edition)*. New York: Cold Spring Harbor Laboratory Press.

Gilboa-Garber, N. & Garber, N. (1992). Microbial lectins. In H.J. Allen & E.C. Kisailus (Eds.), *Glycoconjugates: Composition: Structure, and Function* (pp. 541-592). New York: CRC Press.

Gilboa-Garber, N., Zinger-Yosovich, K.D., Sudakevitz, D., Lerrer, B. & Imberty, A. (2011). The five bacterial lectins (PA-IL, PA-IIL, RSL, RS-IIL, and CV-IIL): interactions with diverse animal cells and glycoproteins. In A.M. Wu (Ed.), *The Molecular Immunology of Complex*

Carbohydrates-3 (pp. 155-211). United States: Springer US.

Gilboa Garber, N., Katcoff, D.J. & Garber, N.C. (2000). Identification and characterization of *Pseudomonas aeruginosa* PAIIL lectin gene and protein compared to PAIL. *FEMS Immunology and Medical Microbiology* **29**: 53-57.

Hazes, B. (1996). The (QxW) 3 domain: a flexible lectin scaffold. *Protein Science* **5**: 1490-1501.

Hazes, B. & Read, R.J. (1995). A mosquitocidal toxin with a ricin-like cell-binding domain. *Nature Structural and Molecular Biology* **2**: 358-359.

Hester, G., Kaku, H., Goldstein, I.J. & Wright, C.S. (1995). Structure of mannose-specific snowdrop (*Galanthus nivalis*) lectin is representative of a new plant lectin family. *Nature Structural and Molecular Biology* **2**: 472-479.

Hirabayashi, J., Dutta, S.K. & Kasai, K. (1998). Novel galactose-binding proteins in Annelida. Characterization of 29-kDa tandem repeat-type lectins from the earthworm *Lumbricus terrestris*. *Journal of Biological Chemistry* **273**: 14450-14460.

Jimbo, M., Koike, K., Sakai, R., Muramoto, K. & Kamiya, H. (2005). Cloning and characterization of a lectin from the octocoral *Sinularia lochmodes*. *Biochemical and Biophysical Research Communications* **330**: 157-162.

Kamjumphol, W., Chareonsudjai, S., Chareonsudjai, P., Wongratanacheewin, S. & Taweechaisupapong, S. (2013). Environmental factors affecting *Burkholderia pseudomallei* biofilm formation. *Southeast Asian Journal of Tropical Medicine and Public Health* **44**: 72-81.

Koh, S.F., Tay, S.T. & Puthucheary, S.D. (2013). Colonial morphotypes and biofilm forming ability of *Burkholderia pseudomallei*. *Tropical Biomedicine* **30**: 428-433.

Lameignere, E., Malinovska, L., Slavikova, M., Duchaud, E., Mitchell, E., Varrot, A., Sedo, O., Imberty, A. & Wimmerová, M. (2008). Structural basis for mannose recognition by a lectin from opportunistic bacteria *Burkholderia cenocepacia*. *Biochemical Journal* **411**: 307-318.

Lameignere, E., Shiao, T.C., Roy, R., Wimmerova, M., Dubreuil, F., Varrot, A. & Imberty, A. (2010). Structural basis of the affinity for oligomannosides and analogs displayed by BC2L-A, a *Burkholderia cenocepacia* soluble lectin. *Glycobiology* **20**: 87-98.

Limmathurotsakul, D., Paeyao, A., Wongratanacheewin, S., Saiprom, N., Takpho, N., Thaipadungpanit, J., Chantratita, N., Wuthiekanun, V., Day, N.P. & Peacock, S.J. (2014). Role of *Burkholderia pseudomallei* biofilm formation and lipopolysaccharide in relapse of melioidosis. *Clinical Microbiology and Infection* **20**: O854-O856.

Loris, R. (2002). Principles of structures of animal and plant lectins. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1572**: 198-208.

McMahon, S.A., Miller, J.L., Lawton, J.A., Kerkow, D.E., Hodes, A., Marti-Renom, M.A., Doulatov, S., Narayanan, E., Sali, A., Miller, J.F. & Ghosh, P. (2005). The C-type lectin fold as an evolutionary solution for massive sequence variation. *Nature Structural and Molecular Biology* **12**: 886-892.

Ngauy, V., Lemeshev, Y., Sadkowski, L. & Crawford, G. (2005). Cutaneous melioidosis in a man who was taken as a prisoner of war by the Japanese during World War II. *Journal of Clinical Microbiology* **43**: 970-972.

Nogueira, L., Cardoso, F.C., Mattos, A.M., Bordignon, J., Figueiredo, C.P., Dahlstrom, P., Frota, C.C., Duarte Dos Santos, C.N., Chalhoub, M., Cavada, B.S., Teixeira, H.C., Oliveira, S.C., Barral-Netto, M. & Báfica, A. (2010). *Mycobacterium tuberculosis* Rv1419 encodes a secreted 13 kDa lectin with immunological reactivity during human tuberculosis. *European Journal of Immunology* **40**: 744-753.

Ramli, N.S.K., Guan, C.E., Nathan, S. & Vadivelu, J. (2012). The effect of environmental conditions on biofilm formation of *Burkholderia pseudomallei* clinical isolates. *PLoS ONE* **7**: e44104.

Sanchez, J.F., Lescar, J., Chazalet, V., Audfray, A., Gagnon, J., Alvarez, R., Breton, C., Imberty, A. & Mitchell, E.P. (2006). Biochemical and structural analysis of *Helix pomatia* agglutinin. A hexameric lectin with a novel fold. *Journal of Biological Chemistry* **281**: 20171-20180.

Sudakevitz, D., Imberty, A. & Gilboa-Garber, N. (2002). Production, properties and specificity of a new bacterial L-fucose- and D-arabinose-binding lectin of the plant aggressive pathogen *Ralstonia solanacearum*, and its comparison to related plant and microbial lectins. *Journal of Biochemistry* **132**: 353-358.

Sudakevitz, D., Kostlánová, N., BlatmanJan, G., Mitchell, E.P., Lerrer, B., Wimmerová, M., Katcoff, D.J., Imberty, A. & Gilboa-Garber, N. (2004). A new *Ralstonia solanacearum* high affinity mannose-binding lectin RSIIL structurally resembling the *Pseudomonas aeruginosa* fucosespecific lectin PAIIL. *Molecular Microbiology* **52**: 691-700.

Sun, Y.Y., Liu, L., Li, J. & Sun, L. (2016). Three novel B-type mannose-specific lectins of *Cynoglossus semilaevis* possess varied antibacterial activities against Gram-negative and Gram-positive bacteria. *Developmental and Comparative Immunology* **55**: 194-202.

Taweechaisupapong, S., Kaewpa, C., Arunyanart, C., Kanla, P., Homchampa, P., Sirisinha, S., Proungvitaya, T. & Wongratanacheewin, S. (2005). Virulence of *Burkholderia pseudomallei* does not correlate with biofilm formation. *Microbial Pathogenesis* **39**: 77-85.

Tielker, D., Hacker, S., Loris, R., Strathmann, M., Wingender, J., Wilhelm, S., Willhelm, S., Rosenau, F. & Jaeger, K.E. (2005). *Pseudomonas aeruginosa* lectin LecB is located in the outer membrane and is involved in biofilm formation. *Microbiology* **151**: 1313-1323.

Wani, W.Y., Boyer-Guittaut, M., Dodson, M., Chatham, J., Darley-Usmar, V. & Zhang, J. (2014). Regulation of autophagy by protein post-translational modification. *Laboratory Investigation* **95**: 14-25.

Wass, M.N., Kelley, L.A. & Sternberg, M.J. (2010). 3DLigandSite: predicting ligand-binding sites using similar structures. *Nucleic Acids Research* **38**: W469-W473.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T. & Arakawa, M. (1992). Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiology and Immunology* **36**: 1251-1275.

Zinger-Yosovich, K., Sudakevitz, D., Imberty, A., Garber, N.C. & Gilboa-Garber, N. (2006). Production and properties of the native *Chromobacterium violaceum* fucose-binding lectin (CV-IIL) compared to homologous lectins of *Pseudomonas aeruginosa* (PA-IIL) and *Ralstonia solanacearum* (RS-IIL). *Microbiology* **152**: 457-463.