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Research article

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Identification of a new family of enzymes with potential O-acetylpeptidoglycan esterase activity in both Gram-positive and Gram-negative bacteria

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Abstract

Background: The metabolism of the rigid bacterial cell wall heteropolymer peptidoglycan is a dynamic process requiring continuous biosynthesis and maintenance involving the coordination of both lytic and synthetic enzymes. The *O*-acetylation of peptidoglycan has been proposed to provide one level of control on these activities as this modification inhibits the action of the major endogenous lytic enzymes, the lytic transglycosylases. The *O*-acetylation of peptidoglycan also inhibits the activity of the lysozymes which serve as the first line of defense of host cells against the invasion of bacterial pathogens. Despite this central importance, there is a dearth of information regarding peptidoglycan *O*-acetylation and nothing has previously been reported on its de-acetylation.

Results: Homology searches of the genome databases have permitted this first report on the identification of a potential family of O-<u>A</u>cetylpeptidoglycan esterases (Ape). These proteins encoded in the genomes of a variety of both Gram-negative and Gram-positive bacteria, including a number of important human pathogens such as species of *Neisseria*, *Helicobacter*, *Campylobacter*, and *Bacillus anthracis*, have been organized into three families based on amino acid sequence similarities with family I being further divided into three sub-families. The genes encoding these proteins are shown to be clustered with Peptidoglycan O-<u>a</u>cetyltransferases (Pat) and in some cases, together with other genes involved in cell wall metabolism. Representative bacteria that encode the Ape proteins were experimentally shown to produce O-acetylated peptidoglycan.

Conclusion: The hypothetical proteins encoded by the *pat* and *ape* genes have been organized into families based on sequence similarities. The Pat proteins have sequence similarity to *Pseudomonas aeruginosa* Algl, an integral membrane protein known to participate in the *O*-acetylation of the exopolysaccaride, alginate. As none of the bacteria that harbor the *pat* genes produce alginate, we propose that the Pat proteins serve to *O*-acetylate peptidoglycan which is known to be a maturation event occurring in the periplasm. The Ape sequences have amino acid sequence similarity to the CAZy CE 3 carbohydrate esterases, a family previously known to be composed of only *O*-acetylxylan esterases. They are predicted to contain the α/β hydrolase fold associated with the GDSL and TesA hydrolases and they possess the signature motifs associated with the catalytic residues of the CE3 esterases. Specific signature sequence motifs were identified for the Ape proteins which led to their organization into distinct families. We propose that by expressing both Pat and Ape enzymes, bacteria would be able to obtain a high level of localized control over the degradation of peptidoglycan through the attachment and removal of *O*-linked acetate. This would facilitate the efficient insertion of pores and flagella, localize spore formation, and control the level of general peptidoglycan turnover.



Figure I

(A) Structure of O-acetylpeptidoglycan and (B) reaction pathway of lytic transglycosylases (LT). A. The O-acetylation of peptidoglycan occurs at the C-6 hydroxyl group of MurNAc residues (shown in red) to generate the corresponding 2-N-6-O-diacetylmuramyl residue (OAcMurNAc). B. The lytic transglycosylases (LT) require a free C-6 hydroxyl group to catalyze the formation of I,6-anhdyroMurNAc products. The R denotes the stem peptide associated with the C-3 lactyl moiety of MurNAc residues.

Background

Peptidoglycan is an essential component of most eubacterial cell walls (the exceptions are the mycobacteria). It provides osmotic stability by serving as an exo-skeleton through which cellular shape is imparted. The tensile strength of this multilayered polymer is provided by two types of covalent linkages. The first consists of β -1,4 glycosidic bonds between N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNaC), while the second occurs through cross-linking of the tetrapeptides associated with MurNAC residues on adjacent glycan strands (Figure 1).

Peptidoglycan biosynthesis occurs in three stages (reviewed in [1]). The first involves the synthesis of soluble precursors in the cytoplasm leading to the production of UDP-linked MurNAc-pentapeptide. This muropeptide is then transferred to the membrane carrier bactoprenol via a pyrophosphate group and a GlcNAc residue is added to produce the membrane-associated, lipid II precursor. By an unknown mechanism, this lipid-linked precursor is translocated to the outer face of the cytoplasmic membrane. Now exposed to the periplasm in this third stage of biosynthesis, the available muropeptide is polymerized into the existing peptidoglycan sacculus, a reaction catalyzed by the transglycosylase domain of the class A highmolecular weight penicillin-binding proteins (PBPs). Once polymerized, the transpeptidase domain of both the class A and class B high-molecular weight PBPs catalyze the formation of cross-links between the peptides of neighboring glycan strands.

The peptidoglycan sacculus which surrounds the entire bacterium is not a static structure as it must permit the growth and division of the bacterial cell [1]. Furthermore, insertion of cell wall structures that transverse the peptidoglycan layer, such as secretion/transport complexes, flagella, and pili, require the sacculus to be constantly remodeled and reinforced [2]. These processes are performed by the coordinated action of peptidoglycan

synthesizing PBPs and the peptidoglycan lytic enzymes. The peptidoglycan cleaving enzymes are numerous and there exists a specific lytic activity for each linkage in peptidoglycan. The amidases cleave the stem peptide from the MurNAc residues, glucosaminidases hydrolyze the β -1,4 linkage between GlcNAc and MurNAc residues, and both the lytic transglycosylases and muramidases cleave the other β -1,4 glycosidic bond that exists between MurNAc and GlcNAc (reviewed in [3]). Collectively, these enzymes are called autolysins because their uncontrolled activity will lead to the lysis of bacterial cells. Given the potential lethal activity of the autolysins, the cell has to control their activity. This is accomplished, at least in part, by associating these enzymes in a holoenzyme complex that includes both the lytic enzymes, which provide sites for the insertion of new peptidoglycan precursors, and the synthesizing PBPs. The major glycolytic enzyme associated with these holoenzyme complexes appears to be the lytic transglycosylases [1]. These enzymes are not hydrolases but cleave the β -1,4 linkage between GlcNAc and MurNAc with the concomitant formation of 1,6-anhydromuramoyl residues [4] (Fig. 1).

Given the inherent importance of peptidoglycan, it is not surprising that lysozyme, one of the most important components of the first line innate immune response of eukaryotic hosts to invading bacteria, targets this polymer. Hydrolysis of the β -1,4 glycosidic linkage between Glc-NAc and MurNAc destabilizes the integrity of the sacculus and ultimately leads to cell lysis. A number of pathogenic bacteria are able to avoid the potentially lethal effects of lysozyme through the addition of an acetate group to the C-6 position of MurNAc (reviewed in [5,6]) (Figure 1). These pathogens include both Gram-positive and Gramnegative bacteria, such as Staphylococcus aureus and Neisseria gonorrhoeae, respectively. Several studies have shown that a direct correlation exists between the extent of Oacetylation and susceptibility to lysozyme-catalyzed hydrolysis of peptidoglycan [7-11]. However, the Oacetylation of peptidoglycan has also been shown to preclude the action of the endogenous lytic transglycosylases [12]. Intuitively, this would be expected given that the mechanism of action of these latter enzymes requires a free C-6 hydroxyl group on MurNAc to produce their reaction product, the corresponding 1,6-anhydro derivative. This has prompted the proposal of two roles for peptidoglycan O-acetylation; one to protect the bacterium from a host immune response, while the second would be to regulate the action of endogenous autolysins [13].

There is a dearth of information concerning the pathway for the O-acetylation of peptidoglycan, and nothing is known about its subsequent removal. All available evidence indicates that O-acetylation is a maturation event, occurring in the periplasm after the cross-linking of newly incorporated muropeptides (reviewed in [5,6]). Two models have been proposed to account for the transfer of acetate from cytoplasmic pools of acetyl-CoA to muramoyl residues in the peptidoglycan sacculus [6]. The first is a two protein system involving an integral membrane protein responsible for the translocation of acetate from the cytoplasm to the periplasm where it is accepted by a second more soluble protein which transfers it specifically to the C-6 hydroxyl of muramoyl residues in peptidoglycan. This system is modeled on the proposed pathway for the O-acetylation of the exopolysaccharide, alginate, produced by the opportunistic human pathogen Pseudomonas aeruginosa [14,15]. The other model invokes a single-enzyme system, exemplified by the action of Nod X for the O-acetylation of the carbohydrate-based nodulation factor by Rhizobium leguminosarum [16], which functions to concomitantly translocate and transfer the acetate from the cytoplasm to peptidoglycan. Recently, an enzyme has been identified within S. aureus that appears to function according to the second pathway [17], but more experimental evidence will be required to bear this out.

Herein, we describe the identification of a putative cluster of ORFs that encode both hypothetical O-acetyltransferases and O-acetyl esterases that we propose act on peptidoglycan to provide a dynamic process for attachment and removal of O-acetate, respectively. This proposal is supported by experimental data which demonstrate that representative bacteria harboring these ORFs do Oacetylate their respective peptidoglycan sacculi. This study thus represents the first to identify the presence of Oacetylpeptidoglycan in a variety of other human pathogens and the proteins involved in both the attachment and removal of this O-linked acetate.

Results and discussion

Initial identification of a peptidoglycan O-acetylation cluster in N. gonorrhoeae

N. gonorrhoeae is known to O-acetylate its peptidoglycan [5,6], however, the genes responsible for this process remain unidentified. Based on the available data, we have postulated that peptidoglycan O-acetylation may follow a similar pathway to that involved with the O-acetylation of the exopolysaccharide alginate produced by Pseudomonas aeruginosa [6]. Thus, to help identify potential genes associated with O-acetylation, the N. gonorrhoeae genome was probed with algI (U50202) [14,15], a P. aeruginosa gene encoding a putative membrane-bound, family 1 O-acetyltransferase [6]. A homologue of AlgI with 33% amino acid identity (51% similarity) was discovered in the raw sequence data of the unfinished genome project of N. gonorrhoeae FA1090 [18] even though this bacterium does not produce alginate. The ORF encodes 478 amino acids and sequence alignments revealed that the hypothetical

Pa	AlgI	235	Y TAQ-	- <mark>L</mark> YF	DF <mark>S</mark> G	<mark>y</mark> sdm	<mark>a</mark> igi	GLM	<mark>g</mark> fre	'ME <mark>1</mark>	<mark>vfn</mark> Q	PYI <mark>8</mark>	5 Q SIT	E <mark>FW</mark> F	RWH	I <mark>SLS</mark>	T <mark>W</mark> L	RDY.	L <mark>Y</mark> IS	3 <mark>L</mark>
Av	AlgI	235	YTAQ-	- <mark>L</mark> YF	DFS <mark>G</mark>	ys dm	<mark>a</mark> igi	GLMI	<mark>g</mark> fre	'ME <mark>1</mark>	<mark>vfn</mark> Q	PYI <mark>S</mark>	5 Q SIT	E <mark>FW</mark> F	RWH	I <mark>SLS</mark>	T <mark>W</mark> L	RDY.	L <mark>Y</mark> IS	3 <mark>L</mark>
Ηр	HP0855	276	YSFQ-	- <mark>L</mark> YF	DFS <mark>G</mark>	<mark>Y</mark> CDM	<mark>A</mark> IG]	IGLFF	NIKI	J <mark>P</mark> I	<mark>IFN</mark> S	py k/	ALN I Q	D <mark>FW</mark> F	RWH	IT <mark>LS</mark>	RFL	KE <mark>Y</mark>	L <mark>Y</mark> II	2 <mark>L</mark>
Τp	TP0566	254	Y CDFS	SGYS	DLAI		<mark>a</mark> vgi	L-F-	<mark>G</mark> FEI	T <mark>P</mark> A <mark>1</mark>	IF KR	PYI <mark>8</mark>	5 <mark>0</mark> SVT	E <mark>FW</mark> F	RWH	I <mark>S</mark> F <mark>S</mark>	Q <mark>W</mark> L	KE <mark>Y</mark>	L <mark>Y</mark> FS	3 <mark>L</mark>
LC	DltB	242	YSGY-	- <mark>L</mark> FF	DFA <mark>G</mark>	YSLF	A VA]	SYLM	<mark>G</mark> IEI	T <mark>P</mark> M	VFNK	₽W- <mark>£</mark>	5HITS	RLL	I <mark>rw</mark> q:	L <mark>SLS</mark>	FWF	RDY	נא <mark>צ</mark> ⊥	RF
Sa	DltB	247	YSLY-	- <mark>L</mark> FF	DFA <mark>G</mark>	YSLF	ai ae	FSYLF	<mark>G</mark> IK]	T <mark>P</mark> P <mark>1</mark>	if dk	PFKA	AKN I K	D <mark>FW</mark> N	RWH	MTL <mark>S</mark>	FWF	RD C	נא <mark>צ</mark> ⊥	RS
Sm	DltB	243	YGLD-	- <mark>L</mark> FF	DFA <mark>G</mark>	YSMF	ai ai	SNFM	GIKS	S <mark>P</mark> T <mark>1</mark>	<mark>IFN</mark> Q	PFK <mark>.</mark>	5 <mark>0</mark> dlk	e <mark>fw</mark> n	RWH	M <mark>SLS</mark>	FWF	<mark>RD</mark> F	VFM	RL
Bs	DltB	234	YSMY-	- <mark>L</mark> FF	DFA <mark>G</mark>	Y TMF	<mark>a</mark> vg\	/SYIM	GIKS	S <mark>P</mark> E <mark>1</mark>	IFNK	PFI <mark>8</mark>	SKNIK	D <mark>FW</mark> N	RWH	M <mark>SLS</mark>	FWF	RDY	VFMI	RF
			*	*	** *	* *	* * *		*	* *	**	* *	*	**	***	* * * *		***	*	*
Ng	PatA	242	YTFQ-	-LFL	DFSG	<mark>YSD</mark> L	VIGN	1 <mark>a</mark> mll	<mark>g</mark> fri	J <mark>P</mark> K <mark>I</mark>	IF SA	PLR <mark>2</mark>	A NIR	A <mark>FW</mark> I	K <mark>WH</mark>	ISLS	TWI	RDY	I <mark>YI</mark> I	P <mark>L</mark>
Nm	Orf	242	YTFQ-	-LFL	DFSG	<mark>YSD</mark> L	VIGN	1 <mark>a</mark> mll	<mark>g</mark> fri	J <mark>P</mark> K <mark>1</mark>	IF SA	PLR <mark>2</mark>	ALNIR	A <mark>FW</mark> I	K <mark>WH</mark>	ISL <mark>S</mark>	TWI	RDY	I <mark>YI</mark> I	2 <mark>L</mark>
Cv	orf	231	YALQ-	-I <mark>Y</mark> L	DFSG	YT <mark>D</mark> L	VTAI	_ <mark>AL</mark> LL	<mark>g</mark> iqi	J <mark>P</mark> R <mark>1</mark>	IF DA	PYL.	LNLR	D <mark>FW</mark> F	RWH	ISL <mark>S</mark>	SWI	RDY	V <mark>YI</mark> I	2 <mark>L</mark>
Pl	orf	234	YA WH-	-I <mark>Y</mark> F	N <mark>FSG</mark>	Y TNL	VTGI	. <mark>AL</mark> LL	gf ri	/ <mark>P</mark> E <mark>1</mark>	IFN A	PYL.	\ E N LQ	A <mark>FW</mark> H	RWH	ISL <mark>S</mark>	EFI	RDY	V <mark>YI</mark> I	2 <mark>L</mark>
Ηh	orf	241	YSVQ-	-I <mark>Y</mark> C	DFSG	Y IHL	VSAE	7 <mark>AL</mark> ML	<mark>g</mark> fsi	פר<mark>ק</mark>ר	VF'NM	PYM <mark>/</mark>	A K N LK	d <mark>fw</mark> n	RWH	ISL <mark>S</mark>	TFI	RDY	I <mark>YI</mark>]	2 <mark>L</mark>
Cj	orf	227	YAIQ-	-I <mark>Y</mark> C	DFSG	YV <mark>D</mark> L	VCAF	7 <mark>AL</mark> ML	<mark>g</mark> fti	ר <mark>9</mark> ם	VF'NM	PYL.	a k n lk	D <mark>FW</mark> A	RWH	ISL <mark>S</mark>	TFI	RDY	I <mark>YI</mark> I	2 <mark>L</mark>
CC	orf	227	YAVQ-	-I <mark>Y</mark> C	DFSG	YV <mark>D</mark> L	VCAF	7 <mark>AL</mark> ML	<mark>g</mark> fti	ר <mark>9</mark> ם	VF'NM	PYL.	a k n lk	E <mark>FW</mark> A	RWH	ISL <mark>S</mark>	TFI	RDY	I <mark>YI</mark> I	2 <mark>L</mark>
Zm	orf	237	YAAQ-	-I <mark>Y</mark> C	DFS <mark></mark> A	YS <mark>D</mark> M	AIGI	L <mark>A</mark> ALL	<mark>g</mark> yre	"PV <mark>1</mark>	VF'NQ	PYR <mark>/</mark>	ASLQ	D <mark>FW</mark> F	RWH	ISL <mark>S</mark>	SWL	RDY	V <mark>YI</mark> V	∕7 <mark>L</mark>
Вj	orf	259	YTFQ-	-L <mark>Y</mark> F	DFSG	YS <mark>D</mark> M	AIG]	IS <mark>L</mark> MF	<mark>G</mark> IF I	J <mark>P</mark> V <mark>1</mark>	IFN S	PY <mark>K</mark> Z	ASIV	E <mark>FW</mark> F	RWH	MT <mark>LS</mark>	QFL	RDY	L <mark>YI</mark>]	2 <mark>L</mark>
Gv	orf	265	YTLQ-	-L <mark>Y</mark> F	DFSG	YS <mark>D</mark> M	AVGI	L <mark>AL</mark> LF	<mark>g</mark> ire	r <mark>p</mark> W <mark>1</mark>	IF DA	PY <mark>K</mark> Z	ASII	D <mark>FW</mark> F	RWH	ITLS	QFL	RDY	L <mark>YI</mark>]	2 <mark>L</mark>
Ba	orf	257	FTFQ-	-L <mark>Y</mark> F	DFSG	YS <mark>D</mark> M	AIG]	I <mark>AL</mark> MF	NIKI	ע <mark>קע</mark> ו	IFN S	PY <mark>K</mark> Z	Avsiq	D <mark>FW</mark> H	RWH	MT <mark>LS</mark>	QFL	TK <mark>Y</mark>	V <mark>YI</mark> S	3 <mark>L</mark>
Bc	orf	257	FTF <mark>Q</mark> -	-L <mark>Y</mark> F	DFSG	YS <mark>D</mark> M	AIG]	I <mark>AL</mark> MF	NIKI	ע <mark>קע</mark> ו	IFN S	PY <mark>K</mark> Z	Avsiq	D <mark>FW</mark> H	RWH	MT <mark>LS</mark>	QFL	TK <mark>Y</mark>	V <mark>YI</mark> S	3 <mark>L</mark>
Pg	orf	269	YALQ-	-I <mark>Y</mark> C	DFSG	YS <mark>D</mark> M	AIG	i <mark>al</mark> vl	<mark>g</mark> fre	INT	IF DS	PYQS	SANIT	E <mark>FW</mark> F	RWH	ISLS	SWL	RDY	L <mark>YI</mark>]	2 <mark>L</mark>
Вt	orf	256	YALQ-	-I <mark>Y</mark> C	DFSG	YS <mark>D</mark> M	AIG	[<mark>AL</mark> LL	<mark>G</mark> FHE	'NL <mark>1</mark>	IFN S	PY <mark>K</mark> S	SASIT	E <mark>FW</mark> F	RWH	ISLS	SWL	RDY	L <mark>YI</mark> S	3 <mark>L</mark>
Bf	orf	258	YALQ-	-I <mark>Y</mark> C	DFSG	YS <mark>D</mark> M	AIG	[<mark>AL</mark> LL	<mark>g</mark> fhe	'NL <mark>1</mark>	NFNS:	PY <mark>KS</mark>	SASIT	E <mark>FW</mark> F	RWH	ISLS	SWL	RDY	L <mark>YI</mark> S	3 <mark>L</mark>

Sequence alignment of N. gonorrhoeae PatA and related hypothetical proteins found in the genome databases with family I O-acetyltransferases. The family of proteins presented in the upper block of sequences includes Algl from both *P. aeruginosa* PAO1 and *Azotobacter vinelandii*, a transporter of acetate involved in the O-acetylation of the exopolysaccharide alginate [6]. The residues in bold face and yellow highlight denote greater than 50% and 80% identity, respectively, within the separate blocks of sequences. The asterisks identify conservation between the two blocks of sequences while the residues in red are invariant amongst all sequences. Abbreviations (accession numbers): *Pa*, *P. aeruginosa* (U50202); *Av*, *Azotobacter vinelandii*, (AF027499); Hp, Helicobacter pylori (AE000596); Tp, Treponema pallidum (AE001232); Lc, Lactobacillus casei, (P35855); Sa, Staphylococcus aureus (D86240); Sm, Streptococcus mutans (AF049357); Bs, Bacillus subtilis (P39580), Ng, N. gonorrhoeae (AAVW89272); Nm, N. meningitidis (NP284200); Cv, Chromobacterium violaceum (NP903736); Pl, Photorhabdus luminescens (NP927855); Hh, Helicobacter hepaticus (NP860615); Cj, Campylobacter jejuni, (NP281794); Cc, Campylobacter coli (ZP00366780); Zm, Zymomonas mobilis (YP162181); Bj, Bradyrhizobium japonicum (NP767015); Gv, Gloeobacter violaceus (NP923893); Ba, Bacillus anthracis (NP843401); Bc, B. cereus (NP977299); Pg, Porphyromonas gingivalis (NP905383); Bt, Bacteroides thetaiotaomicron (NP811593); Bf, Bacteroides. fragilis (YP101412).

protein possesses the signature motifs associated with the family 1 *O*-acetyltransferases [6], including the common F-W-R/N-R-W-H motif in the central region of the proteins (Fig. 2). Given that *N. gonorrhoeae* does not produce alginate, we propose that the hypothetical membrane-protein functions to *O*-acetylate peptidoglycan which is known to occur as a periplasmic, maturation event [5]. Thus, we have named the gene *patA* for <u>peptidoglycan *O*-acetyltransferase A.</u>

Analysis of sequences downstream from the *patA* locus led to the discovery of two more ORFs which, when subjected to BLASTP sequence similarity searches, permitted their tentative assignment of function to be associated also with the metabolism of acetate (Fig. 3). Thus, the third ORF in

this cluster is predicted to be a hypothetical 44,087 Da protein of 397 amino acids having 49% sequence similarity to the catalytic domain of an acetyl xylan esterase (EC 3.1.1.72) from *Ruminococcus flavefaciens* (Fig. 4). This *R. flavefaciens* enzyme belongs to the family 3 carbohydrate esterases (CE3) of the CAZY classification [19]. Each of the other previously known members of family CE3 are also acetyl xylan esterases which aid in the degradation of xylan by removing the blocking O-linked acetate (reviewed in [20]). Although not well characterized, sequence similarity comparisons have permitted the identification of potential catalytic Asp, His, and Ser residues which could form a catalytic triad characteristic of the GDSL and TesA hydrolases [21]. As seen in Figure 4, these invariant residues are also present in the hypothetical *N*.



Gene organizations of O-acetylpeptidoglycan (OAP) cluster composed of *pat* and *ape* genes. Abbreviations for other gene products: cft, ferritin; pfr, ferritin; flgG, flagellar distal rod protein; GuaC, GMP reductase.

Ngi	Apel	71	F <mark>RI</mark> LQI <mark>GI</mark>	<mark>ds</mark> h t a <mark>g</mark> d	FFTDA	L <mark>R</mark> KR <mark>L</mark>	(127)	G <mark>G</mark> IT	VSAN	A <mark>G</mark> IN <mark>G</mark> A	QLTQWS	5 (13)
			hheeeco	cccccc	c hhhhl	hhhhh		ccee	ee hh	hhccc	hhhhh	ı
			eeeeccc	cccccc	c cccci	nnnnn	(ccee	eecccc	cccce	eeeeec	
RÍ	XynB	438	IK <mark>I</mark> LPA <mark>GI</mark>	DSITN <mark>G</mark> D	GEQ GG	Y <mark>R</mark> KY <mark>L</mark>	(25)	N <mark>G</mark> IT	Y D TDHA	A <mark>g</mark> fs <mark>gy</mark>	QIKEVI	2 (26)
Rf	CesA	43	IK <mark>IMPLGI</mark>	DSITY <mark>G</mark> M	ADE GG	Y <mark>R</mark> KY <mark>L</mark>	(29)	QSVK	Y D DNHA	A <mark>g</mark> ys <mark>gy</mark>	TITNL	2 (23)
Nf	Axe	44	VK <mark>IMP</mark> V <mark>GI</mark>	<mark>dsit</mark> f <mark>g</mark> e	GER GG	Y <mark>R</mark> KY <mark>L</mark>	(24)	N <mark>G</mark> IÇ	Y D DNNA	A <mark>g</mark> ys <mark>g</mark> f	'Q i keii	P (26)
Np	Axe	62	I <mark>RI</mark> MPM <mark>GI</mark>	<mark>dsit</mark> f <mark>g</mark> i	GET GG	Y <mark>R</mark> KY <mark>L</mark>	(24)	N <mark>G</mark> IT	F D DNHA	A <mark>g</mark> ys <mark>g</mark> y	TIKNGI	L (26)
An	orf	391	L <mark>R</mark> LLPLGI	<mark>dsit</mark> k <mark>g</mark> s	GSSDDNG	Y <mark>r</mark> rrl	(29)	N <mark>G</mark> NF	'E D RDHÇ) <mark>g</mark> ls <mark>g</mark> k	R I SDI <i>A</i>	A (10)
Nsp	orf	32	I <mark>RI</mark> MPL <mark>GI</mark>	<mark>)S</mark> I <mark>T-</mark> G-	SP G C	W <mark>R</mark> AL <mark>L</mark>	(22)	C <mark>G</mark> VA	HDGDNE	E <mark>G</mark> HG <mark>G</mark> Y	LATNI	A (13)
Gv	orf	106	AKV MPL<mark>G</mark>I	O <mark>SIT</mark> E <mark>G</mark> F	TVS GG	Y <mark>r</mark> td <mark>l</mark>	(20)	PSSL	SDKNH	E <mark>G</mark> HP <mark>G</mark> Y	FIDQIA	A (11)
NC	orf	44	L <mark>RIMPLG</mark> A	A <mark>SIT</mark> Y <mark>G</mark> Q	ASTDGNG	Y <mark>r</mark> nsl	(18)	H <mark>G</mark> TM	IQ D NDVI	E <mark>g</mark> wp <mark>g</mark> y	RIHEVE	H (12)
			:::::*	* * *		* *			~. :	*. *		
Ng 1	Ape1	I	A <mark>D</mark> LVI <mark>L</mark> SY <mark>(</mark>	<mark>JTN</mark> EAFN	N <mark>N</mark> IDI	ADTEOK	(-W <mark>LD</mark>	(78) <mark>E</mark>	G <mark>VH</mark> FSA	40 <mark>gy</mark> rf	RAAEML	(17)
Ng 1	Apel	I	A <mark>D</mark> LVI <mark>L</mark> SY <mark>(</mark> cceeeecc	<mark>3TN</mark> EAFN CCCCCCC	N <mark>N</mark> IDI cchhhl	ADTEQF hhhhhh	(-W <mark>LD</mark> -hhh	(78) <mark>E</mark> c	G <mark>VH</mark> FSI	AQ <mark>GY</mark> RF 1hhhhh	AAEML hhhhh	(17)
Ng 1	Apel	I	A <mark>D</mark> LVI <mark>L</mark> SY <mark>(</mark> cceeeeecc	<mark>3TN</mark> EAFN CCCCCCC	N <mark>N</mark> IDI cchhhl	ADTEQK hhhhhh	(-W <mark>LD</mark> -hhh	(78) <mark>E</mark> c	G <mark>VH</mark> FS <i>I</i> ccchhł	AQ <mark>GY</mark> RF ìhhhhh	RAAEML hhhhh	(17)
Ng 1	Apel		A <mark>D</mark> LVI <mark>L</mark> SY <mark>(</mark> cceeeeecc	<mark>JTN</mark> EAFN CCCCCCC CCCCCCC	N <mark>N</mark> IDI cchhhl cccccccl	ADTEQK hhhhhh hhhhhh	(-W <mark>LD</mark> 1-hhh 1hhhe	(78) <mark>I</mark> c	G <mark>VH</mark> FSA ccchhr	AQ <mark>GY</mark> RF hhhhhh ccccc	AAEML hhhhhh	(17)
Ng Z Rf	Apel XynB		A <mark>D</mark> LVI <mark>L</mark> SY cceeeeecc cceeeeeec PDIILLMI	<mark>JTN</mark> EAFN CCCCCCC CCCCCCC JTND LTA	N <mark>N</mark> IDI cchhhl cccccccl <mark>N</mark> RSMDA	ADTEQK hhhhhh hhhhhh CTAD L F	(-W <mark>LD</mark> 1-hhh 1hhhe RSM LD	(78) c (69) D	G <mark>VH</mark> FS <i>I</i> ccchhr cccccc HL HP N(AQ <mark>GY</mark> RF hhhhhh cccccc GT GYKK	AAEML hhhhh cccee MG NFF	(17)
Ng X Rf Rf	Ape1 XynB CesA	2 c 1 1	A <mark>D</mark> LVI <mark>L</mark> SY CCEEEEEEC CCEEEEEEC PDIILLMI PDIILLOIC	GTNEAFN CCCCCCC CCCCCCC GTNDLTA GTNDVS-	N <mark>N</mark> IDI. cchhhl cccccccl - <mark>N</mark> RSMDA - N GHLDG	ADTEQK hhhhhh hhhhhh CTAD L F SEER L I	(-W <mark>LD</mark> hhhh SMLD KLLD	(78) C (69) (68) D	G <mark>VH</mark> FS <i>I</i> ccchhr cccccc HL HP N(G VHP N <i>I</i>	AQ <mark>GY</mark> RF hhhhhh CCCCCC GT <mark>GY</mark> KK AG <mark>GY</mark> EK	AAEML hhhhh cccee MGNFF	(17) (129) (512)
Ng Z Rf Rf Nf	Apel XynB CesA Axe	2 c I I I I I	A <mark>D</mark> LVI <mark>L</mark> SY cceeeeecc PDIILLMIC PDIILLQIC PDIILLQIC	GTNEAFN CCCCCCC CCCCCCC GTNDLTA GTNDVS- GTNDVS-	N <mark>N</mark> IDI cchhhl ccccccl <mark>N</mark> RSMDA - <mark>N</mark> GHLDG N RSMDA	ADTEQK hhhhhh hhhhhh CTAD L F SEER L F CAND L F	(-W <mark>LD</mark> hhhhe SSM LD KLLD RALLD	(78) c (69) (68) (68) c	G <mark>VH</mark> FS <i>I</i> ccchhr cccccc HL HP NO G <mark>VHP</mark> N <i>I</i> OL HP SO	AQ <mark>GY</mark> RF hhhhhh cccccc GT <mark>GY</mark> KK AG <mark>GY</mark> EK	AAEML hhhhhh ccccee MGNFF MGKYW	(17) (129) (512) (64)
Ng X Rf Rf Nf Np	Apel XynB CesA Axe Axe	2 () 	ADLVILSY cceeeeecc PDIILLMIC PDIILLQIC PDIILLIIC	GENEAFN CCCCCCC CCCCCCC GECCCCC GENDLTA GENDLTA GENDVS- GENDMSG GENEAFN	N <mark>N</mark> IDI cchhhl cccccccl - N RSMDA - N GHLDG - N RSMDA - N RSMDA	ADTEQE hhhhhh CTADL SEERLE CANDLE CTNDLE	K-WLD hhhhe RSMLD HKLLD RALLD	(78) C (69) (68) (68) (68) (66) (66) (66) (66) (66) (66) (66) (66) (66) (66) (66) (76) (77)	GVHFSA ccchhr cccccc HLHPNC GVHPNA QLHPSC KVHPSC	AQ <mark>GY</mark> RE hhhhhh Cccccc GT <mark>GY</mark> KE AG <mark>GY</mark> EE GN <mark>GY</mark> KE GS <mark>GY</mark> KE	AAEML hhhhh ccccee MGNFF MGKYW CIGNFW	(17) (129) (512) (64) (118)
Ng X Rf Rf Nf Np An	Apel XynB CesA Axe Axe orf		ADLVILSY cceeeeecc PDIILLMIC PDIILLQIC PDIILLIIC PDIILLIIC	3TNEAFN CCCCCCC 3TNDLTA 3TNDVS- 3TNDMTA 3TNDMSG 3TNDLDK	N <mark>N</mark> IDI cchhhl - N RSMDA - N GHLDG - N RSMDA - N RSMDA - N RSMDA	ADTEQE hhhhhh CTAD L F SEER L F CAND L F CTND L F	K-WLD h-hhh SMLD KLLD ALLD HDLLD	(78) (69) (69) (68) (68) (66) (66) (66) (66) (66) (66) (66) (66) (66) (66) (66) (66) (76) (78) (7	G <mark>VH</mark> FSA ccchhł cccccc HL HPN O G VHPN A QL HP SO K VHP SO VK HP SO	AQ <mark>GY</mark> RE hhhhhh Cccccc GT <mark>GYKE AGGYEE GNGYKE GSGYKE AO<mark>GY</mark>KE</mark>	AAEML hhhhh ccccee MGNFF MGKYW CIGNFW	<pre>(17) (129) (512) (64) (118) (783)</pre>
Ng X Rf Rf Nf Np An	Apel XynB CesA Axe Axe orf orf		ADLVILSY cceeeeecc PDIILLMIC PDIILLQIC PDIILLIIC PDIILLIIC PDIILLIIC	3TNEAFN CCCCCCC 3TNDLTA 3TNDVS- 3TNDMTA 3TNDMSG 3TNDLDK 3TNDLDK	N <mark>N</mark> IDI cchhhl - N RSMDA - N GHLDG - N RSMDA - N RSMDA - N HSTQS - N HSTQS	ADTEQK hhhhhh CTAD L F SEER L H CAND L F CTND L H APDR L F	A-WLD hhhe SMLD KLLD ALLD ALLD ALLD	(78) C (69) C (68) C (68) C (66) C (66) C	G <mark>VH</mark> FSA ccchhł cccccc HL HP NO G VHP NA QL HP SO K VHP SO YK HP GI G VHP NA	AQ <mark>GY</mark> RF hhhhhh CCCCCC GT <mark>GYKK</mark> AG <mark>GY</mark> EK SN <mark>GYKK</mark> GS <mark>GY</mark> KK AQ <mark>GY</mark> KK	RAAEML hhhhh ccccee MGNFF MGKYW CIGNFW CMGDYF (MAGAW	<pre>(17) (129) (512) (64) (118) (783) (124)</pre>
Ng X Rf Rf Nf Np An Nsp Cy	Apel XynB CesA Axe Axe orf orf orf		ADLVILSY cceeeeecc PDIILLMIC PDIILLQIC PDIILLIIC PDIILLIIC PDIILLIIC PDIILVHVC	3TNEAFN CCCCCCC 3TNDLTA 3TNDVS- 3TNDMTA 3TNDMSG 3TNDLDK 3TNDLDK 3TNDLJFK	N <mark>N</mark> IDI. cchhhl - N RSMDA - N GHLDG - N RSMDA - N HSTQS P N EPVET. - N IAPAT	ADTEQK hhhhhh CTAD L F SEER L H CAND L F CTND L H APDR L F ILSAFT	A-WLD hhhe SMLD KLLD ALLD ALLD ALLD TTLVN	(78) C (69) C (68) C (68) C (66) C (66) C (66) C (66) C	GVHFSA ccchhl cccccc HLHPNO GVHPNA QLHPSO KVHPSO YKHPGI GVHPNA	AQ <mark>GY</mark> RF hhhhhh CCCCCC GT <mark>GYKK</mark> AG <mark>GY</mark> EK SN <mark>GYKK</mark> SS <mark>GYKK</mark> AQ <mark>GY</mark> KK AQDQK	RAAEML hhhhh ccccee MGNFF MGKYW CIGNFW CMGDYF CMGDYF CMAGAW CMSDKW	<pre>(17) (129) (512) (64) (118) (783) (124) (275)</pre>
Ng X Rf Rf Nf Np An Nsp Gv Nc	Apel XynB CesA Axe Axe orf orf orf		ADLVILSY cceeeeecc PDIILLMIC PDIILLQIC PDIILLIIC PDIILLIIC PDIILLIIC PDIILVHVC PDIVVHLC	GECCCCCC GECCCCCC GTNDLTA GTNDVS- GTNDMTA GTNDMSG GTNDLDK GTNDLDK GTNDIEK	N <mark>N</mark> IDI cchhhl - N RSMDA - N GHLDG - N RSMDA - N HSTQS P N EPVET - N IAPAT - N IAPAT	ADTEQK hhhhhh CTAD L F SEER L H CAND L F CTND L H APDR L F ILSAFT APGR L S	A-WLD hhhe SMLD KLLD ALLD ALLD ALLD TTLVN SALID	(78) C (69) C (68) C (68) C (66) C (66) C (66) C (66) C (54) C	GVHFSA ccchhl cccccc HLHPNO GVHPNA QLHPSO KVHPSO YKHPGI GVHPNA TVHPDA	AQ <mark>GY</mark> RF hhhhhh CCCCCC GT <mark>GY</mark> KK AG <mark>GY</mark> EK GN <mark>GY</mark> KK GS <mark>GY</mark> KK AQ <mark>GY</mark> KK AA <mark>G</mark> DQK AE <mark>GY</mark> AK	AAEML hhhhh ccccee MGNFF MGKYW CIGNFW CMGDYF CMGDYF CMAGAW CMSDKW CIADRW	<pre>(17) (129) (512) (64) (118) (783) (124) (275) (46)</pre>
Ng X Rf Rf Nf Np An Nsp Gv Nc	Apel XynB CesA Axe orf orf orf orf	2 с 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	ADLVILSY cceeeeeco PDIILLMIC PDIILLQIC PDIILLIIC PDIILLIIC PDIILLIIC PDVILVHVC PETVLLLIC PNVVLINAC	GECCCCCC GECCCCCC GTNDLTA GTNDVS- GTNDMTA GTNDMSG GTNDLDK GTNDLDK GTNDIEK GTNDAAG	N <mark>N</mark> IDI cchhhl - N RSMDA - N GHLDG - N RSMDA - N HSTQS - N HSTQS - N HSTQS - N HSTQS - N HSTQS - N HSTQS - N HSTZ - N NDPGG	ADTEQK hhhhhh CTAD L F SEER L H CAND L F CTND L H APDR L F ILSAFT APGR L S ASDRMG	A-WLD hhhe SMLD KLLD ALLD ALLD TLVN SALID GAMID	(78) C (69) C (68) C (68) C (66) C (66) C (66) C (66) C (54) C (54) C	GVHFSA ccchhl cccccc HLHPN(GVHPNA QLHPS(KVHPS(YKHPGI GVHPNA TVHPDA STHPTI	AQ <mark>GY</mark> RF hhhhhh CCCCCC GT <mark>GY</mark> KK AG <mark>GY</mark> EK SN <mark>GY</mark> KK SS <mark>GY</mark> KK AQ <mark>GY</mark> KK AE <mark>GY</mark> AK OV <mark>GY</mark> RK	RAAEML hhhhh ccccee MGNFF MGKYW CIGNFW CMGDYF CMGDYF CMAGAW CMSDKW CIADRW CMANIW	<pre>(17) (129) (512) (64) (118) (783) (124) (275) (46)</pre>

Sequence alignment and secondary structure predictions of N. gonorrhoeae Apel and CAZy CE3 carbohydrate esterases (O-acetylxylan esterases). The residues in bold face denote at least 50% identity amongst the CE-3 esterases, while those highlighted in yellow denote identity with N. gonorrhoeae Apel. Invariant residues are identified by the asterisks and the putative catalytic residues are in red. The numbers in brackets denote the length of intervening sequences. Abbreviations (accession numbers): Pa, P. aeruginosa (YP207682); Rf, Ruminococcus flavefaciens (XynB, Q52753; CesA, Q9RLB8); Nf, Neocalli-mastix frontalis (U66253); Np, N. patriciarum (O13497); An, Aspergillus nidulans, (EAA66937); Nsp, Nomomuraea species (Q7WZ50); Gv, Gloeobacter violaceus (Q7NGX3); Nc, Neurospora crassa (XM323878).

gonorrhoeae protein together with associated signature motifs. Given the significant sequence similarity, it follows that the *N. gonorrhoeae* protein also has similar secondary structural elements compared to the family CE3 proteins, as predicted by the Garnier and PSI PRED programs (Fig. 4).

As *N. gonorrhoeae* is not a xylanolytic bacterium and its genome does not appear to encode xylanases, we postulate that the hypothetical family CE3 esterase functions on peptidoglycan to specifically catalyze its de-O-acetylation. This hypothetical protein was thus named Ape1 for *O*-acetylpeptidoglycan esterase 1. It has a high predicted pI value of 8.782 which is typical of periplasmic proteins associated with the metabolism of bacterial cell walls and thus provides further evidence in support of its proposed

function. It should be noted that Ape1 (and all of the Ape proteins described below) do not have any significant sequence homology to either the *Streptococcus pneumoniae* peptidoglycan *N*-acetylglucosamine deacetylase (EC 3.1.1.-) [22] or the recently discovered *N*-acetylmuramic acid deacetylase (EC 3.5.1.-) from *Bacillus subtilis* [23], each of which belong to family CE4.

BLASTP analysis of the second ORF in this potential cluster of genes suggested that its deduced sequence of 335 amino acids is weakly similar to lysophospholipase and related esterases (E value 4e-04). Searches for known domains within its sequence using the CD-Search program indicated that it too resembled the GDSL and TesA hydrolases despite having only 18.6% identity with Ape1. Notwithstanding this low identity, the alignment revealed 53.4% similarity between the two hypothetical proteins and further analysis indicated that the second hypothetical protein does possess some of the Ape1 sequence motifs, including the GDS and Dx(V/T)H motifs containing the potential catalytic Ser, Asp, and His residues, respectively. Hence, this ORF was tentatively labeled *ape2*.

The *patA*, *ape1*, and *ape2* gene sequences discovered on the *N. gonorrhoeae* chromosome were used in BLASTP searches of both the finished and unfinished genome sequences of the NCBI database. These searches led to the identification of 18 other bacteria that possess this cluster of genes (Fig. 3). This list includes some important human pathogens, both Gram-negative and Gram-positive, such as *Campylobacter jejuni, Helicobacter pylori,* and *Bacillus anthracis,* respectively.

Homology of Pat sequences

The recognition of homologs to the acetyltransferase AlgI from P. aeruginosa and their organization into a family has been described previously [6]. In the current study, we extend the list with new family members that include sequences from a variety of human pathogens such as species of Helicobacter, Campylobacter, Neisseria, and Bacillus, in particular, Bacillus anthracis (Fig. 2). All of the additional sequences from these diverse bacteria have at least 49% amino acid similarity to the P. aeruginosa AlgI despite the fact that none produce the exopolysaccharide alginate. In addition, each of these sequences contain the membrane-bound O-acetyltransferase (MBOAT) domain (pfam03062) [24]. However, none of the hypothetical proteins have significant homology to the acyltransferase 3 domain (pfam 01757) in the recently identified peptidoglycan O-acetyltransferase, OatA, from Staphylococcus aureus [17].

Architecture of the hypothetical O-acetylpeptidoglycan esterases (Ape)

The in silico identification of signature sequence motifs within the hypothetical Ape proteins was performed using a variety of programs. Predictions made using PSI PRED indicated that the sequence of secondary structural elements was very similar amongst each of the deduced proteins and that each contained the α/β hydrolase fold associated with the GDSL and TesA hydrolases (data not shown). This analogy was extended using Clustal W alignments and MEME/MAST motif discovery searches which demonstrated the strict conservation of two signature motifs that are also associated with the GDSL and TesA hydrolases (Fig. 5). The first of these, motif I, is characterized by the sequence GDS(H/F) which contains the potential catalytic serine residue, while the Dx(V/T)Hof the other (motif VII) includes both the catalytic aspartyl and histidyl residues. In addition to these common sequence motifs, unique signature motifs were discerned within the intervening regions of the hypothetical proteins which permitted the subdivision of the individual sequences into a total of three separate Ape families, with family 1 being further divided into subfamilies (labeled a, b, and c) (Fig. 6). While the intervening sequences motifs distinguish the three Ape families, they nonetheless maintain the overall α/β fold of the proteins.

The Ape1 family of proteins are characterized by three additional common segments of sequence similarity which exist toward the C-terminal end of the proteins and thus closer to motif VII (Figs. 5 and 6). Three respective consensus motifs, labeled IV, V, and VI, were identified within these regions and hence, the Ape1 sequences are characterized by a total of five common sequence motifs.

The subfamily classification of the family 1 proteins is based on the presence or absence of a large sequence of approximately 100 amino acids immediately following motif I. Ape1a and Ape1b subfamilies contain this segment while it is absent in the family 1c sequences. Within this segment, only one conserved motif could be identified (motif II) and differences within it delineate the two subfamilies 1a and 1b. Although the rest of this extra segment is not highly conserved amongst the Ape1a and 1b homologs, a high amount of β -sheet structure is predicted for each. In an attempt to identify its possible function, the entire segment from each of the 12 hypothetical proteins was subjected to BLASTP searches. The only known protein that could be retrieved with any significance ($E \ge$ 1.0) from the database by this search was penicillin-binding protein 4 (PBP 4) from Bacillus subtilis which was obtained by probing with the segment from B. thetaiotamicron Ape1b. Interestingly, amino acid sequences similar to this N-terminal region of B. subtilis PBP4 are found in the N-terminal regions of a variety of enzymes related to peptidoglycan metabolism, including S. aureus PBP4, Streptocococcus pneumoniae PBP3, Streptomyces K15 Dtranspeptidase, Escherichia coli PBP5, and TEM-1 β-lactamase. In S. aureus PBP4 [25] and Streptococcus pneumoniae PBP3 [26] (1TVF and 1XP4, respectively), the homologous sequences comprise a lobe of four surface exposed α helices as part of the N-terminal, D,D carboxypeptidaselike domain. These helices are positioned on the back side of the active site cleft of the respective enzymes and perhaps participate in protein-protein interactions that contribute to the formation of peptidoglycan biosynthetic complexes.

The family 1a sequences are characterized by the presence of an additional region of sequence homology which is unique to these proteins. Amongst the residues that comprise the consensus motif (motif III) identified within this segment are two of the relatively rare tryptophan residues

	*					
la Ng ApeA 7	'1 FR <mark>I</mark> LQI <mark>GDSH</mark>	TAG <mark>DFF</mark> TDAL <mark>R</mark> KR	LQKTW <mark>G</mark> DGGI	GWVYPANVKGQRM	(19) DFPLGGI LAQTGSG G SMTLT	(69)
Nm orf 7	1 FR <mark>I</mark> LOI <mark>GDSH</mark>	TAG <mark>DFF</mark> TDSLRKR	LOKTW <mark>G</mark> DGGI	GWVYPANVKGQRM	(19) DFPLGGILA HTGSG G SMTLT	(69)
Cv orf 5	1 VRVIOF <mark>GDSH</mark>	TAADY <mark>F</mark> SGEL <mark>R</mark> AR	LOARY <mark>G</mark> DAGI	GWLPPVNVPGORN	(21) DFPLGGYVGIAORAGASITV	(77)
Pl orf 7	1 LHFVOI <mark>GDSH</mark>	TAADFFTGKLRSI	LOORF <mark>G</mark> NAGI	GFVSPINVPGQRN	(23) DFPIGGTIAEPNSOISOLIL	(77)
Ci orf 6	34 IG <mark>i</mark> ríy <mark>gdsh</mark>	MAADFFPRVIRGY	LĨRSNSI	GFAYPLOPKYOON	(23) DFPLGGIIA KAKEK GA KITL	(77)
Cc orf 6	32 IG <mark>I</mark> RIY <mark>GDSH</mark>	TAADFFPRVIRGY	LIKSNSI	GFAYPLOPKYOON	(23) DFPLGGI TAKAKEK GA KITL	(75)
Cu orf 5	9 IG <mark>I</mark> RIY <mark>GDSH</mark>	MAADFFPRVIRDY	LRSNAV	GFAYALOPKYOON	(21) NYPLGGITARADKKGATIKL	(75)
Hh orf 8	7 LTTRTEGDSH	TAGDETSHRLEGI	FKNHST	GFVYPMYPNYHON	(21) EYPMGGVVAKPVELPAHTTL	(77)
consensus	TGDSH	TAADFFLR	TT	GF-YPK-O-N	DFPLGGT-AGA-T-L	(,,,
1b <i>Bt</i> orf 11	4 VRTAVEGDSE	TEADTETADI.REM		GEVTTESMTSGYR	(128) DNESLEGSSGLSLEGTPOOM	
Bf orf 10	17 VRTAVEGDSE	TEADTETADI.REM		GEVTTTSMTSGYR	(128) DNFSLRGSSGLSLRSTPSKM	
Pa orf 14	5 TRTAVIGDSF	TEGDIETDALRAA	LOKREGGNGW	GWMPTTSEVAGER	(114) DNMSTRGNSGTLLKSTDREV	
Zm orf 7		TACDSTSCAWPST		CVI.ADCTDVKCVT	(121) VALSNLGWGSOLTHLOPSS	
				CTTCCVD		
	E VDTIUTCDCU				PN-26-26-111	
DE DE OFF 10	S VRILHIGDSH	VRGHIIPQIIGIL	TTETECATO VTDM			
BI OIL IU		IRGHI F PRIIGAR				
Pg or i e		LSGGIFIRSMSAN		GVPGASISIFSQD-		
consensus	V-T-H-GDSH	G <mark>F</mark>	<mark>Б</mark> <mark>е</mark>	GV-GAIF		
matif		-				
MOLII		1			11	
•						
Ape	CT TT COM					
la Ng ApeA	GIIVSAMG	TNGAQLIQWSKW	ADRMIND-LAQIGA			
Nm ori	GITVSAMG	INGAQLTQWSKW	RADRMIND-LAQTGA	UNITALATO INEAFGI	DNIDIADTEQKWLDIVRQI R DSL	
CV OTI	GVVLDTVG	SNGAELALWRSW	SAAWGRQ-LAARDA	OLVILAYGTNEAFD	AKLDLDEYRAALQGAVNLV R SQL	
PI OTI	GVMLSALG	INGATINMMDKW(DEMIDIPAGWHD	MMILAYGTNEAFN	HSLDLPSYRQQLETKIKQI R NQL	
Cj ort	NVF'LD'I'LA	INGAKSDLWLSWN	NQEVVKKELELLHN	DLI <mark>ILAYG</mark> SNDALFI	KGFEKQKFKNNLKKWISILKTYN	
Cc ort	NAYLDTIA	INGAKSDLWMSWN	NKKISEQELKLLSN	DLILLAYGSNDALFI	KGFEKNKFKKNLKDWIHTLKTYN	
<i>Cu</i> orf	NIFLDTIA	INGARSDLWKDWN	ILDVVKKELGVLKN	DLI <mark>ILAYG</mark> SNDALLI	KDFNAVEFKKNYKDFFKIL R REN	
Hh ort	NNIVENLG	INGARSDIWLKWI	OKELLKHQMSMLPA <mark>I</mark>	DLVVLCYGS <mark>N</mark> DALYI	ONFNESIFLKNYGAFIDLI R QSN	
consensus	G	INGAWW-	<mark>I</mark>	DL-ILAY <mark>G-N</mark> -A	R	
1b Bt orf			LRQFNEQ R P YI	OLIILEY <mark>GLN</mark> VATER	RGRNYDNYQKGLLTSIEHLKNCF	
<i>Bf</i> orf			LQEFNAQ R P YI	<mark>dliil</mark> ey <mark>gln</mark> vatei	RGRNYDPYKKGLLTAINHLKECF	
Pg orf			SHAFASL R N YI	DLI <mark>IL</mark> QY <mark>GLN</mark> VASNI	KQKD Y SN Y AKQMGGV I ET L RNIC	
Zm orf			-DQVIATELKVYHP <mark>I</mark>	<mark>dliv</mark> laf <mark>g</mark> t <mark>n</mark> egfai	PYFDPTAFESV L REQ I SRIRRLS	
consensus			R-Y <mark>I</mark>	<mark>DLIIL</mark> -Y <mark>G</mark> L <mark>N</mark> VA·	YYL I L	
1c Bt orf			-GRIADIAAMKP-H	E <mark>L</mark> L <mark>IL</mark> SF <mark>GTN</mark> ESHNE	RRYNVNLHYNQ M DELVKL L RDS L	
Bf orf			-DRIAA IAALKP-H	E <mark>llil</mark> sf <mark>gtn</mark> eshne	RKYNSNVHYRQ M EELLEL L HDS L	
Pg orf			KYMQQ IK A VEP- <mark>I</mark>	DLV <mark>I</mark> ISL <mark>GTN</mark> DSYCI	FKFSETEMWGN M ETFFGM L RQT L	
consensus			I-AP	- <mark>L-IL</mark> S- <mark>G</mark> T <mark>N</mark> -S	LL	
motif		III		IV		
Аре					* *	
Ape 1a <i>Ng</i> ApeA	PAAG <mark>IL</mark> IIGA <mark>P</mark>	E-SL K N (18) ()rrv <mark>a</mark> rqgq t mfwsi	NQNA <mark>M</mark> GGICSMKN W	(9) <mark>D</mark> G <mark>VH</mark> FSAQ <mark>GY</mark> RRA A EM <mark>L</mark> ADS <mark>L</mark>	(9)
Ape 1a <i>Ng</i> ApeA <i>Nm</i> orf	PAAG <mark>IL</mark> IIGAP PAAG <mark>IL</mark> IIGAP	E-SL K N (18) (E-SL K N (18) (QRRV <mark>A</mark> RQGQ T MFWSV QRRI <mark>A</mark> RQGQ T MFWSV	VQNA <mark>M</mark> GGICSMKNW VQNA <mark>M</mark> GGVCSMKNW	* * (9) <mark>DGVH</mark> FSAQ <mark>GYRRAAEML</mark> ADSL (9) DG <mark>VH</mark> FSAK <mark>GY</mark> QRSAEMLADSL	(9) (9)
Ape 1a Ng ApeA Nm orf Cv orf	PAAG <mark>IL</mark> IIGAP PAAG <mark>IL</mark> IIGAP PQAAILLLGAP	E-SL K N (18) (E-SL K N (18) (D-AARS (24) (QRRV <mark>A</mark> RQGQ T MFWSV QRRI <mark>A</mark> RQGQ T MFWSV QRQL <mark>A</mark> RDNHL L YW D V	IQNA <mark>M</mark> GGICSMKNW IQNAMGGVCSMKNW IQLA <mark>M</mark> GGPCSMRTW	 * * (9) DGWHFSAQGYRRAAEMLADSL (9) DGWHFSAKGYQRSAEMLADSL (9) DMWHFTAPGYTRLGDDLYEGL 	(9) (9) (4)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf	PAAG <mark>IL</mark> IIGAP PAAGILIIGAP PQAAILLLGAP PQTVILIIG-P	E-SL K N (18) (E-SL K N (18) (D-AARS (24) (GDSL K N (20) (QRRV <mark>A</mark> RQGQ T MFWSV QRRI <mark>A</mark> RQGQ T MFWSV QRQL <mark>A</mark> RDNHLLYW D V QQKV <mark>A</mark> QDQH TL FWNV	IQNA <mark>M</mark> GGICSMKNW IQNAMGGVCSMKNW IQLAMGGPCSMRTW IRAY <mark>M</mark> GGKCSIRRW	 * * (9) DGWHFSAQGYRRAAEMLADSL (9) DGWHFSAKGYQRSAEMLADSL (9) DMWHFTAPGYTRLGDDLYEGL (9) DYWHLSAAGYERSAEALYQQL 	(9) (9) (4) (4)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf	PAAG <mark>IL</mark> IIGAP PAAGILIIGAP PQPAILLLGAP PQTVILIIG-P KNAVIMLISPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I	2RRV A RQGQ T MFWSV 2RRI A RQGQ T MFWSV 2RQL A RDNHL LYWD V 2QKV A QDQH TLFW N 2YEV <mark>A</mark> KEEK TL IF D 1	IQNA <mark>M</mark> GGICSMKNW IQNAMGGVCSMKNW IQLAMGGPCSMRTW IRAYMGGKCSIRRW IHQF <mark>M</mark> QDSGGKNKW	 * * (9) DGWHFSAQGYRRAAEMLADSL (9) DGWHFSAKGYQRSAEMLADSL (9) DMWHFTAPGYTRLGDDLYEGL (9) DYWHLSAAGYERSAEALYQQL (9) D-WHLTIKGYELMAKKLLEDL 	(9) (9) (4) (4) (4)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf	PAAGILIIGAP PAAGILIIGAP PQAAILLGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVKKQ (17) I	2RRV <mark>A</mark> RQGQ T MFWSV 2RRIARQGQ T MFWSV 2RQLARDNHLLYWDV 2QKVAQDQHTLFWNV LYEVAKEEK TL IFDN 2VEVAKEEK TLIFD N	NQNAMGGICSMKNW NQNAMGGVCSMKNW NQLAMGGPCSMRTW NRAYMGGKCSIRRW NHQFMQDSGGKNKW	 * * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DYVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLDDL 	(9) (9) (4) (4) (4) (4) (4)
Ape la Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf	PAAGILIIGAP PAAGILIIGAP PQAAILLIGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNALFILISPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVKKQ (17) I TVVKKQ (17) I	QRRV <mark>A</mark> RQGQ T MFWS QRRIARQGQ T MFWS QRQLARDNHLLYWD QKVAQDQH TLFW N YEVAKEEK TLIFD JYEVAKEEK TLIFD YELAKEEK TLIFD	NQNAMGGICSMKNW NQNAMGGVCSMKNW NQLAMGGPCSMRTW NRAYMGGKCSIRRW HHQFMQDSGGKNKW HHQFMQDSGGKNKW	 * * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DYVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLDDL (9) D-VHLSVKGYELMAKKMLDEL 	(9) (9) (4) (4) (4) (4) (4) (3)
Ape la Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf	PAAGILIIGA PAAGILIIGAP PQAAILLIGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNALIFLLISPP POASILLISPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) I TVTQK- (17) J PVVOKV (23) J	QRRV <mark>A</mark> RQGQ T MFWSV QRRIARQGQTMFWSV QRQLARDNHLLYWD QQKVAQDQHTLFWN YEVAKEEKTLIFD YEVAKEEKTLIFD IYELAKEEKTLLFD IXELAKLAKOKOTLLFD	NQNAMGGICSMKNW NQNAMGGVCSMKNW NQLAMGGPCSMRTW NRAYMGGKCSIRRW HHQFMQDSGGKNKW HHEFMQESGTKALW HEFFMQESGTKALW	 * * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DYVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLSVKGYELMAKKLLELL (9) D-VHLSVKGYELMAKKMLDEL (9) D-VHLDPNGYKLVADKLYYEL 	(9) (9) (4) (4) (4) (4) (3) (2)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf consensus	PAAGILIIGAF PAAGILIIGAP PQAAILLGAP PQTVILIIG-P KNAVIMLSPP KNALIMLVSPP KNAIFILSPP PQASILLISPP P-A-ILLIP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) J PVVQK- (17) J PVVQKV (23) J K-	2RRV <mark>A</mark> RQGQ T MFWSV 2RRIARQGQ T MFWSV 2RQLARDNHLLYWDV 2QKVAQDQHTLFWNV 2YEVAKEEKTLIFDD 2YEVAKEEKTLLFDD 12KLAKQKQ TL LFDI 1AKLAKQKQ TL LFDI 	VQNAMGGICSMKNW VQNAMGGVCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW MHQFMQDSGGKNKW MHEFMQDSGGKNKW MHEFMQESGTKALW VEDFINQSGGKKKW M	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DYVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLSVKGYELMAKKLLELL (9) D-VHLLPNGYKLVADKLYYEL D-VHL-GYA-L-LL</pre>	(9) (9) (4) (4) (4) (4) (4) (3) (2)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf consensus 1b Bt orf	PAAGILIIGAF PAAGILIIGAP PQAAILLGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNAIFILISPP PQASILLISP PQASILLISP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVTQK- (17) I TVTQK- (17) J PVVQKV (23) J K	2RRVARQGQTMFWS 2RRIARQGQTMFWS 2RQLARDNHLLYWD 2QKVAQDQHTLFWN 2YEVAKEEKTLIFD 2YEVAKEEKTLLFD 2YELAKEEKTLLFD 1AKLAKQKQTLLFD 	VQNAMGGICSMKNW VQNAMGGVCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW HUQFMQDSGGKNKW HHEFMQESGTKALW HEFINQSGKKKW W FEAMGGECSMANL	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DYVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLSVKGYELMAKKMLDEL (9) D-VHLPNGYKLVADKLYYEL D-VHLGYA-LL (10) DYTHINFRGGKHLAGLLYETL</pre>	(9) (9) (4) (4) (4) (4) (4) (3) (2) (14)
Ape 1a Ng ApeA Nm orf CV orf Cj orf Cc orf Cu orf Hh orf consensus 1b Bt orf	PAAGILIIGAF PAAGLIIGAP PQAAILLIGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNALIMLVSPP PQASILLISPP P-A-ILLI-P PQASLLLSVG PQAGFLLSVG	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) I PVVQKV (23) I K- DRDYKT (17) (DRDYKT (17) (QRRVARQGQTMFWSV QRRIARQGQTMFWSV QRQLARDNHLLYWD QQRVAQDQHTLFWN YEVAKEEKTLIFD YEVAKEEKTLIFD YELAKEEKTLLFD CAKLAKQKQTLLFD ATLD QQNIAAESGIAFWN ONIAAESGIAFWN	NQNAMGGICSMKNW NQLAMGGPCSMRTW NQLAMGGPCSMRTW NRAYMGGKCSIRRW HHQFMQDSGGKNKW HHEFMQESGTKALW MEDFINQSGGKKKW W HFEAMGGEGSMANL FFEAMGGEGSMAGL	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DYVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLLPNGYKLVADKLYYEL D-VHL_PNGYKLVADKLYYEL D-VHL_PGGKHLAGLLYETL (10) DYTHINFRGGRHLAGLLYETL</pre>	(9) (9) (4) (4) (4) (4) (3) (2) (14) (14)
Ape la Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf consensus lb Bt orf Bf orf Pg orf	PAAGILIIGAF PAAGILIIGAP PQAAILLIGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNAIFILISPP PQASILLISPP P-A-ILLIP PQASLLLSVG PQAGFLLLSVG PDSDILLMSVS	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) I PVVQKV (23)] K- DRDYKT (17) (DRDYKT (17) (DRAORT (16) (2)	2RRVARQGQTMFWSV 2RRIARQGQTMFWSV 2RQLARDNHLLYWD 2QKVAQDQHTLFWN YEVAKEEKTLIFD YEVAKEEKTLIFD 1YELAKEEKTLIFD 1XKLAKQKQTLIFD 2QNIAAESGIAFWN 2QNIAAESGIAFWN 2QNIAAESGIAFWN 2QNIAAESGIAFWN	VQNAMGGICSMKNW VQLAMGGPCSMRTW VQLAMGGPCSMRTW VRAYMGRCSIRW VHQFMQDSGGKNKW VHQFMQDSGGKNKW VHQFMQESGTKALW VHEFNQSGGKKKW MW FFEAMGGEGSMANL IFEAMGGEGSMAGL CLRAMHALGGISRW	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DVVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLUPNGYKLVADKLYYEL D-VHL-GYA-LLL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHARGGRELAKKMTEA</pre>	(9) (9) (4) (4) (4) (4) (3) (2) (14) (14) (10)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf Bf orf Pg orf Zm orf	PAAGILIIGAF PAAGILIIGAF PQAAILLGAP PQTVILIIG-P KNAVIMLISPP KNAIFILISPP PQASILLISP PQASILLISVG PQAGFLLSVG PQAGFLLSVG PDSDILMSVS GNVPITMLGAD	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKV (17) I TVVQKV (17) J PVVQKV (17) J PVVQKV (17) (DRDYKT (17) (DRAQRT (13) (DASSRR (13) (2RRVARQGQTMFWSV 2RRIARQGQTMFWSV 2RQLARDNHLLYWDV 2QKVAQDQHTLFWNV 4YEVAKEEKTLIFDD 4YEVAKEEKTLIFDD 1XKLAKQKQTLIFD 1AKLAKQKQTLIFD 1AKLAKQKQTLIFD 2QNIAAESGIAFWNN 2QNIAAESGIAFWNN 2QRUASQIGTAFWN	VQNAMGGICSMKNW VQNAMGGVCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW HHQFMQDSGGKNKW HHQFMQDSGGKNKW HHEFMQESGTKALW HEFINQSGGKKKW M	 * * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DVVHLSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLLPNGYKLVADKLYYEL (9) D-VHLLPNGYKLVADKLYYEL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHLAHRGGRELAKKMEATEAT (10) DYTHLARGGRELAKKMEATEAT 	(9) (4) (4) (4) (4) (3) (2) (14) (14) (10)
Ape 1a Ng ApeA Nm orf Cv orf P1 orf Cj orf Cc orf Cu orf Hh orf consensus 1b Bt orf Bf orf Pg orf Zm orf Cm orf	PAAGILIIGAF PAAGILIIGAF PQAAILLIGAP PQTVILIIG-P KNAVIMLISPP KNALFILISPP PQASILLISPP PQASILLISPG PQAGFLLLSVG PDSDILLSVG PDSDILLMSVS GNVPILMIGAP PLLISVC	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVKKQ (17) I TVVKQ (17) I PVVQKV (23) I K- DRDYKT (17) (DRDYKT (17) (DRDYKT (17) (DRAQRT (16) (DASSRR (13) (DRT C	2RRVARQGQTMFWS 2RRIARQGQTMFWS 2QLARDNHLLYWD 2QKVAQDQHTLFWN 2YEVAKEEKTLIFD 2YEVAKEEKTLLFD 2YELAKEEKTLLFD 2QNIAAESGIAFWN 2QNIAAESGIAFWN 2QRVASQLGIAFWS 2QRVASQLGIAFWS 2QRVASQLGIAFWS	VQNAMGGICSMKNW VQNAMGGVCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW HHQFMQDSGGKNKW HHQFMQDSGGKKKW HHFFMQESGTKALW HEDFINQSGGKKKW FEAMGGEGSMANL IFEAMGGEGSMAGL LTRAMHALGGISRM VHARMGGCG	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DVVHSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLPNGYKLVADKLYYEL D-VHLPNGYKLVADKLYYEL D-VHLLPNGYKLVADKLYYEL (10) DYTHINFRGGRHLAGLLYETL (8) DYTHLAHRGGRELAKKMLEAI (10) DYTHSLGGQILANRLDNDI DYTHRGG-LA-T-E-</pre>	(9) (4) (4) (4) (4) (3) (2) (14) (14) (10) (10)
Ape la Ng ApeA Nm orf Cv orf Pl orf Cc orf Cu orf Hh orf consensus lb Bt orf Bf orf Pg orf Zm orf consensus lc Bt orf	PAAGILIIGAF PAAGILIIGAF PQAAILLIGAP PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNALILISPP PQASILISPP PQASILISPG PQAGFLLISVG PDSDILLMSVG PDSDILLMSVG PDLLISV- PDVPIL.TTPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) I PVVQKV (23) J K- DRDYKT (17) (DRDYKT (17) (DASSRR (13) (DRT GSYESF (21) (21) (21)	2RRVARQGQTMFWSV 2RRIARQGQTMFWSV 2RQLARDNHLLYWD 2QRVAQDQHTLFWN 2YEVAKEEKTLIFD 1YELAKEEKTLIFD 1YELAKEKKTLIFD 1XLAKLAKQKQTLIFD 2001AAESGIAFWN 2001AAESGIAFWN 2001AQCHGIAFWS 2021AQCHGIAFWS 20	VQNAMGGICSMKNW VQLAMGGPCSMKNW VQLAMGGRCSMRTW VRAYMGGKCSIRRW HHQFMQDSGGKNKW HHQFMQESGTKALW HEDFINQSGGKKKW HEDFINQSGGKKKW HFEAMGGEGSMANL HFEAMGGEGSMASL TLRAMHALGGISRW HHARMGGDCAAVHW AMGG-C	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) D-VHLISAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLJVKGYELMAKKLLEDL (9) D-VHLUPNGYKLVADKLYYEL D-VHLGYA-LL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHLNFRGGRLAKKMIEAI (10) DYVHYTSLGQUIANRLDNDI DYTHRGG-LA-L-E 9) DHVHJLPEGYULOGNLIVOA</pre>	(9) (4) (4) (4) (4) (2) (14) (10) (10) (10)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf consensus 1b Bt orf Bf orf Pg orf Zm orf consensus 1c Bt orf Bf orf	PAAGILIIGAF PAAGILIIGAF PQAAILLGAP PQTVILIIG-P KNAVIMISPP KNAIFILISPP PQASILISP PQASILISP PQASILISVG PQAGFLLSVG PQAGFLLSVG PDSDILMSVS GNVPILMIGAP PLLSV-	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) I PVVQKV (23)] K- DRDYKT (17) (DRDYKT (17) (DRAQRT (16) (DASSRR (13) (C)RT (25) GSYESF (21) 1 GSYESF (21) 1	2RRVARQGQTMFWSV 2RRIARQGQTMFWSV 2RQLARDNHLLYWDU 2QKVAQDQHTLFWNV YEVAKEEKTLIFDD YEVAKEEKTLIFDD 1YELAKEEKTLIFDD 1AKLAKQKQTLIFD 1AKLAKQKQTLIFDD 2QNIAAESGIAFWNN 2QALAQQHGIAFWS 2QRVASQLGIAFWS 2QRVASQLGIAFWD 2QRVASQLGIAFWS 2QRVASQLGIAFWS 2QRVASQLGIAFWS 2QRVASQLGIAFWS 2QRVASQLGIAFWS	VQNAMGGICSMKNW VQNAMGGVCSMKNW VQLAMGGRCSIRRW MRAYMGGKCSIRRW MHQFMQDSGGKNKW HHEFMQESGKNKW HEFINQSGGKKKW MW MFEAMGGEGSMANL MFEAMGGEGSMANL MFEAMGGCSMAGL FLRAMHALGGISRM HARMGGDCAAVHW AMGG-G MYDVVGGKRRACTN MYNVVGGRRACTN	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLSVKGYELMAKKLLEL (9) D-VHLSVKGYELMAKKLLEL (10) DYHLNFRGGKHLAGLLYETL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHLNFRGGRHLAGLLYETL (10) DYTHLAHRGGRELAKKMIEAI (10) DYVHTSLGQUIANRLDNE DYTHRGG-LA-L-E (9) DHVHVLPEGYTLQGNLLYQAI (9) DHVHVLPEGYTLQGNLLYAI </pre>	(9) (9) (4) (4) (4) (3) (2) (14) (10) (10) (10)
Ape 1a Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf start bf orf Pg orf Zm orf consensus 1c Bt orf Bf orf Pg orf	PAAGILIIGAF PAAGILIIGAF PQAAILLGAF PQTVILIGAF KNAVIMLISPP KNALIMLVSPP KNAIFILISPP PQASILLSPP PQASILLSVG PQAGFLLSVG PDSDILLSVG PDSDILLSVG PDSDILLSVG PDSDILLSVG PDSDILLSVG PDSDILLTPP PDVPILTTPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVKKQ (17) I TVVKQ (17) I TVVQKV (23) I K- DRDYKT (17) (DRDYKT (17) (DRDYKT (17) (DRAQRT (16) (DASSRR (13) (DRT GSYESF (21) I GSYLESF (21) I SSYLEP (25) (21) I	2RRVARQGQTMFWS 2RRIARQGQTMFWS 2RQLARDNHLLYWD 2QKVAQDQHTLFWN 4VEVAKEEKTLIFD 4VEVAKEEKTLIFD 4VEVAKEEKTLIFD 4000 40	VQNAMGGICSMKNW VQNAMGGVCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW HHQFMQDSGGKNKW HHEFMQESGTKALW HEFINQSGKKKW 	 * * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) DVVHSAAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLTIKGYELMAKKLLEDL (9) D-VHLDNGYKLVADKLYYEL (9) D-VHLDNGYKLVADKLYYEL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYTHUNFRGGRLAGLLYETL (10) DYTHUNFRGGRLAGLLYETL (10) DYTHYSLGGQILANRLDNDI DYTHRGG-LA-LE (9) DHVHYLPEGYILQGNLLYQAI (9) DHVHLPEGYILQGVILQUADA 	(9) (4) (4) (4) (3) (2) (14) (14) (10) (10) (10) (10) (10)
Ape la Ng ApeA Nm orf Cv orf Pl orf Cc orf Cu orf Hh orf consensus lb Bt orf Bf orf Pg orf Zm orf consensus lc Bt orf Bf orf pg orf	PAAGILIIGAF PAAGILIIGAF PQAAILLIGAF PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNALILISPP PQASILISPP PQASILISVG PQAGFLLISVG PDSDILLSVG PDSDILLSVG PDVPILNTPP PDVPILNTPP PDVPIL	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) J PVVQKV (23) J K DRDYKT (17) (DRDYKT (17) (DRAQRT (16) (DASSRR (13) (DRT C GSYESF (21) J PSYLRR (35) J	2RRVARQGQTMFWSV 2RRIARQGQTMFWSV 2RQLARDNHLLYWD 2QKVAQDQHTLFWNU .YEVAKEEKTLIFD 1YELAKEEKTLIFD 1YELAKEKTLIFD 2QNIAAESGIAFWNN 2QALAQQHGIAFWS 2QRVASQLGIAFWD 2QALAQCHGIAFWS 2QRVASQLGIAFWD 2QALAQCHGIAFWS 2QRVASQLGIAFWD 2QALAQCHGIAFWS 2QRVASQLGIAFWD 2QALAQCHGIAFWS 2QRVASQLGIAFWD 2Q	VQNAMGGICSMKNW VQLAMGGPCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW HHQFMQDSGGKNKW HHQFMQDSGGKNKW HHQFMQESGTKALW HEDFINQSGGKKKW HEDFINQSGGKKKW HFAMGGEGSMADL TLRAMHALGGISRM HHARMGGDCAAVHW AMGG-C YDVVGGKRACTN YDVVGGLRACKN JSAAMGGEAETRQW 	<pre>* * (9) DGVHFSAQGYRRAAEMLADSL (9) DGVHFSAKGYQRSAEMLADSL (9) DMVHFTAPGYTRLGDDLYEGL (9) D-VHLISAGYERSAEALYQQL (9) D-VHLTIKGYELMAKKLLDDL (9) D-VHLJVKGYELMAKKLLDDL (9) D-VHLUPNGYKLVADKLYYEL D-VHLGYA-LL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHINFRGGRHLAGLLYETL (10) DYTHINFRGGRLAGLLYETL (10) DYUHYTSLGQUIANRLDNDI DYTHRGG-LA-L-E 9) DHVHYLPEGYTLQGELLYEAI (8) DHIHYTVGGYTRHGGLVADAL DWUY2Y=Y</pre>	(9) (9) (4) (4) (4) (3) (2) (14) (10) (10) (10) (10) (17)
Ape la Ng ApeA Nm orf Cv orf Pl orf Cj orf Cc orf Cu orf Hh orf consensus lb Bt orf Bf orf Zm orf consensus lc Bt orf Bf orf pg orf consensus	PAAGILIIGAF PAAGILIIGAF PQAAILLIGAF PQTVILIIG-P KNAVIMLISPP KNALIMLVSPP KNAIFILISPP PQASILLISVG PQAGFLLLSVG PDSDILLMSVS GNVPILMIGAP PLLSV- PDVPILMTPP EDVPFVLTPP -DVPIL-TPP	E-SLKN (18) (E-SLKN (18) (D-AARS (24) (GDSLKN (20) (TVVQKQ (17) I TVVQKQ (17) I TVTQK- (17) I PVVQKV (23)] K- DRDYKT (17) (DRDYKT (17) (DASSRR (13) (DRT (16) (GSYESF (21)] GSYESF (21)] PSYLRR (35)] -SY 1	2RRVARQGQTMFWSV 2RRIARQGQTMFWSV 2RQLARDNHLLYWDI 2QKVAQDQHTLFWNV YEVAKEEKTLLFDN YEVAKEEKTLLFDN YYELAKEEKTLLFDN 2YEVAKEEKTLLFDN 2QNIAAESGIAFWNN 2QNIAAESGIAFWNN 2QALAQQHGIAFWSV 2QALAQQHGIAFWSV 2QRVASQLGIAFWDV 2QALAQHIAFWSV 2QRVASQLGIAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2QRVASQLAFWDV 2Q	VQNAMGGICSMKNW VQLAMGGVCSMKNW VQLAMGGPCSMRTW VRAYMGGKCSIRRW MHQFMQDSGGKNKW HHQFMQDSGGKNKW HHEFMQESGTKALW HEFINQSGGKKKW MGGEGSMANL FEAMGGEGSMANL FEAMGGEGSMANL FIRAMHALGGISRM HARMGGDCAAVHW AMGG-G MYDVVGGSLRACKN JSAAMGGEAETRQW MGG	 * * 9 DGVHFSAQGYRRAAEMLADSL 9 DGVHFSAKGYQRSAEMLADSL 9 DMVHFTAPGYTRLGDDLYEGL 9 DYVHISAAGYERSAEALYQQL 9 D-VHLTIKGYELMAKKLLEDL 9 D-VHLTIKGYELMAKKLLEDL 9 D-VHLSVKGYELMAKKLLEDL 9 D-VHLSVKGYELMAKKLLEL 10 DYTHINFRGGKHLAGLLYETL 10 DYTHINFRGGKHLAGLLYETL 10 DYTHINFRGGRHLAGLLYETL 10 DYTHINFRGGRHLAGLLYETL 10 DYTHLARFGGRELAKKMIEAI 10 DYTHLARFGGRELAKKMIEAI 11 DYVHYTSLGQQIIANRLDNDI 12 THRGG-LA-L-E 9 DHVHYLPEGYTLQGRLLYQAI 18 DHIHYTVGGYTRHGGLVADAL 18 DHIHYTVGGYTRHGGLVADAL 	(9) (9) (4) (4) (4) (3) (2) (14) (10) (10) (10) (10) (17)

Sequence alignment of family 1 O-acetylpeptidoglycan esterases (Ape) and identification of consensus motifs. The residues in bold face and yellow highlight denote greater than 50% identity amongst the subfamilies (1A, 1B, 1C) and the entire family, respectively, while those in red are invariant amongst all sequences. The numbers in brackets denote the length of intervening sequences and the potential catalytic residues are identified with the red asterisks. Abbreviations (accession numbers): Ng, N. gonorrhoeae (AAW89270); Nm, N. meningitidis (NP284202); Cv, Chromobacterium violaceum (NP903734); Pl, Photorhabdus luminescens (NP927853); Cj, Campylobacter jejuni, (NP281792); Cc, Campylobacter coli (ZP00366778); Cu, Campylobacter upsaliensis (ZP00369960); Hh, Helicobacter hepaticus (NP860613); Bt, Bacteroides thetaiotaomicron (1b, NP811595; 1c, NP811594); Bf, Bacteroides. fragilis (1b, YP101414; 1c, NP811594); Pg, Porphyromonas gingivalis (1b, NP905385; 1c, NP905384); Zm, Zymomonas mobilis (1b, YP162180).



Distribution of consensus sequence motifs within the three families of Ape proteins. The motifs of the family I Apes are colour coded, and the asterisks denote the locations of the catalytic serine and aspartyl/histidyl residues of motifs I and VII, respectively. The numbers at the beginning of each sequence denote the length of the respective N-terminal sequences.

present in these proteins. This segment may thus contribute to the proteins binding specificity given the prominent role that tryptophanyl residues play in the binding subsites of carbohydrate-active enzymes through stacking interactions.

The sequences of the family 2 Ape proteins are highly homologous (Fig. 7) and, like the family 1a proteins, are characterized by having five distinct segments of high similarity separating the proposed catalytic motifs I and VII (Fig. 7). However, the consensus motifs identified are unique to this family (Fig. 7). Whereas the GDS(H/F) sequence is conserved in motif I, the conserved residues separating the catalytic D and H of consensus motif VII are distinctively G and I/V. As with the family 1 proteins, the family 2 sequences are encoded within the genomes of a number of important human pathogens, such as species of *Neisseria, Campylobacter* and *Helicobacter*.

The family 3 proteins have very little in common with either the family 1 or family 2 proteins (Fig. 6). In fact, the GDS(H/F) motif is replaced with G(T/S)S and there does not appear to be any consensus in residues separating the

catalytic D and H residues of motif VII (Fig. 8). Whereas two other segments of some similarity are present between the catalytic motifs, there is a large region with minimal sequence similarity. This variation seen within the grouping suggests that further division of this family may occur once more genome sequences become available. Nonetheless, again this family does include sequences from some important pathogens such as *Helicobacter pylori* and *Bacillus anthracis*.

Predicted cellular localization of Ape

The SignalP, PSORT and DAS prediction programs indicated that almost all of the hypothetical Pat and Ape protein sequences have an N-terminal localization signal. The only exception appears to be with the Ape sequence from *H. hepaticus*. Family 1 proteins from *H. hepaticus*, *P. luminescnes*, *Z. mobilis* and *P. gingivalis* may have a cytoplasmic membrane association due to the retention of an α -helical transmembrane anchor. However, the other Ape1 sequences contain a predicted signal peptidase I cleavage site that should release the expressed proteins from the cytoplasmic membrane. In the case of the Ape2 homologs, the *Campylobacter* sp. and *P. luminescens*

<i>Ng</i> Ape2	124	GDKVFFAGDSLMQGVAPFVQKSLKQQY	JIESAN L<mark>SKQ</mark>S<mark>TGL</mark>	S-YPSFFDWPK-T]	LEE TL K K H P -E <mark>I</mark>	SV <mark>L</mark> AV-F <mark>LG</mark> P <mark>NDPWD</mark> F
Nm orf	116	GDKVFFAGDSLMQGVAPFVQKSLKQQY	GIESVNL <mark>SKQ</mark> S <mark>TGL</mark>	S- <mark>Y</mark> PSF <mark>FDW</mark> PK-TJ	LEE TL KKHP-E <mark>I</mark>	SV <mark>L</mark> A <mark>V</mark> -F <mark>LG</mark> P <mark>NDPWD</mark> F
Cv orf	128	GLP VL LAGDSMMQGVALHLLPPLFRQH	QVKAIDI <mark>SKQ</mark> S <mark>TGL</mark>	T- <mark>Y</mark> PDF <mark>F</mark> N <mark>W</mark> P-A <mark>T</mark> J	LAERQLANP-KT	Q- <mark>L</mark> L <mark>V</mark> MFV <mark>GAND</mark> T <mark>WD</mark> M
Pl orf	164	K D K VLF A <mark>GDS</mark> M MQG I <mark>A</mark> PH L KRR <mark>L</mark> YQDYS	SISSINL <mark>SKQ</mark> S <mark>TGL</mark>	A- <mark>Y</mark> P <mark>S</mark> F <mark>F</mark> NWPE-TJ	INK TL EA N H-D <mark>I</mark>	K- <mark>LVV</mark> IF <mark>LG</mark> P <mark>NDPWD</mark> M
Cj orf	129	GDEFLFIGDSLMQGVAIALNRDLRNLN·	-LKVTD L<mark>SKQ</mark>N<mark>TGL</mark>	S- <mark>Y</mark> KSY <mark>FDW</mark> SKATN	JEAFI- KN S-N <mark>I</mark>	KY <mark>LVV</mark> L- <mark>LG</mark> ANDPWDI
Cc orf	118	GDEFLFIGDSLMQGVAIALNRDLIDLG	-LKANDL <mark>SKQ</mark> N <mark>TGL</mark>	S- <mark>Y</mark> KSY <mark>FDW</mark> AKA <mark>T</mark> F	(EAFA- KNP -N <mark>I</mark>	KY <mark>LVV</mark> L- <mark>LGANDPWD</mark> I
<i>Cu</i> orf	116	NEEFLLI <mark>GDS</mark> LMQGVAVALNKDLKNLN	-LKVVDL <mark>SKQ</mark> N <mark>TGL</mark>	S- <mark>Y</mark> KSY <mark>FDW</mark> AKE <mark>T</mark> A	AK-TLQNNK-K <mark>I</mark>	KY <mark>LVV</mark> L- <mark>LGANDPWD</mark> I
Hh orf	211	GSSVLLIGDSMMQGVAPYVLKTFKKVN·	-LRGINL <mark>SK</mark> HS <mark>TGL</mark>	T- <mark>Y</mark> KHY <mark>F</mark> N <mark>W</mark> EAALF	(D-TFA KNP -D <mark>I</mark>	ALV VV L- <mark>LGANDPW</mark> GM
Zm orf	114	PLR V GVF <mark>GDS</mark> YGD <mark>G</mark> LWSALYRLLPAKE	GYRVLKF <mark>S</mark> Q Q S <mark>TG</mark> F'	TR <mark>Y</mark> Q <mark>S</mark> LNVEE K AKS	SD-LV-SQPVD <mark>i</mark>	A V ICF- <mark>G</mark> ANDVQGV
consensu	S	GD-VLF- <mark>GDS</mark> L <mark>MQGVA</mark> L <mark>L</mark>	L <mark>SKQ</mark> -TGL	S- <mark>Y</mark> -S-FDW-K-T		LVVL <mark>G</mark> ANDPWD
motif		I		II		III
Na Anol					עד אמ דע מ	
Ng Apez			EAAHIHKVQVVWLG	TDYMERARDCOM		
Nui OII						
			ASAGARAVAVLWLG		KYIDGI YOGH W	AAGGG-RFISIREIL
			I EARNHGADIIWVA		NILKSLIUSE-V	I KAGE-I IVSVND
			KIAKKHKAKVFWFE	TPPVKKEDLNKKI	QVLNKIISDE-I	
CC OTI		-KKGG-VYHRFGSDSWIDIYTYRVNELI	NIAKQHHAKILWFE		QILNKIYSEE-I	LKNKQ-IFINTKLFF
Hn ori		KNISFKSVRWEETYIQRIEELL	NVAHSIGARVVWIE	VPSVSSKSLNDKI	VYLNSLYERV	VKEEGEYFLQSNGII
Zm ori		LV G HHAAPLL <mark>S</mark> AE <mark>W</mark> RQVIGD <mark>R</mark> IESFV	SMLRQKGIS <mark>v</mark> Y <mark>w</mark> VG	L <mark>P</mark> VMRKADFDASI	SSMINSF <mark>Y</mark> AA <mark>E</mark> -M	A <mark>k</mark> ln-vpf1e1r-sl
consensu	g	$\mathbf{G} = \mathbf{Y} = \mathbf{F} = \mathbf{S} = -\mathbf{W} = -\mathbf{Y} = -\mathbf{R}\mathbf{V}$	V -W	$-\mathbf{P}-\mathbf{M}-\mathbf{K}\mathbf{I}\mathbf{N}$	YT.N-I.YR	- <mark>K</mark> -KTF <mark>T-T</mark>
motif	5			V	v	T T
				•	•	-
			* *			
Ng Ape2		SGGKGR <mark>Y</mark> T-D-SVNV <mark>N</mark> GKPVRY <mark>R</mark> S	K <mark>DGIHFT</mark> AE <mark>G</mark> OKLL	AEKIMEK <mark>I</mark> VFEPS'	TOPSSTOP	
Nm orf		SGGKDR <mark>Y</mark> T-D-SVNV <mark>N</mark> GKPVRY <mark>R</mark> S	K <mark>DG</mark> I HFT AE <mark>G</mark> ÕKLL	AAKIMEK <mark>I</mark> VFEPS'	TÕPSSTÕP	
Cv orf		G SODDSFNKFMTLPDOGEVAV <mark>R</mark> T	A <mark>DGVHFT</mark> RO <mark>G</mark> ÕLLL	ARRV L AELRFE	~ ~	
Pl orf		IFKYHÄND <mark>Y</mark> S-DY-VGD <mark>N</mark> ŠNKIKL <mark>R</mark> S	g <mark>dg</mark> i hf slř <mark>g</mark> ooti	SDNIFSL <mark>I</mark> KFIOE	PAKONENS	
Ci orf		SVNDEYSA <mark>Y</mark> IK-DE <mark>N</mark> NRSIKV <mark>R</mark> T	D <mark>DGVHFT</mark> PS <mark>G</mark> AREM	I S KLL L EH <mark>I</mark> KLKĒE!	NASK	
Cc orf		SVNDEFSTYIK-NENNKSIKMRT	D <mark>DGIHFT</mark> SN <mark>G</mark> AKEM	SKLLLOH <mark>I</mark> TIKEE	NAN	
Cu orf		SKNDAYSA <mark>Y</mark> IK-DENNKSIKVRS	D <mark>DGVHFT</mark> PS <mark>G</mark> AKEM	I S KLLLEY <mark>I</mark> KLKDN	NASF	
Hh orf		TOG G K <mark>Y</mark> SAFIK-NA <mark>N</mark> GKSVOVRI	D <mark>DGVHFT</mark> AR <mark>G</mark> YOIM	ANIF L NALEIIPO	VKELDVOEDSIH	SLPAODVEYOIKEHLWE
Zm orf		TVDEN G OYAP <mark>Y</mark> LMDGTNDATK K MTLMRA	N <mark>DGVH</mark> MSMT <mark>G</mark> YVRL	SRGLASO <mark>I</mark> RHFAD	OHRPKLPATSSE	TASLVASOKEVSR
		~		~	~ ~~~	
consensu	s	<mark>N</mark> -KR-	- <mark>DG</mark> V <mark>H</mark> FT <mark>G</mark>			
motif		VI	I			

Sequence alignment of family 2 O-acetylpeptidoglycan esterases (Ape2) and identification of consensus motifs. The residues in bold face and yellow highlight denote greater than 50% and 80% identity, respectively, while those in red are invariant amongst all sequences. The numbers in brackets denote the length of intervening sequences and the potential catalytic residues are identified with the red asterisks. Abbreviations (accession numbers): Ng, N. gonorrhoeae (AAW89271); Nm, N. meningitidis (NP284201); Cv, Chromobacterium violaceum (NP903735); Pl, Photorhabdus luminescens (NP927854); Cj, Campylobacter jejuni, (NP281793); Cc, Campylobacter coli (ZP00366779); Cu, Campylobacter upsaliensis (ZP00369961); Hh, Helicobacter hepaticus (NP860614); Zm, Zymomonas mobilis (1b, YP162179).

sequences are predicted to associate with the cytoplasmic membrane, while the *Neisseria* sp. and *C. violaceum* proteins would be cleaved by signal peptidase I releasing them to the periplasm. The family 3 Ape from *H. pylori* is also predicted to remain associated with the cytoplasmic membrane. These predictions are thus consistent with the proposed function of the expressed proteins as participating in the *O*-acetylation of peptidoglycan as a maturation event occurring within the periplasm [5].

Genomic organization of the OAP cluster

Despite their distant phylogenic relationship, the *patA* and *ape* genes are closely associated on respective chromo-

somes and comprise a small cluster we have called \underline{O} acetylpeptidoglycan (OAP) (Fig. 3). However, different gene organizations were observed which appear to be specified by the type of *ape* genes present. Thus, species containing the Ape1a proteins possess the strict gene order of *pat* immediately followed by *ape2* and then *ape1a*. In contrast, the *ape1b* gene always appears to be located upstream of *pat*, sometimes with the addition of an intervening *ape1c* gene. The only *ape2* not located downstream of *pat* is found in *Zymomonas mobilis* where instead it is positioned upstream together with an *ape1b* gene. All of the other *ape1b* genes are situated at the start of the OAP cluster and are followed in order by an *ape1c* and then *pat*. *

Hp orf66Bj orf64Gv orf62Ba orf66Bc orf66	ILIGTSISLNF-SLK-DIHDLLGLKDPIKLT-VSGM-NI AFIGTSLAIHFRQSDIDRA-LGVRSLKLAMTGSNSRQQ YIFGSSRTMYYAAAAADRA-SGERYRFFNFGVSLEN VILGSSRT-TFIN-ENDFTGLNA-FNYAVSSTDPF VILGSSRT-TFIN-ENDFTGLNA-FNYAVSSTDPF	GEILSLLPFAFKHQPVNTVAMDLAFFEFIL- NK ESKYFFEYPYF GFVLEQAIDR-GARRVIWAMDDFSFVDAAG-IEAD-PY- GFGI-RRKLEWLIRQKRIQK-ALIGLDFDIQS-VPTD-P LEYDAYIEYAKERKGSELSSIVIGLDFLGTNKNREIGFEKPEV LEYDAYIEYAKERKGSELSSIVIGLDFLGTNKNREIGFEKPEV
consensus	I- <mark>GSS</mark> RTFDGLFNVS	I-IGL <mark>DF</mark>
motif	I	II
Hp orf	TGGFVY LY NFGVLKNALFYFSTN Y L K IKEKTFCK	LNS WFQLYDFCNSVL N RYDFDFMFSH N NPYDYSLDNVK <mark>R</mark>
Bj orf	LSVD LY RRTAKGIAAYLFSGPMA K ESLFALLRSVF	PLREP L-SR AAPFLPVKFALSDVD-DI Y ALPRDFDVARAYNA E R
Gv orf	LD L LRQDHPL VSGD SALGF <mark>Y</mark> G K YLLFQPRIIAI	FVAENLDSRKA-YGFETWDRGN-DNGPQAL-YPIGRPFDP E R
Ba orf	YINEAQSL LY P-IKSY VSMD GIT Y	SRNTIQNSIHH N K-TIDV Y NRDNIKSMREYTKE E R
Bc orf	YINEAQSL LY P-IKSY VSMD GIT Y	SRNTIQNSIHH N K-TID UY NRDNIKSMREYTKE <mark>E</mark> R
consensus motif	LYVS-DY-K III	
Hp orf	S-Y Y LS <mark>AL</mark> KNPNQ-LA-IDEENA Y KITKQI	EDFIQKHSDKHFILWTRTDSLLKYKVYNHTILTRNLN-TIHNAL
Bj orf	TRRAFAYITDPVRSSF L GEGYERDAMIRHFERDAASLIT	RHP-DVTFDVY-FPPYSILQFVAMRDASPATLTIVTDLTARIAQ
Gv orf	LYAVSAG <mark>ALA</mark> LDIAR-SNRKPPRPVAASGLE	EYGRSVALLDAAGVARVLVVPPFSLEKFRSYDIDAYLSWLA
Ba orf	D-TQI A -A-QVDKFSREIYGGNYEYQNMK	EILGTVKSHNPNTKFYIFTTPVSEPLFRTMIQSGRWEDYKRWLH
Bc orf	D-TQI A -A-QVDKFSREIYGGNYEYQNMK	EILGTVKSHNPNTKFYIFTTPVSEPLFRAMIQSGRWEEYKRWLH
consensus motif	* *	
Hp orf	KTLLKYPNVEIHDLRTM-PLAKEIKRYKDIRHY-DPIGS	KEVLQAIASKKYLLT <mark>P</mark> NNIDSFKQKLIQTIENYQ <mark>I</mark> PKEIQN
Bj orf	RLTTQLPNVRLHDFRAIKAVTHDLNNYGDVIHH-SPAVC	DAMVLKWLASGEYRVDP-K-APLASLEELKAQVEAYRVER
Gv orf	ETVRVGGRVWDFAGVNSVTADGRNFGDPVHFLKP-VG	DMVLRRVLDPNPTGLPADFGVLLTSQNLAARLVQLRAQHRKLQL
Ba orf	DAVDVFGEVHHFMYLNSVTKNLDNYMDGHHF-RPEVG	KIIAHKVVNEEDRNVPKDFGIVITKDNIDEVLEQIHASFQ
Bc orf	DAVDVFGEVHHFMYLNSVTKNLDNYMDGHHF-RPEVG	KIIAHKVVNEEDRNVPKDFGIVITKDNIDEVLEQIHASFQ
consensus	V-G- <mark>V</mark> HDFNS <mark>VT</mark> K-L- <mark>NY</mark> -DHFP-VG	K-VL R <mark>P</mark> DFGTNE-L- <mark>Q</mark> I- <mark>A</mark>
motif	IV	V

Figure 8

Sequence alignment of family 3 O-acetylpeptidoglycan esterases (Ape3) and identification of consensus motifs. The residues in bold face and yellow highlight denote greater than 50% and 80% identity, respectively, while those in red are invariant amongst all sequences. The numbers in brackets denote the length of intervening sequences and the potential catalytic residues are identified with the red asterisks. Abbreviations (accession numbers): Hp, Helicobacter pylori (NP207650); Bj, Bradyrhizobium japonicum (NP767014); Gv, Gloeobacter violaceus (NP923892); Ba, Bacillus anthracis (NP843402); Bc, B. cereus (NP977300).

Interestingly, the family 3 Ape appears to be capable of replacing the function(s) of both the family 1 and 2 proteins because its gene is situated alone immediately downstream of *pat*. Thus, this gene arrangement more closely resembles that of distantly related organisms like *Bacillus anthracis, Bradyrhizobium japonicum* and *Gloeobacter violaceus* than that of *H. hepaticus*.

Extent of peptidoglycan O-acetylation in Pat- and Apeproducing bacteria

With the exception of *Agrobacterium tumefaciens*, none of the bacteria listed in Fig. 3 are xylanolytic suggesting that the discovered genes may be used by the respective bacteria for the modification of their peptidoglycans as proposed for *N. gonorrhoeae*. However, of these listed bacteria, only *N. gonorrhoeae* was previously known to *O*-acetylate its peptidoglycan [5,6,27]. Hence, we investi-

gated the presence of this modification in a selection of the bacteria possessing the OAP cluster of genes.

Following the isolation and purification of the peptidoglycans from cultures of the respective cells grown to late exponential phase, the quantification of base-labile, esterlinked acetate was determined by the HPLC-based method previously described [27]. Of the nine species tested, eight were found to possess *O*-acetylated peptidoglycan (Table 1). The levels of this *O*-acetylation were not stoichiometric, consistent with previous observations made with each of the other known bacteria that modify their peptidoglycan in this manner [5,6,27]. Thus, the extent of *O*-acetylation ranged from 14.7 % to 65.6 % relative to muramic acid content. These data thus provide preliminary support for the ascribed function of the OAP cluster of genes for the *O*-acetylation of peptidoglycan.

Species	Strain	% O-Acetylation ¹
Agrobacterium tumefaciens	A6	3.44 ± 0.79
	371	1.52 ± 0.36
Bacillus cereus	ATCC 10702	24.7 ± 0.09
Bacteroides fragilis	ATCC 25285	69.4 ± 1.2
Bacteroides thetaiotamicron	ATCC 29741	27.0 ± 1.2
Bradyrhizobium japonicum	532 C	18.5 ± 2.2
Campylobacter jejuni	ATCC 700819	62.7 ± 5.6
	NCTC 11168	55.7 ± 1.4
Chromobacterium violaceum	ATCC 12472	14.7 ± 2.5
Helicobacter pylori	ATCC 700392	45.6 ± 3.2
Photorhabdus luminescens	ATCC 29999	65.6 ± 1.6
Ruminococcus flavefaciens	007	33.4 ± 3.4

Table I: Extent of	f peptidoglycan	O-acetylat	ion in bacteria	possessing OAP	gene clusters
Table I. Extent of	peptidogiyean	O-acceyiae	aon in bacceria	possessing Ori	gene clusters

I. Average ± s.d.(n = 3)of base-labile acetate content relative to muramic acid concentrations in isolated and purified peptidoglycan.

With *A. tumefaciens*, only minimal levels of peptidoglycan *O*-acetylation were detected suggesting that the "Ape" proteins produced by this xylanolytic bacterium may function as authentic xylan esterases. Alternatively, environmental signals may be required by this plant pathogen to induce the *O*-acetylation of its peptidoglycan.

Conclusion

A new cluster of genes putatively involved in regulating Olinked acetate levels has been identified in a number of bacteria. These O-acetylpeptidoglycan clusters all consist of an O-acetyltransferase and at least one O-acetyl esterase. The O-acetyltransferases demonstrate high amino acid similarity to members of the MBOAT family of acetyltransferases [24] while the O-acetyl esterases share similarity to the CAZy family 3 esterases (CE3). The previously recognized members of family CE3 are all acetyl xylan esterases; no de-N-acetyltransferases belong to this family. Acetyl xylan esterases aid in the degradation of xylan by removing the blocking O-linked acetate and thereby permitting its subsequent hydrolysis by xylanases (reviewed in [9]). As many of the bacteria identified with these O-acetyl esterases are not xylanolytic and their genomes do not appear to encode xylanases, we postulated that these hypothetical esterases function on peptidoglycan to specifically catalyze de-O-acetylation. Consequently, they have been termed Ape proteins for their hypothetical O-acetylpeptidoglycan esterase activity.

Recognition of different domain structures for each of the Ape proteins prompted us to divide them into three distinct families. Of the families, only Ape3 appears to lack a clear conserved domain structure and this may represent a group that needs more members to permit its definitive subdivision. However, each of the bacteria possessing an Ape3 protein contain the unique cluster order of an *O*-

acetyltransferase followed by a single O-acetyl esterase. The other Ape proteins are associated with an O-acetyltransferase, but always seem to be found in pairs consisting of Ape1b and Ape1c or Ape1a/1b and an Ape2. This ORF organization may translate into the evolutionary development of the Ape families. In fact, evolutionary divergence seems plausible for the Ape1 and Ape2 proteins, where duplication of an Ape1a/1b protein and divergence into an entirely new protein could have easily occurred. However, the ORF organization in Helicobacter species makes this theory far more confounded. Alternatively, differences between the Ape families may be linked to separate cellular functions. Some of the Ape proteins, especially those associated with either the cytoplasmic membrane and cytoplasm, may be involved in peptidoglycan recycling, while those in the periplasm and/or outer membrane of the gram-negative bacteria may be involved in the general maintenance of the peptidoglycan sacculus.

Regardless of their specific role in peptidoglycan metabolism, the activity of Ape proteins would be essential for the removal of acetate which would otherwise preclude the lysis of peptidoglycan by endogenous enzymes. Indeed, the major lytic enzymes associated with both the biosynthesis and maintenance of peptidoglycan are the lytic transglycosylases, enzymes that require a free C-6 hydroxyl group on muramoyl residues to produce their 1,6-anhydro product. Given that the bacteria harboring genes apparently involved with this activity were demonstrated to produce O-acetylated peptidoglycan, Ape activity would be required to function in advance of the lytic transglycosylase, many of which act processively along peptidoglycan strands [1], to provide appropriate sites for cleavage. Although strictly conjecture at this point, it is likely that these Ape proteins would be associated with the peptidoglycan 'replicase' holoenzyme complexes [1]

which have been shown to include both the lytic transglycosylases and penicillin-binding proteins [28-30]. In this regard, it should be noted that many of the ape genes are in close proximity to other genes encoding enzymes involved in cell wall associated functions. For example, the three Campylobacter sp. contain a ferritin gene (cft and pfr) immediately upstream, while both C. coli and C. upsaliens also possess genes encoding a potential ABC transporter and an outer membrane protein directly downstream of the OAP cluster. Similarly, both Bacteroides sp. encode a putative cation-efflux transporter upstream of their OAP clusters, while the Z. mobilis chromosome encodes a nearby cell wall hydrolase. Hence, through a dynamic process of removal and attachment of O-acetate, the bacteria would be able to obtain a high level of localized control over the degradation of peptidoglycan. This would facilitate, for example, the efficient insertion of pores and flagella, localize spore formation, and control the level of general peptidoglycan turnover.

It is difficult to determine whether or not a correlation exists between O-acetylation levels and the type or number of O-acetyl esterases present in a particular organism. In the case of the Ape1b/1c and Ape3 this may be because so few organisms have been tested for peptidoglycan O-acetylation. However, even Neisseria sp., C. violaceum, and the Campylobacter sp., which all possess Ape1a and Ape2, contain O-acetylation levels that vary significantly. This suggests that the level of O-acetylpeptidoglyesterase may be regulated can activity by compartmentalization or at the level of transcription/ translation. The Ape family 1a/1b proteins are different from the Ape1c proteins due to the presence of an extra amino acid segment. The variation in each of these segments raises questions of substrate specificity and function that must be proven, but it is interesting to note that one of the segments has partial similarity to PBP 4, a D,D carboxypeptidase. These proteins are peptidoglycan maintenance proteins, and therefore suggest that the extra protein segment found in the Ape1a/b proteins may be a peptidoglycan specific fragment or one directing proteinprotein interactions. This would certainly explain its absence from the O-acetylxylan esterase CE3 family, but not necessarily from the other Ape families.

Two models of peptidoglycan *O*-acetylation have been proposed in a recent review [6]. The first model involves the sequential action of two proteins. The first protein would be located within the cytoplasmic membrane and facilitates the transfer of acetate from acetyl-CoA to the periplasm, where a second protein accepts the acetate and attaches it to peptidoglycan. The second model consists of a single membrane bound *O*-acetyltransferase that performs both the transport of acetate across the cytoplasmic membrane and the peptidoglycan *O*-acetylation steps. A peptidoglycan O-acetyltransferase recently identified in S. *aureus* [17] is expected to act by the second peptidoglycan O-acetylation pathway. This O-acetyltransferase bears no resemblance to the O-acetyltransferases identified in the present study. This strongly suggests that, as with the O-acetyl esterases together with the lytic transglycosylases [31] and the PBPs [32], different classes of peptidoglycan O-acetyltransferases exist.

In summary, experiments are still required to conclusively demonstrate that the *O*-acetyltransferases and Ape proteins identified in this study are actually involved in controlling peptidoglycan *O*-acetylation. However, it is important to note that all the organisms that demonstrated some level of peptidoglycan *O*-acetylation also contain the putative *O*-acetylation cluster. Verification of peptidoglycan *O*-acetylation in the other bacteria containing the *O*-acetylation cluster are in progress, and should further strengthen the link between these two events. In addition, experiments characterizing the gene product of *N. gonorrhoeae* Ape1a have been initiated and preliminary evidence supports its function as an *O*-acetylpeptidoglycan can esterase.

Methods

Chemicals and reagents

DNase I, RNase A, and pronase were purchased from Roche Molecular Biochemicals (Laval, PQ), while Fisher Scientific (Nepean, ON), provided Luria Bertani (LB) growth medium. All other growth media were purchased from Difco Laboratories (Detroit, MI), and all other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO).

Bacterial strains and growth media

Agrobacterium tumefaciens and Photorhabdus luminescens were both cultured in Tryptic Soy Broth (TSB) for 48 h at ambient temperature with shaking (200 rpm) while Bacillus cereus was grown in Penassay Broth as previously described [33]. Chromobacterium violaceum was cultured in LB broth with shaking (200 rpm) for 48 h at ambient temperature. Bacteroides fragilis and B. thetaiotaomicron were grown at 37°C under strict anaerobic conditions using Bacteroides Bile Esculin Broth (BBE) supplemented with 100 mg/mL gentamicin while Ruminococcus flavefaciens was cultured anaerobically using Dehority's Complete Artificial Medium as previously described [34]. Helicobacter pylori and Campylobacter jejuni were cultured at 37 °C under microaerophilic conditions (10% (vol/vol) O₂, 5% (vol/vol) CO2, and 85% (vol/vol) N2) in Brain Heart Infusion Broth supplemented with 10% irradiated horse serum and TSB supplemented with 0.6% (w/vol) yeast extract, respectively. Bradyrhizobium japonicum was grown in Yeast Extract Mannitol broth at ambient temperature as previously described [35].

Peptidoglycan isolation and determination of extent of O-acetylation

O-Acetylated peptidoglycan was isolated and purified from the various bacteria, taking the necessary precautions to prevent the facile hydrolysis of *O*-acetyl groups as previously described [36]. Quantification of *O*-acetyl content was performed by both the HPLC-based organic acid analysis using an Aminex HPX-87H Bio-Rad column as previously described [27] and using the Megazyme Acetic Acid Assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The extent of *O*-acetylation is presented as a percentage of muramic acid content as determined by quantitative aminosugar analysis [37].

Identification and genomic analysis of a putative cluster of peptidoglycan O-acetylation ORFs

The raw nucleotide sequence data of the N. gonorrhoeae FA1090 genome project [18] was searched for hypothetical proteins involved in acetate transport using the Pseudomonas aeruginosa protein sequence for AlgI as the probe (algI encodes a putative acetate translocator involved with the biosynthesis of alginate [14,15]). Identification of open reading frames, protein translations, and isoelectric points (pI) were performed using Clone Manager 5. Signal sequence and cellular localization predictions were performed using signalP version 3 [38], PSORT [39], and the DAS Transmembrane Prediction Server [40]. Searches for homologs to the characterized N. gonorrhoeae ORFs in the NCBI database were carried out using the BLASTP search engine to tentatively identify functions for each of the ORFs in the cluster [41]. Sequences with an (E) value threshold of 1e-06 were used to generate multiple alignments using ClustalW Version 1.8 software [42]. Manual correction of these alignments was performed based on the original BLASTP results in conjunction with the MEME/ MAST Version 3.0 motif discovery tool [43]. Predictions of secondary structure were performed using the GOR IV Secondary Structure Prediction method [44] and PSI PRED [45], while searches for known domains were performed using CD-Search [46] at NCBI.

Authors' contributions

JTW and AJC conceived the study, participated in the sequence alignments and analyses, and wrote the manuscript. JMP performed the quantification of peptidoglycan *O*-acetylation, and all authors read and approved the final manuscript.

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References

- Höltje J-V: Growth of the stress-bearing and shape-maintaining murein sacculus of Escherichia coli. Microbiol Mol Biol Rev 1998, 62:181-203.
- Koraimann G: Lytic transglycosylases in macromolecular transport systems of Gram-negative bacteria. Cell Mol Life Sci 2003, 60:2371-2388.
- Shockman GD, Höltje J-V: Microbial peptidoglycan (murein) hydrolases. In New Comprehensive Biochemistry, Bacterial Cell Wall Volume 27. Edited by: Ghuysen J-M, Hakenbeck R. Amsterdam: Elsevier BV; 1994:131-166.
- Höltje J-V, Mirelmen D, Sharon N, Schwarz U: Novel type of murein transglycosylase in Escherichia coli. J Bacteriol 1975, 124:1067-1076.
- Clarke AJ, Dupont C: O-Acetylated peptidoglycan: Its occurrence, pathobiological significance and biosynthesis. Can J Microbiol 1992, 38:85-91.
- Clarke AJ, Strating H, Blackburn NT: Pathways for the O-acetylation of bacterial cell wall polymers. In *Glycomicrobiology* Edited by: Doyle RJ. New York: Plenum Publishing Co. Ltd; 2000:187-212.
- Dupont C, Clarke AJ: Dependence of lysozyme-catalyzed solubilization of Proteus mirabilis peptidoglycan on the extent of O-acetylation. Eur J Biochem 1991, 195:763-769.
- 8. Swim SG, Gfell MA, Wilde CE III, Rosenthal RS: Strain distribution in extents of lysozyme resistance and O-acetylation of gonocccal peptidoglycan determined by high-performance liquid chromatography. Infect Immun 1983, 42:446-452.
- Rosenthal RS, Folkening WJ, Miller DR, Swim SC: Resistance of Oacetylated gonococcal peptidoglycan to human peptidoglycan-degrading enzymes. Infect Immun 1983, 40:903-911.
- Rosenthal RS, Blundell JK, Perkins HR: Strain-related differences in lysozyme sensitivity and extent of O-acetylation of gonococcal peptidoglycan. Infect Immun 1982, 37:826-829.
- Blundell JK, Smith GJ, Perkins HR: The peptidoglycan of Neisseria gonorrhoeae: O-acetyl groups and lysozyme sensitivity. FEMS Microbiol Lett 1980, 9:259-261.
- Blackburn NT, Clarke AJ: Characterization of soluble and membrane-bound family 3 lytic transglycosylases from Pseudomonas aeruginosa. Biochemistry 2002, 41:1001-1013.
- Payie KG, Strating H, Clarke AJ: The role of O-acetylation in the metabolism of peptidoglycan in Providencia stuartii. Microb Drug Resist 1996, 2:135-140.
- Nivens DE, Ohman DE, Williams J, Franklin MJ: Role of alginate and its O acetylation in formation of Pseudomonas aeruginosa microcolonies and biofilms. J Bacteriol 2001, 183:1047-105.
- Franklin MJ, Ohman DE: Identification of algl and algl in the Pseudomonas aeruginosa alginate biosynthetic gene cluster which are required for alginate O-acetylation. J Bacteriol 1996, 178:2186-2195.
- Firmin J, Wilson KE, Carlson RW, Davies AE, Downie JA: Resistance to nodulation of cv. Afghanistan peas is overcome by nodX, which mediates an O-acetylation of the Rhizobium leguminosarum lipo-oligosaccharide nodulation factor. Mol Microbiol 1993, 10:351-360.
- Bera A, Herbert S, Jakob A, Vollmer W, Gotz F: Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of Staphylococcus aureus. Mol Microbiol 2005, 55:778-787.
- 18. Neisseria gonorrhoeae Genome Sequencing Strain FA 1090 [http://www.genome.ou.edu/gono.html]
- CAZy Carbohydrate-Active enZYmes [http://afmb.cnrsmrs.fr/CAZY]
- 20. Clarke AJ: Biodegradation of Cellulose: Enzymology and Biotechnology. Boca Raton: CRC Press; 1997.
- Upton C, Buckley JT: A new family of lipolytic enzymes? Trends Biochem Sci 1995, 20:178-179.
- 22. Vollmer W, Tomasz A: The pgdA gene encodes for a peptidoglycan N-acetylglucosamine deacetylase in Streptococcus pneumoniae. J Biol Chem 2000, 275:20496-20501.
- 23. Fukushima T, Kitajima T, Sekiguchi J: A polysaccharide deacetylase homologue, PdaA, in *Bacillus subtilis* acts as an N-acetylmuramic acid deacetylase in vitro. *J Bacteriol* 2005, 187:1287-1292.

- 24. Hofmann K: A superfamily of membranebound O-acyltransferases with implications for Wnt signalling. Trends Biochem Sci 2000. 25:111-112.
- 25. Rajashankar KR, Ray SS, Bonanno JB, Burley SK: Crystal structure of penicillin-binding protein 4 (Pbp4) from Staphylococcus aureus. PDB: ITVF [http://www.ncbi.nlm.nih.gov/Structure/mmdb/ mmdbsrv.cgi?form=6&db=t&Dopt=s&uid=28361].
- 26. Morlot C, Pernot L, Le Gouellec A, Di Guilmi AM, Vernet T, Dideberg O, Dessen A: Crystal structure of a peptidoglycan synthesis regulatory factor (PBP3) from Streptococcus pneumoniae. J Biol Chem 2005, 280:15984-15991. Clarke AJ: Extent of peptidoglycan O-acetylation in the tribe
- 27 Proteeae. J Bacteriol 1993, 175:4550-4553.
- 28. Alaedini A, Day RA: Identification of two penicillin-binding multienzyme complexes in Haemophilus influenzae. Biochem Biophys Res Commun 1999, 264:191-195.
- 29. Vollmer W, von Rechenberg M, Höltje J-V: Demonstration of molecular interactions between the murein polymerase PBPIB, the lytic transglycosylase MItA, and the scaffolding protein MipA of Escherichia coli. | Biol Chem 1999, 274:6726-6734.
- 30. von Rechenberg M, Ursinus A, Höltje J-V: Affinity chromatography as a means to study multienzyme complexes involved in murein synthesis. Microb Drug Resist 1996, 2:155-157.
- 31. Blackburn NT, Clarke AJ: Identification of four families of peptidoglycan lytic tranglycosylases. J Mol Evol 2002, 52:78-84
- 32. Goffin C, Ghuysen JM: Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. Microbiol Mol Biol Rev 1998, 62:1079-1093.
- 33. Zipperle GF, Ezzell JW, Doyle RJ: Glucosamine substitution and muramidase susceptibility in Bacillus anthracis. Can J Microbiol 1984, 30:553-559
- 34. Scott HW, Dehority BA: Vitamin requirements of several cellulolytic rumen bacteria. J Bacteriol 1965, 89:1169-1175.
- 35. Vincent JM: A Manual for the Practical Study of Root Nodulating Bacteria Oxford: Blackwell Scientific; 1970.
- 36. Strating H, Clarke AJ: Differentiation of bacterial autolysins by zymogram analysis. Anal Biochem 2001, 291:149-154.
- 37. Clarke AJ: Compositional analysis of peptidoglycan by highperformance anion-exchange chromatography. Anal Biochem 1993, 212:344-350.
- SignalP 3.0 Server [http://www.cbs.dtu.dk/services/SignalP/] PSORT Prediction [http://psort.nibb.ac.jp/form.html] 38
- 39
- "DAS" Transmembrane Prediction server 40. [<u>http://</u> www.sbc.su.se/~miklos/DAS/]
- NCBI BLAST [http://www.ncbi.nlm.nih.gov/BLAST/] 41
- ClustalW [http://www.ebi.ac.uk/clustalw] 42.
- THE MEME/MAST SYSTEM Motif Discovery and Search 43. Version 3.0.14 [http://meme.sdsc.edu/meme/website/intro.html]
- GOR IV Secondary Structure Prediction Method 44. [http:// abs.cit.nih.gov/gor]
- The PSIPRED Protein Structure Prediction Server [http:// 45 bioinf.cs.ucl.ac.uk/psipred/]
- NCBI Conserved Domain Search [http://www.ncbi.nlm.nih.gov/ 46. structure/cdd/wrpsb.cgi]

