REVIEW ARTICLE Polyester synthases: natural catalysts for plastics

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Polyhydroxyalkanoates (PHAs) are biopolyesters composed of hydroxy fatty acids, which represent a complex class of storage polyesters. They are synthesized by a wide range of different Gram-positive and Gram-negative bacteria, as well as by some Archaea, and are deposited as insoluble cytoplasmic inclusions. Polyester synthases are the key enzymes of polyester biosynthesis and catalyse the conversion of (R)-hydroxyacyl-CoA thioesters to polyesters with the concomitant release of CoA. These soluble enzymes turn into amphipathic enzymes upon covalent catalysis of polyester-chain formation. A self-assembly process is initiated resulting in the formation of insoluble cytoplasmic inclusions with a phospholipid monolayer and covalently attached polyester synthases at the surface. Surface-attached polyester synthases show a marked increase in enzyme activity. These polyester synthases have only recently been biochemically characterized. An overview of these recent findings is provided. At present, 59 polyester synthase structural genes from 45 different bacteria have been cloned and the nucleotide sequences have been obtained. The multiple alignment of the primary structures of these poly-

INTRODUCTION

Polyester [polyhydroxyalkanoate (PHA)] synthases are the key enzymes of PHA biosynthesis and catalyse the stereo-selective conversion of (R)-3-hydroxyacyl-CoA substrates to PHAs with the concomitant release of CoA [1] (Scheme 1). These polyesters are deposited as water-insoluble inclusions by eubacteria and Archaea when a carbon source is available in excess, and other nutrients are growth-limiting. When carbon starvation occurs, the polyester serves as reserve polymer and is mobilized by intracellular PHA depolymerases [2]. More than 59 different PHA synthases have been cloned and assigned [1,3,4]. The multiple alignment of the primary structures of these PHA synthases showed an overall identity of 8-96% with only eight strictly conserved amino acid residues [5]. PHA synthases comprise a new family of enzymes with unique features, particularly considering the functional role in biogenesis of the water-insoluble subcellular structures called PHA granules, and the association with a phospholipid monolayer. These enzymes can be divided into four classes, which will be discussed below.

BIOPOLYESTERS

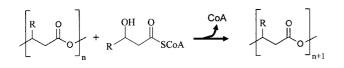
PHAs comprise a rather complex class of polyoxoesters that are synthesized by most genera of eubacteria and members of the family Halobacteriaceae of the Archaea [1,6]. Only recently, a PHA synthase gene was also identified in the genome of ester synthases show an overall identity of 8–96% with only eight strictly conserved amino acid residues. Polyester synthases can been assigned to four classes based on their substrate specificity and subunit composition. The current knowledge on the organization of the polyester synthase genes, and other genes encoding proteins related to PHA metabolism, is compiled. In addition, the primary structures of the 59 PHA synthases are aligned and analysed with respect to highly conserved amino acids, and biochemical features of polyester synthases are described. The proposed catalytic mechanism based on similarities to α/β -hydrolases and mutational analysis is discussed. Different threading algorithms suggest that polyester synthases belong to the α/β -hydrolase superfamily, with a conserved cysteine residue as catalytic nucleophile. This review provides a survey of the known biochemical features of these unique enzymes and their proposed catalytic mechanism.

Key words: biopolyester, biopolymer, catalytic mechanism, polyester synthase, polyhydroxyalkanoate (PHA), PHA synthase.

an uncultivated Crenarchaeota [7]. Most of these prokaryotes synthesize poly(3-hydroxybutyric acid) [poly(3HB)], and other PHAs as reserve material and deposit these polyesters as waterinsoluble inclusions in the cytoplasm. Meanwhile, approximately 150 different hydroxyalkanoic acids are now known to occur as constituents of PHAs (Figure 1). These water-insoluble PHAs exhibit relatively high molecular masses, thermoplastic and/or elastomeric features and some other interesting physical and material properties (Table 1). Therefore, and since they are biodegradable [8], they are considered for several applications in the packaging industry, medicine, pharmacy, agriculture and food industry or as raw materials for the synthesis of enantiomerically pure chemicals and the production of paints [9]. Recently, it was found that some eubacteria are able to synthesize polythioesters using mercaptoacids as carbon source and presumably employing PHA biosynthesis enzymes [10]. Many prokaryotic and eukaryotic organisms are able to produce low-molecularmass PHB (polyhydroxybutyrate) molecules that are complexed with other biomolecules such as e.g. polyphosphates and that occur at concentrations which are three to four orders of magnitude less than storage PHAs in the cells [11-15]. Evidence has been provided that these complexes form ion channels in the cytoplasmic membrane and play a role in acquisition of competence in Escherichia coli [16-18]. A still intriguing question is how these PHB molecules are synthesized. So far no enzyme could be identified and no gene could be assigned in E. coli, the genome of which has been sequenced, that is involved

Abbreviations used: CVFF, consistent-valence force field; 3HB, 3-hydroxybutyric acid; PHA, polyhydroxyalkanoate; PhaC etc., PHA synthase subunits(s); PHB, polyhydroxybutyrate; R386C etc., mutation of Arg-386 to cysteine etc.

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Scheme 1 Reaction catalysed by polyester synthase

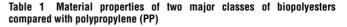
R, alkyl chain of 1-11 carbon atoms.

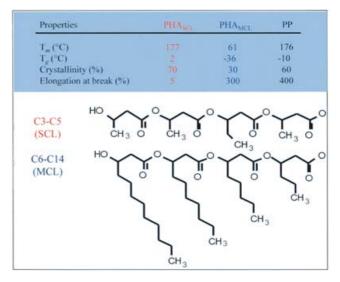
in synthesis of the low-molecular-mass PHB. Obviously, these biosynthesis enzymes must differ significantly from the highly processive polyester synthases discussed in this review. A few eukaryotic micro-organisms such as *Aureobasidium pullulans* are able to synthesize the water soluble polyester polymalic acid which is not synthesized by prokaryotes [19].

POLYESTER SYNTHASES, A FAMILY OF ENZYMES

Meanwhile, the nucleotide sequences of 59 PHA synthase genes from 45 different bacteria have been obtained. With respect to the primary structures deduced from these sequences, the substrate specificities of the enzymes and the subunit composition, four major classes of PHA synthases can be distinguished (Table 2).

Class I and class II PHA synthases comprise enzymes consisting of only one type of subunit (PhaC) with molecular masses between 61 kDa and 73 kDa [20]. According to their *in vivo* and *in vitro* substrate specificity, class I PHA synthases (e.g. in Ralstonia eutropha) preferentially utilize CoA thioesters of various (R)-3hydroxy fatty acids comprising 3 to 5 carbon atoms, whereas class II PHA synthases (e.g. in *Pseudomonas aeruginosa*) preferentially utilize CoA thioester of various (R)-3-hydroxy fatty acids comprising 6 to 14 carbon atoms [5,21–24].





Class III PHA synthases (e.g. in *Allochromatium vinosum*) comprise enzymes consisting of two different types of subunits: (i) the PhaC subunit (molecular mass of approx. 40 kDa) exhibiting amino acid sequence similarity of 21-28% to class I and II PHA synthases and (ii) the PhaE subunit (molecular mass of approx. 40 kDa) with no similarity to PHA synthases. These PHA synthases prefer CoA thioesters of (*R*)-3-hydroxy fatty acids comprising 3 to 5 carbon atoms [25,26].

Class IV PHA synthases (e.g. in *Bacillus megaterium*) resemble the class III PHA synthases, but PhaE is replaced by PhaR (molecular mass of approx. 20 kDa) [27].

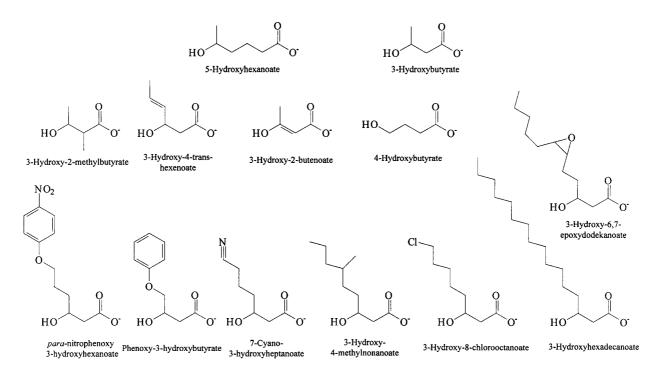
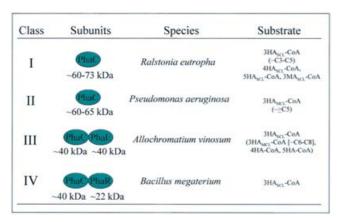


Figure 1 Representative constituents found in biopolyesters

The respective CoA thioesters of these constituents are considered as substrates for polyester synthases.

Table 2 Polyester synthases can be divided into four classes



Exceptions to this classification are the synthases from Thiocapsa pfennigii (two different subunits with strong similarity to the PhaC subunit of approx. 85 % identity to class III PHA synthases), from Aeromonas punctata (one type of subunit with strong similarity to class I PHA synthases of approx. 45%) and from Pseudomonas sp. 61-3 (PhaC1 and PhaC2 with strong similarity to class II PHA synthases of approx. 80% identity) with respect to the substrate specificity [28-30]. The T. pfennigii PHA synthase is characterized by broad substrate specificity comprising CoA thioesters of short-chain-length (3 to 5 carbon atoms) and medium-chain-length (6 to 14 carbon atoms) 3-hydroxy fatty acids. The A. punctata synthase catalyses synthesis of a copolyester of 3-hydroxybutyrate and 3-hydroxyhexanoate. Generation of hybrid class III PHA synthases by interchanging the PhaE and PhaC subunits from Aeromonas vinosum and T. pfennigii indicated that the PhaC subunit, respectively, mediates the substrate specificity [29]. Furthermore, the PHA synthases PhaC1 and PhaC2 from Pseudomonas sp. 61-3 catalyse the polymerization of a copolyester of 3-hydroxybutyrate and medium-chainlength 3-hydroxy fatty acids [30]. Accordingly, studies about the in vivo substrate specificity of the R. eutropha PHA synthase produced in recombinant E. coli showed in principle that this class I synthase accepts medium-chain-length 3-hydroxy fatty acid-CoA thioesters as substrate [31,32]. All these findings indicated that the PHA synthases generally show a rather broad substrate specificity.

Extensive comparison of the 59 PHA synthases from various bacteria revealed that these enzymes exhibit strong similarity (8–96% identical amino acids) (Figure 2). With respect to amino acid sequence regions with stronger similarity, six conserved blocks could be identified, whereas the N-terminal region (approx. 100 amino acids relative to class I PHA synthases) is highly variable (Figure 3). The N-terminal region is also dispensable for a functionally active enzyme as revealed by the analysis of truncated R. eutropha PHA synthases that lacked 36 or even 100 amino acids, whereas the more conserved C-terminal region is required for enzyme activity [4,33]. Overall, eight amino acid residues are identical in all the known 59 PHA synthases, suggesting an important role for these residues in enzyme function (Figure 2). A phylogenetic tree was constructed, based on the multiple alignment, which supports the classification of PHA synthases (Figure 4). However, among the class I PHA synthases a rather strong diversity exists, indicating that this class might exhibit more diverse enzymological properties. Comparative hydrophilicity-plot analysis, according to Kyte and

Doolittle [34], clearly revealed that the hydrophilicity profiles of all classes of PHA synthases showed a similar pattern, indicating a similar topology for the respective proteins. Comparison of the hydrophilicity profiles from class I and class II PHA synthases showed only one difference at positions 100-130 (R. eutropha PHA synthase) or 80-110 (P. aeruginosa PHA synthase), suggesting that this region might contribute to the substrate specificity (Figure 3). Interestingly the C-terminus (approx. 40 amino acid residues) appears to be conserved and hydrophobic among all class I and II PHA synthases, suggesting that this region might function as binding domain attaching the synthase to the hydrophobic polyester core. In the PhaC subunits of class III and class IV synthases no hydrophobic C-terminus is present. However, in class III and class IV synthases the second subunit, PhaE or PhaR respectively, possess a hydrophobic C-terminus, which might exert a similar function as proposed for the C-terminus of class I and class II synthases. Nevertheless, the hydrophilicity profile alignment of the PhaE/PhaR subunit of the class III and class IV synthases with class I PHA synthases suggests that PhaE/PhaR might also functionally replace the N-terminus of class I PHA synthases. Both are dispensable without completely abolishing PHA synthase activity. However, PhaC from A. vinosum showed only very weak activity (<1% of wild-type activitiy) in the absence of PhaE [35]. The same situation was found in class IV enzymes, with PhaR functionally related to PhaE and required for synthase activity [27,36]. Although both synthase subunits PhaE and PhaR show only 18% amino acid sequence identity, the hydrophilicity profiles show a significant overlap at the hydrophobic C-terminus. Thus, as indicated above, the hydrophobic C-terminus might be functionally important for synthase activity, perhaps by mediating contact with the hydrophobic polyester core.

Only recently the PHB synthase from an extremely halophilic archaebacterium was identified and characterized, which might constitute a new class of synthases [37]. This enzyme was stable up to 60 °C and still exhibited approx. 90% of the maximum enzyme activity, which was obtained at 40 °C. The soluble archaeal PHB synthase was only active at high salt concentration, whereas the granule-bound PHB synthase was almost independent of the salt concentration.

GENETICS OF POLYESTER SYNTHASES

The PHA synthase genes and genes for other proteins related to the metabolism of PHA are often clustered in the bacterial genomes [1,3] (Figure 5). In R. eutropha, which has been studied in detail [38], the genes for class I PHA synthase (phaC), β -ketothiolase (*phaA*) and NADP-dependent acetoacetyl-CoA reductase (phaB) constitute the phaCAB operon [22-24]. Besides the frequently found genetic organisation of R. eutropha among PHB-accumulating bacteria, some bacteria show a different gene order but, at least, the synthase gene is colocalized with other PHB biosynthesis genes (see [3] for review). However, PHB-accumulating bacteria belonging to the α -proteobacteria, such as Caulobacter crescentus, Azorhizobium caulinodans, Rhizobium etli, Sinorhizobium meliloti, Paracoccus denitrificans and Methylobacterium extorquens, contain the class I PHA synthase gene not colocalized with other PHA biosynthesis genes [20, 39-43]. Only a few exceptions, such as Zoogloea ramigera (β -proteobacterium), Aeromonas punctata (γ -proteobacterium) and Gordonia rubripertinctus (a firmicute), not belonging to α -proteobacteria, have been described that do not contain colocalized PHA biosynthesis genes. Some species, such as

r. violacea	MIDIQEDRSTING	LLATGODVYLIDWGYPDQAD	RALTLDDYINGYIDRCVDYLREAH SALTLDDYINGYIDSCVDYLCETH	EVD 140	A. vinosum F. violacea	OWNER	GAFSLMYSALHPDK		
r. pfennigii	MIDIQEDRSTING	LLATGODVYLIDWGYPDQAD	RALTLDDYINGYIDRCVDYLRETH	WD 142 1	T. pfennigii	CONTLACTOR	CAFSLCYTALHSEN		
. shaposhnikovii Svnechocvstis sp.			RFLTMDDYLNGYLDRCVDEICRRH RMLTLEDYLSGYLNNCVDIICQRS	4LD 140	5. shaposhnikovii	KINILGI	GGTFSLCYSAMHPEK	**************	***********
renarchaeota			KYITVDDYVNLFIYECVEYIKNIEN		Synechocystis sp.	KITLLGV Q	GOTFSLCYASLFPDK		***********
. megaterium	ILDLTPGNSLVEY.	LINRGFDVYLLDWGTPGLED	SNMKLDDYIVDYIPKAAKKVLRTSH	CSP 144	Crenarchaeota B. megaterium	DLSVLGY	GTMTSI FAALNEDLP		
cillus sp.			SHLKFDDFVFDYIAKAVKKVMRTA	CSD 143	Bacillus sp.	EISLLGY	GUTLTSIYAALHPHMP		
lcaligenes sp. . acidovorans	TLDLOPONSLIPT	AVEQUERTEEVSWENPDUSL AVEQUERTEVMSWENPDUSL	AHRTWDDYVEDGAMAAIDVVQNITC ARRTWDNYIEDGVLTGIRVAREIAC		Alcaligenes sp. D. acidovorans	QINALGPOS	GOTILSNALAVLAARGD		
. latus	ILDLOPDNSLIRY	TVAQGHRVFVVSWRNPDASV	AGKTWDDYVEQGVIRAIRVMQQITC	HE 258	A. latus	QINVLGE VI	COTMLSTALAVLOARHDREHO	(VAAPAAKAPAAKIAA	AGSIKSAARTSTAF
urkholderia sp.	ILDLQPENSLVAR	ALSOGHQVFLVSWRNADASV	AHRTWDDYMNEGLLAAIDAVQQVSC	RE 346 1	Burkholderia sp.	QINTLOP V	GTILSTALAVLAARGE		
. eutropha . ramigera	ILDLOPESSLVRM ILDLOPENSLVRY	VVEQGHTVFLVSWRNPDASM AVEOUNTVFL 1 SWENDDAST	AGSTWDDYIEHAAIPAIEVARDISC AGTTWDDYVEQGVIEAIRIVQDVSC		R. eutropha	KINVLGP	GTIVSTALAVLAARGE		
. sp. 61-3 (PhbC)			AGTTWDDLIELGVIDGLQVAREISC		I. ramigera P. sp. 61-3 (PhbC)	KLNMFGF	GTIVATALAVLAARGQ		
. violaceum	LMDLQPENSMVRH	FVAQGYRVFLISWRSAVAEM	DHFTWETYIEKGVFAAAEAVQKITH	KQP 284	C. violaceum	THEVLOR	GUILTTALCVAOASGL		
. cepacia . ruber			RDLSLDDYRQLGVHAALDTVSKIV	PDH 324	B. cepacia	KIHATGY L	OGTLLSIAAAMAGSGDN		
. chlororaphis (C1)			RHITMDDYYVDGIATALDVVEEITC REWGLSTY-IEALKEAVDVVTAITC	and and	R. ruber	KIEVLSICL	G <mark>GAMAAMAAARAFA</mark> VGDK G <mark>G</mark> ITCTALLGHYAALGEK		
, sp. 61-3 (C1)	VFDLSPDKSLARF	CLSNNQQTFIVSWRNPTKAQ	REWGLSTY-IDALKEAVDVVSAITC	1017 D.0.0	P. chlororaphis (Cl) P. sp. 61-3 (Cl)	DINMLGA SI	GITCTALLGHYAALGEK		
. caryophylli (C1)	VFDLSPEKSLARP	CLRSNVQTFIVSWRNPTKAQ	REWGLSTY-IDALKEAVDAVLAITC	CK 288 ;	B. caryophylli (C1)	DLNMLOACS	GITCTALLGHYAALGEN		
. putida (C1) . oleovorana (C1)			REWGLSTY-IDALKEAVDAVLAITC	X5K 287	P. putida (C1)	DIMMLGACS	GITCTALVGHYAAIGEN GITCTALVGHYAAIGEN	*************	
. mendocina (C1)			REWGLSTY-IDALKEAVDAVLAITC REWGLSTY-IEALKEAIDVICAITC		P. oleovorans (C1)	DLNMLGALS	GITCTALVGHYAALGEN GITTASLLGHYAALGQP		
. pseudoalcaligenes Cl	VFDLSPEKSLARF.	LLRSQVQTFVVSWRNPTKAQ	REWGLSTY-IEALKEAIDVICAITC	SK 288	P. mendocina (Cl) P. pseudoalcaligenes Cl	DVNMLGACS	GLTTASLLGHYABLGOO		
. stutzeri (C1)			REWGLSTY-IAALKEAIEVICAITC	ASIK 200	P. stutzeri (C1)	TUNMT CLUMP	COLUMN STATISTICS OF STATISTIC		
. resinovorans (Cl) . aeruginosa (Cl)			REWGLSTY-IEALKEAIDVILKITC REWGLTTY-IEALKEAIEVVLSITC	1014 0.00	P. resinovorans (C1)	DINILGALS	GITTVALLCHYQAIGET	**************	
putida BM01 (C2)			REWGLSSY-VQALEEALNACRSISC		P. aeruginosa (Cl) P. putida BM01 (C2)	DINLIGA SI	GUTTATLVGHYVASGEK		
. oleovorans (C2)	IFDLSSTNSFVQY	MLKNGLQVFMVSWRNPDPRH	REWGLSSY-VQALEEALNACRSISC	MR 288	P. oleovorans (C2)	DPNLMGA A	GLTMAALOGHLOAKHOLR		
. putida (C2)			REWGLSSY-VQALEEALNACRSISC	NR 286	P. putida (C2)	DPNLMGA A	GLTMAALOGHLOANHOLR	*************	
 caryophylli (C2) nitroreducens (C2) 			REWGLSSY-VQALEEATNACRAISC REWGLSSY-VQAVEEAVDACRAISC	10H 100	 caryophylli (C2) 	DVNLMGA	GUTHAALQGHLQAKRQLR		
. pseudoalcaligenes C2	IFDLSNDKSFVQY.	ALKNGLQTFMISWRNPDPRH	REWGLSSY-VOAVEEAVDACRAISC	35K 288	P. nitroreducens (C2) P. pseudoalcaligenes C2	DVNLLGARA	GLTIAALOGHLQARRQLR		
, mendocina (C2)	IFDLSNDKSFVQY	ALKNGLQTYMISWRNPDPRH	REWGLSSY-VOAVEEAVDACGTITC	3GK 288	P. mendocina (C2)	DUTLICH	COLTTANTOCHLOAPBOLS		
. stutzeri (C2) . resinovorans (C2)			REWGLSSY-VOAVEQAVDACRAITC		P. stutzeri (C2)	DVNLLGA	GLTIAALOGHLQAKROLR		
. chlororaphis (C2)	IFDLSPTNSFVQT	ALKNGLOTFI I SWRNPDARH	REWGLSTY-VOALEEAFEACRAITC REWGLSSY-VEAVEEAMNVCRAITC		P. resinovorans (C2)	EVNLIGA	GLTIAALQGHLQARRQLR GLTIAALQGHLQAKRQLR		**********
. sp. 61-3 (C2)	IFDLSPGNSFVQY.	ALKNGLOVFVVSWRNPDVRH	REWGLSSY-VEALEEALNVCRAITC	AR 288	P. chlororaphis (C2) P. sp. 61-3 (C2)	DVNLMGA M	GLTIAALOGHLOAKROLR		
. aeruginosa (C2)	IFDLSPEKSFVQY.	ALKNNLQVFVISWRNPDAQH	REWGLSTY-VEALDQAIEVSREITC	35R 288	P. aeruginosa (C2)	SVNLAGA	GLTVAALLGHLOVRROLR		
. rubripertinctus . cholerae			GOWDFDTY-AGRVIRAIDEVREITC AQLNFEDYVLEGVVKAVNAIESITC	25D 287	rubripertinctus	DVNLIGF M	GGI LATTVLNHLAAQGDT	*************	
. parahaemolyticus			AQVEFGDYVLEGVVKAVNALES ITC AQVEFGDYVTEGVAKAVTALEDVTC		V. cholerae	QINAAGY IC	GTVLATTIAYYAAKRMKK GOTVLASTVAYYAAKRMKK		
cinetobacter sp.	VIDIREQNSIVNW	LROOGHTVFLMSWRNPNAEO	KELTFADLITQGSVEALRVIEEITC	ZEK 316	V. parahaemolyticus Acinetobacter sp.	EANCIGY	GUTLLAATOAYYVARRLKN		
- punctata			AQIDLDDYVVDGVIAALDGVEAATO	ZER 311 /	A, punctata	RVHGTGV 14	COTAL ST AMONT A SPROKO		
. hydrophila . etli	TLDLNPOKETIKW	LVAUGOTVPH15WRNP5VAQ CVDOGOTVPV15WVNPDCBH	AQIDLDDYVVDGVIAALDGVEAATG AEKDWAAYAREGIDFALETIEKATG		 hydrophila 	EVHGIGY	GTALSLAMOWLAARROKO		
. meliloti			ASKDWEAYAREGIGFALDIIEQATO		R. etli S. meliloti	EVNAVGY	GTLLAATLALHAKE-KNK		
. tumefaciens	ILDLNPQKSFVGW	CLEQGHSVFHVSWINPDAGL	ANKOWDDYINEGIDFALDTIEERTO	EK 368	A. tumefaciens	OINAIGY W	GUTLLSSALALHAOO-GNE		
. caulinodans . extorguens			ADRGFDDYMRDGIFAALDAVERATO RDRDFESYMREGIETAIDMIGVATO	JEH 312	A. caulinodans	OANTICYUM	GTLLAVTLAYMANT-GDD		
. crescentus			AAKTFEDYMVEGIYDAAQQVMTQCC		 extorquens crescentus 	DVAAAGY W	GTLLAVTLAYOAAT-GNR		
. rubrum HA	ILDLEPHNSFIKM	AIEQGRSVFVISWVNPEADL	AAKSFEDYMVEGPLAALDIVKTITC	ED 324	R. rubrum HA	SVNAIGY	GTLLOCVLSYLEAKGQSD		
. rubrum ATCC25903	ILDLTERNSLIKY	MVDQGFSVFVISWVNPDAGL	AETRFEDYLSOGPLAAMEVMTEITC	2QR 401	R. rubrum ATCC25903	ALGLVGY IC	GTLTACTLAVLARE-RDH		
. prowazekii (PhbC2) . denitrificans			SKRGFEDYLKEGILAPFEYVKN-LC GDTGMDDYVS-AYLEVMDRVLDLTI		R. prowazekii (PhbC2)	KIDFVGYCM	GOMPLAIIIAYPKVK-RID	*************	
. capsulatus	ILDLNPQNSLIRW	IVDQGFTLFVVSWRNPDRSY	ADVGMEDYVRDGYLAAIEEVKAITC	EK 323	P. denitrificans R. capsulatus	KLNAVGY L	AGTTLALTPVVLKORGDDR		
. sphaeroides	ILDLKPQNSLLKW.	LVDQGFTVFVVSWVNPDKSY	AGIGMDDYIRDGYMRAMAEVRAITE	NOK 327	R. sphaeroides	QINAVGY	AGTTLTLTLAHLQKAGDPS		
. prowazekii (PhbCl)	. : *	1 1	YCLDDYVNEIIEVIDIL	GK 130 1	R. prowazekii (PhbCl)	DINLICH	GONLAIAANVLMPQF		
violacea	VIONLVT	MVTPVDFKTPGNLLSAM	VQNVDIDLAVDTMGNI VQNVDIDLAVDTMGNI 	PG 205 T.	. vinosum . violacea . přenpigli	ELLNWTFLSL	KPFSLTGOKYVNMVDLLDDPD KPFSLTGOKYVNMVDMLDDPD KPFSLTGOKYVNMVDLLDDED	KVIONELEMENNI EDSE	PDOAGETFROFTH
. violacea . pfennigii . shaposhnikovii ynechocystis sp.		MVTPVDPKTPGNLLSAM MVTPVDPQTPGNLLSAM MVTPVDPQTPDNILSHM MVAPVDPEQPGTLLNARGGG TAPIVDAEKDKSVIKM		PG 205 T. PG 207 T. PG 205 E. PG 227 S: PY 212 C.		ELLNWTFLSL ELLNWTFLSL ELLNWTFLNL DYLNLEFLML		KVIOPLEMEIONI POSE KVIOPLEMEIONI POSE KLONPLEMEIONI POSE KLLNPLEMEIONI POSE	PDQAGETFRQFTK PDQAGETFRQFIK PDQVGBTYRQFIK PDQAGETYRQFIK
. violacea . pfennigii . shaposhnikovii ymechocystis sp. remarchaeota . megaterium		MVTPVDFKTPGNLLSAM INVTPVDFQTFGNLLSAM INVTPVDFQTFGNLLSAM MVAPVDFQPGTLLNARGGG ILAPIVDAEKOKSVINM INTSPFDFSDTGLYGAFLD	VQNVDIDLAVDTMGNI VQNVDVDLAVDTMGNI VRHVDIDTLVDTMGNV TLGAEAVDIDLMVDAMGNI AEHMDIDLMVLSHHENI DRYFNLDKAVDTFGNI	PG 205 T PG 207 T PG 205 E PG 205 E PG 227 S PY 212 C PP 211 B	. violacea . přennigii . shaposhnikovii ynechocystis sp. renarchaeota . megaterium	ELLNWTFLSL ELLNWTFLSL ELLNWTFLNL DYLNLEFLML ELLYLVYASL EMIDFGNHML	KPFSLTGGKYVNNVDMLDDPDI KPFSLTGGKYVNNVDLDDEDI KPYQLMGGKYLDMVEVLQDKDI KPLQLGYQKYLDVPDIMGDEAI KPFNGGYNKYYNLFNNFEDESI KPITNFYGPYVTLVDRSENQRI	KVENFLEMENT FOSF KVENFLEMENT FOSF KLENFLEMENT FOSF KLENFLEMENT FOSF FVONFLEIENKLYDT FVESWICLMONVADGI	PDQAGETFRQFT PDQAGETFRQFI PDQVGBTYRQFI PDQAGETYRQFI PDQAGETFRQWY IPFAGEAYRQWI
. violacea . pfennigii . shapoahnikovii ymechocystis sp. renarchaeota . megaterium ncillus sp.		MVTPVDPKTPGNLLSAM MVTPVDPQTPGNLLSAM MVTPVDPQTPGNLLSAM MVTPVDPCTPGNLSAM MVAPVDPEQPGTLLNARGGC TIAPIVDAEKOKSVINOM MTSPPDPSDTGLYGPLD MTSPPDPSETGLYGPLD	VQNVDIDLAVDTMGNI VQNVDVDLAVDTMG	PG 205 T. PG 207 T PG 205 E PG 227 S PY 212 C. PP 211 B PP 210 B	. violacea . přennijli . shaposhnikovii ynechocystis sp. renarchaeota . megaterium acillus sp.	ELLNWTFLSL ELLNWTFLSL DYLNLEFLML ELLYLVYASL EMIDFGNKML EMIDFGNKML	KPPSLTQCKYVNWDMLDDPDI KPPQLWQCKYLDWVDLDDPDI KPLQLGYQCKYLDWVDLDDDDI KPLQLGYQCKYLDVPDIMGDBA KPIQCGYNKYYNLFINFIDDES KPITNFYGPYVTLVDRSENQRI KPITNFYGPYVTLVDRSENERI	KVINPLEMENGI POSE KVINPLEMENGI POSE KLINPLEMENGI POSE KLINPLEMENGI POSE VLINPLEMENGI POSE PVESHGLAQNOVADGI PVESHGLAQNOVADGI	PDQAGETFROFT PDQAGETFROFT PDQAGETYROFT PDQAGETYROFT PTAGETFROW IPFAGEAYROWT IPFAGEAYROWT
violacea pfennigii shaposhnikovii nechocystis sp. renarchaeota megaterium toillus sp. lcaligenes sp.	VIORLVI VKNLVI VKNLVI IKNLVI IKNLIF EPVASATF	NVTPVDPKTPGNLLSAM		PG 205 T PG 207 T PG 205 E PG 205 E PY 212 C PP 211 B PP 210 B VG 361 A.	 violacea pfennigii shaposhnikovii ynechocystis sp. renarchaceta megaterium acillus sp. lcaligenes sp. 	ELINWTFLSL ELINWTFLSL ELINWTFLNL DYLNLEFIMI, ELLYLVYASL EMIDFGNIML EMIDFGNIML QDLASTFSFL	KPPSLTGQKYVNWVDMLDDPDI KPPSLTGQKYVNWVDLDDPDI KPYQLMGQKYLDMVEVLQDKDI KPFKQGVKYLDVFDIMODPA KPFKQGVKYLDVFDIMODPA KPFKQGVKYVTLVRSENGE KPITNFYGPYVTLVRSENGE RPNDLVWFYVVGNYLKGETP	KVKNPLEMEKKI FDSE KVKNPLEMEKKI FDSE KLLNPLEMEKKI FDSE KLLNPLEMEKKI FDSE FVQNPLEI EKKLYDT FVSSRKLMQRVADG3 FVPSSRKLMQRVADG3 	PDQAGETFRQFT PDQAGETFRQFI PDQAGETYRQFI PDQAGETYRQFI PDIAGETYRQWY I PFAGEAYRQWI I PFAGEAYRQWI INLFGPFYAMYLF
violacea pfennigii shaposhnikovii nmechocystis sp. remarchaeota .megaterium toilus sp. tosligenes sp. acidovorans latus	VIGILVI VVIDILVI VVIDILVI VVIDILVI INILVI INILI INILI POPASATI APAGVPFPVASVTI OPAASLTI	INVERVERTEGNILISAN INVERVERVERSILISAN INVERVERVERSILISAN INVERVERVERSILISAN INFORMERSISSI INFORMERSISSI INFORMERSISSI INFORMERSISSI INFORMATION INTERFORMERSISSI INTELEPSIDG ILLOWID INTELEPSIDG ILLOWID INTELEPSIDG ILLOWID		PG 205 T PG 207 T PG 205 E PG 227 S; PY 212 C PP 211 B PP 210 B NG 361 A NG 425 D NG 334 A	 violacea pfennigii shaposhnikovii ymechocystis sp. renarchaeota megaterium acillus sp. lcaligenes sp. acidovorans latus 	ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ELINFGNIMML ENIDFGNIMML QDLASTFSFL QDLASTFSFL KELATTFSFL	KPFSLTGGKYVNMVDHLDDED KPFSLTGGKYLDWFVLQDKD KPYGLMGGKYLDWFVLQDKD KPLGLAVGKYLDVFDIMGDEJ KPIGGYKRYNLBYRFEDES KPIGTYKPYVLDRSENGE RPNDLVWFYVGNYLKGEP RPNDLVWFYVGNYLKGEP RPNDLVWFYVGNYLKGEP	KVKNPLEMEKTIFDSE KVKNPLEMEKTIFDSE KLLNFLEMEKTIFDSE KLLNFLEMEKTIFDSE VVNPLEIEKTVDFF VVSNRLNQGVADGI FVESKRLVQGVAGGI 	PDQAGETFROFTI PDQAGETFROFTI PDQAGETFROFTI PDQAGETFROFTI PTAGETFROWTI IPFAGEATROWTI IPFAGEATROWTI INLPGPTAMYLI TNLPGPTAMYLI TNLPGPTAMYLI TNLPGPTAMYLI
. violacea . přennigii . shaposhnikovii mechocystis sp. renarchsecta . megaterium crillus sp. lealigenes sp. . latus urkholderia sp.	VIGLVT VINLVY VINLY VINLY V	INT FVD FKT FGKLLSAM- INT FVD FQT FGKLLSAM- INT FVD FQT FGKLLSAM- INT FVD FQT FGKLSAM- INT FVD FEG FGTLIAARGO INF FPD FST GLAGELLD- INT FTD FST GLAGELLD- INT FTD FST GLAGELLD- LIT FTD FST GLAUPFID- LIT FTD FST GLAUPFID- LIT FTD FST GULDYFTD- LIT FTD FST GVLD FTD- LIT FLOF ST GVLD FTD- LIT FLOF ST GVLD FTD-	VUNIVDI DLAVDTMS	PG 205 T PG 205 T PG 205 K PG 227 S5 PF 212 C PP 211 B PP 211 B PP 210 S RG 361 A RG 425 D NG 334 A RG 422 B	. violacea . pfennigli . shaposhnikovii ymechocystis sp. renarchaeota . megaterium acillus sp. lealigenes sp. . latus urkholderia sp.	ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL DYLNLEFIML ELLYLVYASL EMIDFGNIML QDLASTFSFL QDLASTFSFL VEFANTFSFL	NEFSITGONYUNNUHLDEDED NEFSITGONYUNNULLDEDED NEFSITGONYUNNULLDEDED NEFINGOVIKYINLFKOREDES NEFINFXOFYVTLUDREDES NEFINFXOFYVTLUBREDES REINDUNNIYUVGAYLKOEFF REINDUNNIYUVGAYLKOEFF REINDUNNIYUVGAYLKOEFF REINDUNNIYUVGAYLKOEFF REINDUNNIYUVGAYLKOEFF	KVPNFLEMENT FDSF KVFNFLEMENT FDSF KVFNFLEMENT FDSF KLINFLEMENT FDSF FVCNFLEREKKLTDFF FVCNFLEREKKLTDFF FVCSNFLMQROVADGI FVCSNFLMQROVADGI FVCSNFLMQROVADGI FPFPLLYNNSDST 	PDQAGETFRQFTI PDQAGETFRQFTI PDQAGETYRQFTI PDQAGETYRQFTI IPFAGEAYRQWI IPFAGEAYRQWI INLPGPYAWYLI INNAGPMYCWYLI INNAGPMYCWYLI
<pre>violacea pfennigi shaposhnikovii nmechecystis ap. remarchaeota megaterium cicilus sp. lcaligenes ap. acidovorans latus urkholderia sp. eeutropha</pre>	VIOLVY VIOLY VIOLY VIOLVY VIOL	IMT PYOPATEGNILSAM- INTPYOP OF CONLESAM- INTPYOP OF CONLESAM- INTARYOPE (IGTILIAAGGO INTARYOPE CONTANGO INTRY OF CONTANGO INTERPOSE CONCELLONG INTERPOSE CON	VUNVDIDLAVDYMS NIL VUNVDVDLAVDYMS NIL VUNVDLAVDYMS NIL VILABAVDIDLAVDYMS NIL ABBYDIDKVLAYHE NIF DEYYNLDKAVDTYG NIL ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM ELYVVFPEMJMER CGLM	PG 205 T PG 205 T PG 205 S PG 227 S PF 212 C PP 211 B PP 210 B NG 361 A NG 425 D NG 334 A RG 422 Bi RG 387 F	 violacea pfennigii shaposhnikovii ymechocystis sp. renarchaeota megaterium acillus sp. lcaligenes sp. acidovorans latus 	ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL DYLNLEFIMI ELLYLVYASL EMIDFGNIOL QDLASTFSFL QDLASTFSFL VEFANTFSFL VEFANTFSFL	KPFSLTGGKYVNMVDHLDDED KPFSLTGGKYLDWFVLQDKD KPYGLMGGKYLDWFVLQDKD KPLGLAVGKYLDVFDIMGDEJ KPIGGYKRYNLBYRFEDES KPIGTYKPYVLDRSENGE RPNDLVWFYVGNYLKGEP RPNDLVWFYVGNYLKGEP RPNDLVWFYVGNYLKGEP	NYAYELARENI POST KVENFLARENI POST KUENFLARENI POST KLIAFLARENI POST PVONFLAIENU POT PVONFLAIENU POT PVESHCHORVADGI PVESHCHORVADGI - PPFDLLYNSDS - APFDLLYNSDS - APFDLLYNSDS - VFFDLLYNSDS	PDQAGETFRQFTI PDQAGETFRQFTI PDQAGETYRQFTI PDQAGETYRQFTI IPFAGEAYRQWTI IPFAGEAYRQWTI INLPGPYAWYLI INLPGPYAWYLI INLPGPYAWYLI INLPGPWXCWYLI
violacea přennígli shaposhnikovii ymechocystis ap, recarchaeota . negaterium totilus sp, colligues ap, a cládovorans . acidovorans . latus urkholderia sp, . eutropha . ramigera . sp, 61-3 (PhbC)	VIOLUY VIOLUY VIOLUY INUN INULY INUL	INT TPUP FOR GRALLSAM INT TPUP FOR FORLLSAM INT TPUP FOR FORLLSAM INT TPUP FOR FORLLSAM INT SPM FOR TALKARS INT SPM FOR TALKARS INT SPM FOR TALKARS INT SPM FOR STALKARS INT SPM FOR STALKARS	VUINVDIDLAVDTMS MI VUINVDVLAVDTMS MI VUINVDVLAVDTMS MV VILABAVDIDLAVDTMS MV MENDIDLAVDTMS M	RG 205 T RG 301 A RG 422 B RG 303 T RG 307 T RG 3070 T RG 306 P	 violacea přennigii shaposhnikovii prenarchaeota negaterium aeiilus sp. lealigenes sp. aeidovorans latus ramigera ramigera aps. (3 (PhC) 	ELINWTFLSL ELINWTFLSL ELINWTFLSL DYINLEFIMI ELINWTFLSL EMIDFGNOM QDIASTFSFL QDIASTFSFL VEFANTFSFL VEFANTFSFL RDIASTFSFL EDMINTFSL	REPSICOGNYUNHYUNLLOPPI REPSICOGNYUNHYULLODED REPSICOGNYUNHYULLODED REPSICOGNYUNHYULDHO REPLOLOYONYUNHI REPNOVHYYUNHI REITNYYEPYYUNHI REITNYYEPYYUNHYUNHYUNHYU REPNOVHYYVUNYUNHYUNHY REPNOVHYYVUNYUNHYUNHY REPNOVHYYVUNYUNHYUNHY REPNOVHYVUNYUNHYUNHY REPNOVHYVUNYUNHYUNHY REPNOVHYVUNYUNHYUNHY REPNOVHYVUNYUNHYUNHY	NYNFLEADEN I FOS NYNFLEADEN I FOS KLONFLEMEN I FOS KLONFLEMEN I FOS KLONFLEMEN I FOS NYNFLEMEN KONTOG PPFOLLYNSDS - PPFOLLYNSDS - PPFOLLYNSDS - NFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS - YFFOLLYNSDS	PDQAGETFROFI PDQAGETFROFI PDQAGETFROFI PDQAGETFROFI PDQAGETFROFI PFAGEAVROWI FFAGEAVROWI TRLPGPYYAWYLI TRLPGPYYAWYLI TRLPGPYYCWYLI TRLPGPYYCWYLI TRLPGPYYCWYLI
<pre>violacea , violacea , shaposhnikovii , shaposhnikovii prenarchaeota</pre>	VIOLUY VOLUY VOLUY VIOL	IMT PUP FOR FORLEAM INT PUP FOR ILLEAM INT PUP FOR ILLEAM INT PUP FOR FORLLEAM MAPPO FER FORLING ILLEAM INT POST ILLEAM INT POST ILLEAM	VUNIVDICLAVETWS	RG 205 P RG 207 T RG 205 E RG 227 S PY 212 C PP 210 B RG 361 A NG 361 A RG 387 R RG 387 R RG 366 P RG 366 P RG 366 P RG 365 S	 violacea přenniji shaposhnikovii prenarchaeota menarchaeota nempiterium acilius ap. latus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum 	ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ELLINVASL EMIDFGNNOL QDLASTFSFL RELATTFSFL VEFANTFSFL ELINTTFSFL EDMONTFSFL EDMONTFSFL EDMONTFSSL	REPELOGIEYUNNYUKULDUPU REPELOGORYUNNYULDUPU REPELOGORYUNNYULDUPU REPELOGORYUNNYULPUPU REPECONNYUNUPUPU REPECONNYUNUPUPUPU REPECONNYUNUPUPUPUPUPUPUPUPUPUPUPUPUPUPUPUPUPU	NYNYLLAMENT FOS NYNYLLAMENNI FOS NYNYLLAMENNI FOS KLIAFLAMENNI FOS FYLSAKLANDAVADG FYLSAKLANDA	PODAGETPROFT PODVGETPROFT PODVGETPROFT PODVGETPROFT PODVGETPROFT PODVGETPROFT PIAGETPROFT PIAGETPROFT PAGEATRONG PTAGETPROFT INLGOPYCAVIL INLGOPYCAVIL INLGOPYCAVIL INLGOPYCAVIL INLGOPYCAVIL
<pre>violacea pfennigii shaposhnikovii phennigii shaposhnikovii precharystis ap, recarchaeota . negaterium tcilius sp, cilius sp, cacidovorans . latus urkholderia sp, . eutropha ramigera sp, 61-3 (PhbC) violaceum . cepacia</pre>	VIOLUM VIOLUM VIOLUM VIOLUM INOLUM	INT PUP OF FREE GALLEAN INT PUP OF PORTILLEAN INT PUP OF PORTILLEANC WALPUP SECONDERTILLEANCON IT AP 10 ARKONST INOM MIS PUP SECONDERLID ITT PUP SECON	VUINVDIDLAVDTMS MIL VUINVDVLAVDTMS MU VUINVDVLAVDTMS MV VUINVDVLAVDTMS MV VILABAXVDIDLAVDTMS MV MENDDIDKVLSTHS MV DEVTHLOKKVDTFO MIL EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJMER GGLM EXVVLFREMJS GGLM EXVVLFREMJS GGLM EXVVLFREMJS GGLM EXVVLFREMJS GGLM EXVVLFREMJS GGLM EXVVLFREMJS GGLM EXVVLFREMJS GGLM	RG 205 T RG 207 T RG 205 E RG 361 A RG 362 D RG 362 E RG 367 E RG 366 P SG 355 C SG 355 B	 violacea přennigii shaposhnikovii prenarchaeota negaterium aeiilus sp. lealigenes sp. aeidovorans latus urkholderia sp. eutropha ramigora ap. 61-3 (PhbC) violaceum cepacia 	ELLINGTFLSL ELLINGTFLSL ELLINGTFLSL DYINLEFINI, ELLINGTFLSL EMIDFGNIGE, QDIASTFSFL KELATTFSFL LELANTFSFL LELANTFSFL KEIGFTFSL KEIGFTFSL KEIGFTFSL	REPSICQUEYUNRUMULDEPDI REPSICQUEYUNRULDEPDI REPSICQUEYUNRULDING REPLOLOUNRUVULDIND REPICQUEYUNUTUNIPUNE REPICUNRUVUNIPUN REITINYGEYUNUNSUURSI REITINYGEYUNUNSUURSI REITINYGEYUNUNSUURSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RENDUNRUVUNIYUNSI RANDUNRUVUNIYUNSI RANDUNRUVUNIYUNSI	NYNFLEMENT FOS NYNFLEMENT FOS KLINFLEMENT FOS NLINFLEMENT FOS PYNFLEMENT FOS FYNFLEMENT FOS PFPDLLYNNSDS - PFPDLLYNNSDS - PFPDLLYNNSDS - AFPDLLYNNSDS - AFPDLLYNNSDS - AFPDLLYNNSDS - AFPDLLYNNSDS - PFDLLYNNSDS - PFDLLYNNSDS	PODAGET PROFIN PODAGET PROFIN PODAGET PROFIN PODAGET PROFIN PODAGET PROFIN I PPAGEAY ROWIT I PPAGEAY ROWIT I PPAGEAY ROWIT I INL GOPY CAVILI INL GOPY CAVILI INL GOPY CAVILI INL GOPY CAVILI INL GOPY CAVILI
<pre>violacea pfennigii shaposhnikovii phennigii shaposhnikovii precharystis ap, recarchaeota . negaterium tcilius sp, colius sp, acidovorans latus urkholderia sp, eutropha ramigera sp, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl)</pre>	VVRLVY VVRLVY VVRLVY VVRLVY VVRLVY VVRLVY VVRLVY VVRLVY VVRLVY VVRLVY VVRLV VVRV V V V V V V V V V V V V V V V V V V V	INT PPUP FREGRILLSAM- INT PPUP FOR FORLILSAM- INT PPUP FOR FORLILSAM- MADPUP FREGRILLSAME MADPUP FREGRILLSAME INT PPUP FREGRILLSAME MADPUP FREGRILLSAME MADPUP FREGRILLSAME INT PPUP FREGRILLSAME INT PUP FREGRIL	VQINVDIDLAVDYMS NIL VQINVDVLAVDYMS NIL VQINVDVLAVDYMS NIL VRADIDLAVDYMS NIL ARHODIDLAVDYMS NIL ARHODIDKVLSYNS NIL REVYNLAKAVDYTG NIL EXVYLFREMGMER GGLM ESVVLFREMGMER GGLM ESVVLFREMGMER GGLM ESVVLFREMGMER GGLM EXAVLERFENGEN GGLA ELAVALERFENGEN GGLA EDVVLFREMGNER GGVA ELEVANDERTIGGVAC PIGLF ESVVLRESOULD GGL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL ESVVLRESOULS GGVL	RG 205 T RG 207 T RG 205 E RG 205 E RG 227 S PY 212 C RG 321 B RG 425 D RG 427 B RG 344 A RG 625 D SG 357 B SG 355 B SG 355 B SG 357 R SG 357 P	 violacea přenniji shaposhnikovii prenarchaeota negaterium aeiilus sp. lealigenes sp. aeidovorans latus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum cepacia ruber chlororaphis (C1) 	ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL ENLIPTON EMIDFGNNEL GDLASTFSFL VEFANTFSFL VEFANTFSFL ELMITFSFL EDMANTFSFL HUMAGAPOLL NEIMATFASL HUMAGAPOLL	KR P5 LTGGKYVNNYULLUDPO KR P5 LTGGKYVNNYULLUDPO KR P5 LTGGKYVLDPO KR P5 LGGKYKVTNUPNYULDPO KR P1 LGKYKVTVUPNYULDPO KR P1 TNYYGP YVALVORSESNIR KR 11 TNYYGP YVALVORSESNIR F7 NDUVNYVVGYYLGYLKGKT F7 NDUVNYVVGYYLKGKT F7 NDUVNYVVGYYLKGKT F7 NDUVNYVVGYYLKGKT F7 NDUVNYVVDYYLKKR F7 NDUVNYVVDYLLKGKT F7 NDUVNYVVDYLLKGKT F7 NDUVNYVVDYLLKGKT F7 NDUVNYVVDYLKKGKT F7 NDUVNYVVDYLKGKT F7 NDUVNYVDYLKGKT F7	NYNFLEADER I FDS NYNFLEADEN I FDS NLOFLEADEN I FDS NLOFLEADEN I FDS NYNFLEALEN I FDS NYNFLEALEN I FDS NYNFLEALEN I FDS PFPDLL NYSDS AFPDLL NYSDS	POAGETPROFT POQUETPROFT POQUETPROFT POQUETPROFT POQUETPROFT POAGETYROFT IPPGESYROWI IPPGESYROWI IPPGESYROWI INLGOPYYANYLI INLGOPYYANYLI INLGOPYYCHYLI INLGOPYCHYLI INLGOPYCHYLI INLGOPYCHYLI INLGOPYCHYLI INLGOPYCHYLI ITMPAGESYLI ISMARZESSYLI ISMARZESSYLI
<pre>violacea , violacea , shaposhnikovii , shaposhnikovii , shaposhnikovii , shaposhnikovii , seqaterium nclilus sp, latus , latus , latus , latus , latus , latus , sp, 61-3 (PhC) , violaceum , cepacia , cuber , chororaphis (Cl) , sp, 61-3 (Cl)</pre>	VIORUM VI	IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FORLEAM INT PUP FORLEAM INT PUP FORLEAM INT PUP FOR FORLEAM INT PUP FORLE	VUNIVDIDLAVDTMS	RG 205 ? ? RG 205	 violacea přenniji shaposhnikovii prenarchaeota nenarchaeota nenarchaeota acilius sp. latus urkholderia sp. atus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum cuberia cuberia	ELLINWTFLSL ELLINWTFLSL ELLINWTFLSL DYLNLEFLML EMIDFGNNHL EMIDFGNNHL EMIDFGNNHL EMIDFGNNHL KELATTFSFL KELATTFSFL LELANTFSFL EDMONTFSLL KEIGFTFSL KEIGFTFSL KENAGSFDHI RDMAGAFOLL KEMAGSFDHI RDMAVFAMM	KP F3LTGGKYVNNYULLDDPDI KP F3LTGGKYVNNYULLDDDDI KP YQLAWGKYLLDWYULQDRDI KP YQLAWGKYLLDWYULQDRDI KP YQLAYGWYNYULPPDIMCDBA KP YTAYGYYVLDPSINGBS KP ITNYYGYYVLDPSINGBS KP ITNYYGYYVLGYLLMGET PROLVMYYVGYLLMGET PROLVMYYVGYLLMGET PROLVMYYVGYLLMGET PROLVMYYVGYLLMGGKP PROLVMYYVDYLLMGGKP PROLVMYYVDYLLMGGKP PROLVMYYVDYLLMGER PROLVMYYVNYULGURSER PROLVMYYVNYULGURSER PROLVMYYVNYULGURSER PROLVMYYNNYLLMGEP	VNNITLANGKIT FOS KLONTLANGKIT FOS KLONTLANGKIT FOS VNNITLAR EXAMPLE VNNITLAR EXAMPLE VNNITLAR EXAMPLE VNNITLAR EXAMPLE PPTOLL VNSSHIT VPFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VFFDLL VNSSHIT VNSS	PEDAGET PROPT PDDAGET PDDAGET PROPT PDDAGET PDDAGET PDDA
<pre>violacea pfennigii shaposhnikovii nechocysti sp, recarchaeota negaterium cclilus sp, cclilus sp, cclidovorans latus trkholderia sp, eutropha ramigera sp, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) sp, 61-3 (Cl) caryophyli (Cl)</pre>	VIOLUM VIOLUM VIOLUM VIOLUM VIOLUM II	INT TPU FOR GRALAM INT TPU FOR GRALAM INT FU FOR FORLAM INT FU FOR GRALAM INT FU FOR GRALAM INT SPH F	VUINVDIDLAVDTMS MI VUINVDULAVDTMS MI VUINVDULAVDTMS MV VILABAVDIDLAVDTMS MV MENDIDLIVUS MS MENDIDLIVUS MS MENDIDLI	RG 205 T RG 207 T RG 205 E RG 205 E RG 227 S PY 212 C SG 361 A NG 425 D NG 334 A RG 427 B RG 367 F RG 370 Z RG 355 C GG 355 B GS 355 B GS 357 R R 357 P ZG 359 P ZG 359 P	 violacea přenniji shaposhnikovii prenarchaeota negaterium aeiilus sp. lealigenes sp. aeidovorans latus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum cepacia ruber chlororaphis (C1) 	ELLINGTPLSL ELLINGTPLSL ELLINGTPLSL ELLINGTPLSL EMIDFGNOL GULASTFSFL GULASTFSFL KELATTFSFL LELANTFSFL LELANTFSFL LELANTFSFL EDMANTFSSL EDMANTFSSL EDMANTFSSL EDMANTFAM	KR P5 LTGGKYVNNYULLUDPO KR P5 LTGGKYVNNYULLUDPO KR P5 LTGGKYVLDPO KR P5 LGGKYKVTNUPNYULDPO KR P1 LGKYKVTVUPNYULDPO KR P1 TNYYGP YVALVORSESNIR KR 11 TNYYGP YVALVORSESNIR F7 NDUVNYVVGYYLGYLKGKT F7 NDUVNYVVGYYLKGKT F7 NDUVNYVVGYYLKGKT F7 NDUVNYVVGYYLKGKT F7 NDUVNYVVDYYLKKR F7 NDUVNYVVDYLLKGKT F7 NDUVNYVVDYLLKGKT F7 NDUVNYVVDYLLKGKT F7 NDUVNYVVDYLKKGKT F7 NDUVNYVVDYLKGKT F7 NDUVNYVDYLKGKT F7	NINITLABER 1103 KLINTLABER 1103 KLINTLABER 1103 NINITLABER 110	PEQAGET FROM TO CONSTRUCT THE POLY POLY AND AND AND AND AND AND AND POLY AND
violacea pfennigii shaposhnikovii mechecystis ap, recarchaeota mechecystis ap, recaligenes ap, acidovorans latus ramigera ap, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) caryophyli (Cl) putida (Cl) oleovorans (Cl)	VIOLUM VIOLUM VIOLUM VIOLUM VIOLUM II	INT PUP FOR FORLEAM INT PUP FORLEAM	VQINVDIDLAVDYMS	RG 205 T RG 207 T RG 205 E RG 205 E RG 227 S PY 212 C RG 237 A NG 341 A NG 342 B RG 422 B RG 342 B RG 355 C RG 355 B RG 355 B RG 359 P RG 358 P RG 358 P	 violacea přenniji shaposhnikovii prenarchaeota negaterium aeiilus sp. lealigenes sp. aeidovorans latus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum cepacia ruber chlororaphis (C1) sp. 61-3 (C1) caryophyli(C1) putda (C1) oleevorans (C1) 	ELLINNTFLSL ELLINNTFLSL ELLINNTFLSL ELLINNTFLSL ELLINNTFLSL EMIDFGNNOL QDLASTFSFL EMIDFGNNOL QDLASTFSFL ELAITFSFL ELAITFSFL EDMARTFSL EDMARTFSL EDMARTFSL EDMARTFSSL EDMARTFSSL EDMARTFSSL EDMARTFSSL EDMARTFSSL EDMARTFSS EDMARTF	KR PS LTGGKYVNNYULLODPO KR PS LTGGKYVNNYULLODD KR PS LTGGKYVLOPNO KR PS LTGGKYVNYULDRD KR PS LTGGKYVNYULDRD KR PTNYURYUN PS NOB KR PTNYURYUN PS NOB KR PTNYURYUN STULDRE FNDUVNYYVVGYYLKKRT FNDUVNYYVVGYYLKKRT FNDUVNYVVGYYLKKRT FNDUVNYVVGYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVDYLKKRT FNDUVNYVVNYLLKRT FNDUVNYVVNYLLKRT FNDUVNYVNYLLKRT FNDUVNYVVNYLLKRT FNDUVNYVVNYLLKRT FNDUVNYVNYLLKRT FNDUVNYVNYLLKRT FNDUVNYVNYLLKRT FNDUVNYVNYLLKRT	WINITLABER (TIDS) KLINTLABER (TIDS) KLINTLABER (TIDS) KLINTLABER (TIDS) VINITLABER (TIDS) VINITLABER (TIDS) PFPOLI, VISION PFPOLI, VISION AFPOLI, VISION VISI	DECAMET FROM TO AND A CONSTRUCTION DECAMET FROM DECAMET FROM DECAMET FROM DECAMETER DE
<pre>violacea pfennigii shaposhnikovii mechocystis ap. renarchaeota</pre>	VIOLUM VI	IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FORLEAM IMT PUP FORLEAM IT PUP FORLEAM IT PUP FOR FORLEAM IT PUP FORLEAM IT PUP FORLEAM IT PUP FORLEAM INT PUP FO	VUNNTDILAVETMES	RC 205 P RC 205 F RC 205 F RC 205 F RC 212 C RC 212 C RD 210 B PP 211 B PP 210 B RC 365 D NC 334 A RC 365 D RC 367 F RC 367 F RC 368 P RC 368 P RC 359 B RC 359 P RC 359 P <td><pre>violacea . violacea . shaposhnikovii . shaposhnikovii ymechocysti sgp. renarchaeota . estidovorans . actidovorans . latus utholderia sp. . eutropha . etaligene . etalige</pre></td> <td>ELIANTILES ELIANTILES ELIANTILES ELIANTILES ELIANTILES ELIANTIAS ELIANTIAS ELIANTISE NELATITSE VERMITSE ELIANTISE VERMITSE ELIANTISE ELI</td> <td>KP F3LTGGKYVNNYLLDDPDI KP YGLAGKYLDHYXVLGDBDI KP YGLAGKYLDHYXVLGDBDI KP YGLAGKYNYLDHYDNYLDDB HC YGGYNKYVNLFNYLDBS KP TINYGPYALYDBSSKIP F7 TINYGPYALYDBSSKIP PRIOLYMYYVGYYLLMGST PRIOLYMYYVGYYLLMGST PRIOLYMYYVGYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST</td> <td>VNORTLAGENT FOS KLONTLAGENT FOS KLONTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT PEPDIL VNORT VESENTAGENT VE</td> <td>PEQAGET FROM TO CONSTINCT TO CONSTINCT PEQAGET FROM TO PEDAGET</td>	<pre>violacea . violacea . shaposhnikovii . shaposhnikovii ymechocysti sgp. renarchaeota . estidovorans . actidovorans . latus utholderia sp. . eutropha . etaligene . etalige</pre>	ELIANTILES ELIANTILES ELIANTILES ELIANTILES ELIANTILES ELIANTIAS ELIANTIAS ELIANTISE NELATITSE VERMITSE ELIANTISE VERMITSE ELIANTISE ELI	KP F3LTGGKYVNNYLLDDPDI KP YGLAGKYLDHYXVLGDBDI KP YGLAGKYLDHYXVLGDBDI KP YGLAGKYNYLDHYDNYLDDB HC YGGYNKYVNLFNYLDBS KP TINYGPYALYDBSSKIP F7 TINYGPYALYDBSSKIP PRIOLYMYYVGYYLLMGST PRIOLYMYYVGYYLLMGST PRIOLYMYYVGYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST	VNORTLAGENT FOS KLONTLAGENT FOS KLONTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT FOS VNORTLAGENT PEPDIL VNORT VESENTAGENT VE	PEQAGET FROM TO CONSTINCT TO CONSTINCT PEQAGET FROM TO PEDAGET
<pre>violacea pfennigii shaposhnikovii nechocysti sp, recarchaeota negaterium tcilius sp, caligenes sp, acidovorans latus trkholderia sp, eutropha ramigera sp, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) sp, 61-3 (Cl) caryophyli (Cl) putida (Cl) oleovorans (Cl) mendocina (Cl) endocina (Cl) </pre>	VIOLUM VI	INT PV PY FORELLAM INT PV PY FORENLLAM INT PV PY FORENLLAM INT PV PY FORENLLAM INT PV PY FORENLLAM INT PV PS FORENLLAM INT PS PS FORENLLAM	VUINVDIDLAVETMS MI VUINVDULAVETMS MI VUINVDULAVETMS MI VIERVDIDTLVETMS MV VIERVDIDTLVETMS MV MENDEDEVELAVETMS MV MENDEDEVELAVETMS NEITABALEN DEVELAVETMENGMEN GGLM ESVVFFEMJONEN GGLM ESVTERAMEN GGLAVET ESTELAVETMEN GGLAVET ESTELAVETMEN GGLAVET ESTELAVETMEN GGLAVET ESTELAVETMEN GGLAVETMEN ESTELAVETMEN GGLAVETMEN ESTELAVETME	RG 205 T RG 207 T RG 205 E RG 205 E RG 227 S PY 212 C SG 361 A NG 425 D NG 344 A RG 427 B RG 627 B RG 55 C RG 355 C RG 355 B RG 359 P RG 359 P RG 358 P RG 359 P	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . latus urkholderia sp. . acidovorans . acidovorans</pre>	ELINGTILES ELINGTILES	REPSICOGNYUNNYULLOBED REPSICOGNYUNNYULLOBD REPSICOGNYUNNYULDB REPLOLOGNYUNNYULDB REPLOLOGNYUNYUNUNSUN REPINYUNYUNYUNYUNYU REINYUNYUNYUNYUNYUNYU REINYUNYYUNYUNYUNYU REINUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYUNYU RENDUNYUNYUNYU RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET RENDUNYUNYUNYULUBET	WINITLANDER TO SAL	PEQAGET FRUET POLYMET FRUET POLYMET FRUET PEQAGET FRUET PEDAGET FRUET PEDAGET FRUET PEDAGET FRUET PEDAGET FRUET PEDAGET POLYMET PEDAGET PEDAGET PEDAGET PEDEAL POLYMET PEDEAL POLYMET PEDE
<pre>violacea pfennigii shaposhnikovii mechocystis ap. renarchaeota</pre>	VVOLUM VV	IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FORLEAM IMT PUP FORLEAM INT PUP	VUINTDILAVETHS	RG 205 2 RG 205 F RG 205 F RG 205 F RG 227 S; PY 212 C: RG 227 S; PP 211 B PP 211 B PP 210 B RG 361 A NG 362 D NG 364 P RG 366 P RG 366 P RG 366 P RG 355 C GG 355 P RG 359 B RG 359 P RG 359 P <	<pre>violacea . violacea . shaposhnikovii . shaposhnikovii ymechocysti sgp. renarchaeota . estidovorans . actidovorans . latus utholderia sp. . eutropha . etaligene . etalige</pre>	ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATISE ENIDATIS	KP F3LTGGKYVNNYLLDDPDI KP YGLAGKYLDHYXVLGDBDI KP YGLAGKYLDHYXVLGDBDI KP YGLAGKYNYLDHYDNYLDDB HC YGGYNKYVNLFNYLDBS KP TINYGPYALYDBSSKIP F7 TINYGPYALYDBSSKIP PRIOLYMYYVGYYLLMGST PRIOLYMYYVGYYLLMGST PRIOLYMYYVGYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYVDYYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST PRIOLYMYYNNYLLMGST	VNORTLARGENT FOS KLONTLARGENT FOS KLONTLARGENT FOS VNORTLARGENT FOS VNORTLARGENT FOS VNORTLARGENT FOS VNORTLARGENT FOS VNORTLARGENT PEPDIL VNORVOG PEPDIL VNOS VDFDLLFNORT ANFOLL FOSDS VDFDLLFNORT PUPDIL FONDT PUPDIL FONDT	PEQAGET FROM TO CONSTINCT TO PERSON PERSON AND ADDRESS TO PERSON PERSON AND ADDRESS TO CONSTITUCT PERSON AND ADDRESS TO CONSTITUCT PERSON AND ADDRESS TO CONSTITUCT THE PERSON ADDRESS TO CONSTITUCT T
<pre>violacea pfennigii .shaposhnikovii mechocystis ap, renarchaeota .equatorium tolilus ap, loling ap, .acidovorans .acidovorans .acidovorans .atus .utus .eutropha .capacia .capacia .capacia .cuber .chorcraphis (Cl) .sp. 61-3 (Cl) .caryophylli (Cl) .pseudoalcaligenes Cl .stutteri (Cl) .resinovorans (Cl) .resinovorans (Cl)</pre>	VIORUM VI	INT PUP FOR FORLEAM INT PUP	VUNIVDI DLAVDYNG	RG 205 ? ? RG 205	<pre>violacea . violacea . shaposhnikovii . shaposhnikovii ymechocysti sgp. renarchaeota . megaterium aeiilus sp. . acidovorans . acidovorans . acidovorans . tamigora . tamigora . tamigora . dp. 61-3 (Cl) . capschi . capacia . cubercaphis (Cl) . aps. 61-3 (Cl) . caryophylli (Cl) . puttda (Cl) . puttda (Cl) . puttda (Cl) . resinovorans (Cl) . resinovorans (Cl) . resinovorans (Cl)</pre>	ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATISE ELINATISE ESTORAGIO ODASTISE ESTORAGIO ODASTISE ESTORATISE ESTORATISE ESTATISE E	KP F3LTGGKYVNNYULLDDPDI KP F3LTGGKYVNNYULLDDPDI KP YQLAGGKYLDMYUVULDDPDI KP YQLAGGKYLDMYUVULDKD KP YQLAGYGWKYVNLPSPLME KP YQGYNKYVNLPSPLME KP YNGYYALYDBSENG KP TINYYGPYYALYDBSENG KP TINYYGPYYALYDBSENG F3 TINYYGPYYALYDBSENG F3 TINYYGPYYALYDBSENG F3 TINYYGPYYALYDBSENG F3 TINYYGPYYALYDBSENG F3 TINYYGPYYALYDBSENG F3 TINYYGPYYLDSE F3 TINYYGPYYLBSE F3 TINYYGPYYLBSE F	VNORTLARGENT FOR VNORTLARGENT FOR ULINFLARGENT FOR VLINFLARGENT FOR VNORTLARGENT FOR VNORTLARGENT FOR VNORTLARGENT FOR VNORTLARGENT VNORTLARGENT PEPDIL VNORTLARGENT VFFDLL PRODUCT PEPDIL VNORT PEPDIL PRODUCT PVFDLL PRODUCT PVFDL	PEQAGET FRUET PEQAGET FRUET PEQAGET FRUET PEDALET FRUET PEDALET FRUET PEDALET FRUET PEDALET FRUET PEDALET FRUET PEDALET FRUET PEDALET FRUET PEDALET FLUET PEDEALES FUT TILL GENTLATT FLUET PEDEALES FUT FLUE
<pre>violacea pfennigii shaposhnikovii mechocystis sp. recarchaeota mechocystis sp. recaligenes sp. acidovorans latus ranigera sp. 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) sp. 61-3 (Cl) caryophyli (Cl) putida (Cl) oleovorans (Cl) mendocina (Cl) speudoalcaigenes Cl stutzeri (Cl) resinovorans (Cl) ereinovorans (Cl) ereinovorans (Cl) putida BMD (C2)</pre>	VIOLUM VI	INT TV PY FORELLAM INT TV PY FORENLLAM INT TV PY FORENLLAM INT PY PY FORENLLAM INT PY FORENLLAM INT PY FORENLLAM INT PY FORENLING INT PY FORENLING I	VUINVDIDLAVETMS MI VUINVDULAVETMS MI VUINVDULAVETMS MI VUINVDULAVETMS MI MENDIDLIVUTMS MI MENDIDLIVUTMS MI MENDIDLIVUTMS MI MENDIDLIVUTMS MI MENDIDLIVUTMS MI MENDIDLIVUTMS MI MENDIDLIVUTMS MI ENVVERMIMMENT GENAMARSKI ENVVERMIMMENT GENAMARSKI ENVVERMIMMENT GENAMARSKI ENVVERMIMMENT GENAMARSKI ENVVERMIMMENT ENVVERMIMMENT ENVVERMIMMENT MENDIDLIVUTMS	RG 205 T RG 227 S PY 212 C SG 361 A NG 425 D NG 425 D RG 422 B RG 346 P SG 355 C SG 355 B SG 355 P SG 359 P <td><pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . celacidovorans . celacidovorans . celacidovorans . celacidovorans . colocorans . caryophyli(cl) . mendocina (cl) . setucidovorans (cl) . aetoropinosa (cl)</pre></td> <td>ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTISE ELINYTISE ODASTRIJE VERANTISE ELINYTISE</td> <td>KR PSIJCQKYVNNYULLODPO KR PSIJCQKYVNNYULDOD KR PSIJCQKYULDWJVUDOD KR PSIJCQKYVNYULDWJVUDOD KR PSIJCQKYVNYULDWJVUDOD KR PINYUPYUPYUDOSENIR KR INNYGPYULUORSENIR PSINUUWRYVVGYULWSKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVDIYUKUKIT PRINUWRYVDIYUKUKIT PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKUKE PRINUWRYVNIYUKUKUKUKUKE PRINUWRYVNIYUKUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKE PRINUWRYVNIYUKUKE PRINUKUKE PRINUKUKYVNIYUKUKE PRINUKUKYVNIYUKUKE PRINUKUKYVNIYUKUKE PRINUKUKYVNIYUKUKE PRIN</td> <td>WNIFLENGEN TO SI KLINFLENGEN TO SI KLINFLENGEN TO SI KLINFLENGEN TO SI VINFLENGEN TO SI VINFLENGEN TO SI VINFLENGEN VARG PFPOLL VINSIS PFPOLL VINSIS AFPOLL VINSIS AFPOLL VINSIS AFPOLL VINSIS AFPOLL VINSIS PFPOLL VINSIS PFPOLL VINSIS PFPOLL VINSIS VFPOLL FINIT PFID LENNIT PFID LENNIT PFVD LENNIT</td> <td>PEQAGET FPOPT COMMETTROPTING COMMETT</td>	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . celacidovorans . celacidovorans . celacidovorans . celacidovorans . colocorans . caryophyli(cl) . mendocina (cl) . setucidovorans (cl) . aetoropinosa (cl)</pre>	ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTISE ELINYTISE ODASTRIJE VERANTISE ELINYTISE	KR PSIJCQKYVNNYULLODPO KR PSIJCQKYVNNYULDOD KR PSIJCQKYULDWJVUDOD KR PSIJCQKYVNYULDWJVUDOD KR PSIJCQKYVNYULDWJVUDOD KR PINYUPYUPYUDOSENIR KR INNYGPYULUORSENIR PSINUUWRYVVGYULWSKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVGYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVVDYUKUKKIT PRINUWRYVDIYUKUKIT PRINUWRYVDIYUKUKIT PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKUKE PRINUWRYVNIYUKUKUKUKUKE PRINUWRYVNIYUKUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKUKE PRINUWRYVNIYUKE PRINUWRYVNIYUKUKE PRINUKUKE PRINUKUKYVNIYUKUKE PRINUKUKYVNIYUKUKE PRINUKUKYVNIYUKUKE PRINUKUKYVNIYUKUKE PRIN	WNIFLENGEN TO SI KLINFLENGEN TO SI KLINFLENGEN TO SI KLINFLENGEN TO SI VINFLENGEN TO SI VINFLENGEN TO SI VINFLENGEN VARG PFPOLL VINSIS PFPOLL VINSIS AFPOLL VINSIS AFPOLL VINSIS AFPOLL VINSIS AFPOLL VINSIS PFPOLL VINSIS PFPOLL VINSIS PFPOLL VINSIS VFPOLL FINIT PFID LENNIT PFID LENNIT PFVD LENNIT	PEQAGET FPOPT COMMETTROPTING COMMETT
<pre>violacea pfennigii shaposhnikovii mechocystis ap, recarchaeota mechocystis ap, recarchaeota collum sp, collum sp, acidovorans latus ranigera sp, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) opusida (Cl) oleovorans (Cl) sputida (Cl) esinovas (Cl) sputida (Cl) eleovorans (Cl) sputida (Cl) oleovorans (Cl) sputida (Cl) oleovorans (Cl) sputida (Cl) oleovorans (Cl) sputida (Cl) oleovorans (Cl) sputida (Cl) oleovorans (Cl) sputida (Cl) oleovorans (Cl) sputida (Cl)</pre>	VIOLUM VI	INT PV PY FORMLEAM INT PV PS FORMLEAM INT PV	VUINVDIDLAVETMS MI VUINVDULAVETMS MI VUINVDULAVETMS MI VUINVDUDLAVETMS MI ARMODIDLVUINS MI ARMODIDLVUINS MI ARMODIDKVLSYIE MF DEVYHLOKKVDTFO MI EXVVIEMMMCH GGUM ESVVIEMMMCH GGUM ESVVIEMMMCH GGUM ESVVIEMMCH GGUM ESVV	RG 205 T RG 212 S PY 212 G RG 227 S PY 211 B RG 227 S RG 326 A RG 425 D RG 427 R RG 326 P RG 326 P RG 325 B RG 355 B RG 355 P RG 355 P <td> violacea přenniji shaposhnikovii prenarchaecta nespiterium aciilus sp. lealigenes sp. acidovorans latus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum cepacta ruber chlororaphis (C1) sp. 61-3 (C1) caryophyli(C1) putida (C1) oleovorans (C1) speudoalogiosa (C1) speutoalogiosa (C1) aresinovans (C2) oleovorans (C2) </td> <td>ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTISE ELINYTISE ELINYTISE ELINYTISE UOLASTISE VERANTISE ELINYTISE</td> <td>KR PSIJCQKYVNNYULLODPO KR PSIJCQKYVNNYULDODD KR PSIJCQKYVLOPNOLLODDD KR PSIJCQKYVNYULDRD KR PSIJCQKYVNYULDRD KR PINTYGPYVLOPSINCE KR INTYGPYVLOPSINCE KR INTYGPYVLOPSINCE KR INTYGPYVLOPSINCE RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTU</td> <td>WINITLABER I TOSS KLINTLABER I TOSS KLINTLABER I TOSS KLINTLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS PEPDIL I VISSE PEPDIL I VISSE PEPDIL I VISSE AFDEL I VISSE VIS</td> <td>PEQAGET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET POLYMET PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PLOFT POLYT PO</td>	 violacea přenniji shaposhnikovii prenarchaecta nespiterium aciilus sp. lealigenes sp. acidovorans latus urkholderia sp. eutropha ramigera sp. 61-3 (PhbC) violaceum cepacta ruber chlororaphis (C1) sp. 61-3 (C1) caryophyli(C1) putida (C1) oleovorans (C1) speudoalogiosa (C1) speutoalogiosa (C1) aresinovans (C2) oleovorans (C2) 	ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTILSE ELINYTISE ELINYTISE ELINYTISE ELINYTISE UOLASTISE VERANTISE ELINYTISE	KR PSIJCQKYVNNYULLODPO KR PSIJCQKYVNNYULDODD KR PSIJCQKYVLOPNOLLODDD KR PSIJCQKYVNYULDRD KR PSIJCQKYVNYULDRD KR PINTYGPYVLOPSINCE KR INTYGPYVLOPSINCE KR INTYGPYVLOPSINCE KR INTYGPYVLOPSINCE RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUVGYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYULMRTI RFNDUVNYUMYUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTUMTU	WINITLABER I TOSS KLINTLABER I TOSS KLINTLABER I TOSS KLINTLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS PEPDIL I VISSE PEPDIL I VISSE PEPDIL I VISSE AFDEL I VISSE VIS	PEQAGET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET FPLOFT POLYMET POLYMET PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PFAGELY POLYT PLOFT POLYT PO
<pre>violacea pfennigii shaposhnikovii mechocystis ap, renarchaeota</pre>	VVOLUM VVVOLUM VVVVOLUM VVVVOLUM VVVOLUM VVVOLUM VVVVVVV VVVVVVV VVVVVVVVVVVVVVVVVVVV	IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FORLEAM INTS PUP FORLEAM INTS PUP FORLEAM INTS PUP FORLEAM INTS PUP FORLEAM INTS PUP FORLEAM INTS PUP FORLEAM INTER FOR FORLEAM INTER FOR FORLEAM INTER FORLEAM	-VUNVDICLAVETWES	RG 205 ? ? RG 205	<pre>violacea . violacea . shaposhnikovii . shaposhnikovii sphencysti sp. renarchaeota . megaterium aetilus sp. . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . esp. 61-3 (C1) . caryophylli (C1) . puttda (C2) . caryophylli (C2)</pre>	ELINTYTLSI ELINTYTLSI ELINTYTLSI ELINTYTLSI ELINTYTLSI ELINTYTLSI ELINTYSL ENIDYTLSI ENIDYTLSI ENIDYTLSI ENIDYTSL ENIDYTSL ENISTYSL EDISTYL EDIST	KP F3LTGQKYVNNYULLDBPI KP F3LTGQKYVNNYULDBPI KP YQLAGACKULDWYULDBPI KP YQLAGACKULDWYULDBPI KP YQLAGACKULDWYULDPPI KP YQLAGACKULDWF KP YQLAGACKULDWF YC YC Y	VNORT-DREEKT POS KLONT-DREEKT POS KLONT-DREEKT POS VNORT-DREEKT POS VNORT-DREEKT POS VNORT-DREEKT POS VNORT-DREEKT POS VNORT-DREEKT POS VNORT-DREEKT POS PPFOLL VNOS PPFOLL VNOS VNORT-DREEKT PPFOLL VNOS VNORT-DREEKT VPFOLL POS VNORT-DREEKT PVFOLL POS VNORT-DREEKT PVFOLL POS VNORT-DREEKT PVFOLL POS VNORT-DREEKT PVFOLL POS VNORT-DREEKT PVFOLL POS VNORT-DREEKT PVFOLL VNOS VNORT-DREEKT VNORT-DREE	POJAGET FRUPT POJAGET FRUPT POJAGET FRUPT POJAGET POJAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDAGET POJAGET PEDEAGET POJAGET POJAGET PEDEAGET
<pre>violacea pfennigii shaposhnikovii nechocystis ap, renarchaeota negaterium tcilius sp, calidovorans latus ranigera sp, 61-3 (PhbC) violaceum celarceum cepacia ruber chlororaphis (Cl) aputida (Cl) oleovorans (Cl) mendocina (Cl) estucteri (Cl) redinovans (Cl) aeruginesa (Cl) aerugine</pre>	VIOLUM VI	INT TPU FOR FORLEAM INT TPU FOR FORLEAM INT FUT FOR FORLEAM INT FORLEAM	VUINTDILAVETMS MI VUINTDILAVETMS MI VUINTDILAVETMS MI VIINTDILAVETMS MI VIINTDILVENS MI MENDIDIL	RG 205 T RG 227 S PY 212 C SG 361 A NKG 425 D ARG 422 B RG 366 P RG 366 P SG 355 C GA 395 B SG 355 P SG 355 P SG 359 P </td <td><pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . violaceum . violaceum . cepacia . ruber . chloeroraphis (Cl) . optida (Cl) . sp. 61-3 (Cl) . mendocina (Cl) . speudoalesligenee (L) . stutzeri (Cl) . aeruophyli (Cl)</pre></td> <td>ELIMITILES ELIMITILES ELIMITILES ELIMITILES ELIMITILES ELIMITILES ELIMITIES ELIMITIES ENIDORINE ENIDORISE</td> <td>REPSICOGNYUNNYULUOPPIN REPSICOGNYUNNYULUOPPIN REPSICOGNYUNNYULUOPPIN REPSICOGNYUNYULUOPPIN REPINOVNYUNIYUUNYUNYUUNYUNYUNYUNYUNYUNYUNYUNYUNYUNYU</td> <td>WINITLABER I TOSS KUNTLABER I TOSS KUNTLABER I TOSS KUNTLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS PEPDIL I VISIS PEPDIL VISIS AFFOLI VISIS</td> <td>POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET PO</td>	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . violaceum . violaceum . cepacia . ruber . chloeroraphis (Cl) . optida (Cl) . sp. 61-3 (Cl) . mendocina (Cl) . speudoalesligenee (L) . stutzeri (Cl) . aeruophyli (Cl)</pre>	ELIMITILES ELIMITILES ELIMITILES ELIMITILES ELIMITILES ELIMITILES ELIMITIES ELIMITIES ENIDORINE ENIDORISE	REPSICOGNYUNNYULUOPPIN REPSICOGNYUNNYULUOPPIN REPSICOGNYUNNYULUOPPIN REPSICOGNYUNYULUOPPIN REPINOVNYUNIYUUNYUNYUUNYUNYUNYUNYUNYUNYUNYUNYUNYUNYU	WINITLABER I TOSS KUNTLABER I TOSS KUNTLABER I TOSS KUNTLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS PEPDIL I VISIS PEPDIL VISIS AFFOLI VISIS	POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET TPOPT POLYMET PO
<pre>violacea pfennigii shaposhnikovii nechocystis ap, renarchaeota</pre>	VVOLUM VVVOLUM VVVVOLUM VVVOLUM VVVVOLUM VVVVVVV VVVVVVVVVVVVVVVVVVVVVVVVVVVV	IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM IMT PUP FOR FORLEAM INTS PUP FOR FORLEAM INTEL FORLEA		RG 205 ? ? RG 205	<pre>violacea . violacea . shaposhnikovii . shaposhnikovii sprenarchaeota . sequentia sp. renarchaeota . acidovorans . acidovora</pre>	ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATISE ELINATISE ENIDERNA OLASTISE ENIDERNA VERANTISE ELINATISE ELI	KP F3LTCQKYVNNYULLDDPD KP F3LTCQKYVNNYULLDDPD KP YQLAGACKYLDWYULQDRD KP YQLAGACKYLDWYULQDRD KP YQLAGACKYLDWYULDPDIMCDBA KP YQCAVNKYVNIYULDPDIMCDBA KP YQCAVNKYVNIYULGKET P PRIDLIWYUVAIYUKGKET P PRIDLIWYUVAIYULGKET P PRIDLIWYUVAIYULGKAP	VNORT-DREEM FORS (LONT-DREEM FORS (LONT-DREEM FORS (LONT-DREEM FORS VNORT-DREEM FORS VNORT-DREEM FORS VNORT-DREEM FORS VNORT-DREEM FORS VNORT-DREEM FORS PFPCL VNORT PFPCL VNORT PFPCL VNORT PFPCL VNORT PFPCL PNORT PFPCL VNORT PFPCL VNORT	PEQAGET FROPT PEQAGET FROPT PEQAGET FROPT PEQAGET PEQAMET PEDAGET PEQAMET PEDAGET PEQAMET PEDAGET PEQAMET PEDAGET PEQAMET INLEGPPCWLINE INLEGPPCWLINE INLEGPPCWLINE INLEGPPCWLINE INLEGPPCWLINE PEDAGET PEDAGE
<pre>violacea pfennigii shaposhnikovii nechocysti sp. renarchaeota nechocysti sp. renarchaeota sp. cillus sp. ciclus sp. acidovorans latus ranipera sp. 61-3 (PhbC) violaceum cepacia ruber putcha(cl) putch(cl) putch(c</pre>	VIDEUV VI	INT PUP FOR FORLLAAM- INT PUP OF FORLLAAM- INT PUP OF FORLLAAM- INT PUP OF FORLLAAM- MAP UP OF BORLLAAM- MAP UP OF BORLLAAM- MAP UP OF BORLLAAM- MAP UP OF BORLLAAM- INT PUP STORY MAP IN MAP UP OF BORLLAAM- MAP UP OF BORLLAAM- INT PUP STORY MAP IN LITTLE OF STORY LAWY PUP LITTLE OF STORY PUP LITTLE OF STORY LAWY PUP LITTLE OF STORY LAWY PUP LITTLE OF STORY LAWY PUP LITTLE OF STORY PUP LITTLE OF STORY LAWY PUP LITTLE OF STORY LAWY PUP LITTLE OF STORY PUP LAWY PUP LITTLE OF STORY PUP LAWY PUP LITTLE OF STORY PUP LAWY PUP PUP LAWY PUP LAW	VUINDI DLAVDTNG	RG 205 2 RG 205 T RG 205 F RG 205 F RG 227 S; PY 212 C: RG 227 S; PY 212 C: RG 327 B; RG 425 D RG 425 C RG 422 B; RG 422 B; RG 425 C RG 422 B; RG 5370 Z RG 5355 C RG 360 P RG 36355 C RG 355 C RG 355 P RG 359 P </td <td><pre>violacea , violacea , pfennigli , shaposhnikovii ymechocystis sp. renarchaeota , megatorium aciilus sp. i.acidovorans . acidovorans . (cl) . paetudoalcoligenes . (cl) . acivophylli (cl) . acidovorans . (cl) . acidovorans</pre></td> <td>ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYS: ELINTYS: ELINTYS: ELINTYS: ELINTYS: VERATYS: VERATYS: ELINTYS: EL</td> <td>REPSICO(RYVNRYURLDDPD) REPSICO(RYVNRYURLDDDD) REPSICO(RYVNRYULD) REPSICO(RYVNRYURLDPD) REPSICO(RYVNRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYRRY) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYRRYR) REPSICO(RYVRRYRRYRRYR) REPSICO(RYVRRYRRYRRYRRYRRYRRYRRYRRYRRYRRYRRYRRYR</td> <td>VNORT-BREEKT FOS LLONT-BREEKT FOS LLONT-BREEKT FOS LLONT-BREEKT FOS VNORT-BREEKT FOS PEDILLARS LEART DES PEDILLARS LEART DES PEDILLARS DES PEDILLARS DES PEDILLARS DES PEDILLARS DES PEDILLARS DES PEDILLARS DES APPOLLARS DES APPOLLARS DES APPOLLARS DES PEDILLARS DES PED</td> <td>POAGET FROM POAGET FROM POAGET FROM POAGET POET POAGET POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT PEA</td>	<pre>violacea , violacea , pfennigli , shaposhnikovii ymechocystis sp. renarchaeota , megatorium aciilus sp. i.acidovorans . acidovorans . (cl) . paetudoalcoligenes . (cl) . acivophylli (cl) . acidovorans . (cl) . acidovorans</pre>	ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYS: ELINTYS: ELINTYS: ELINTYS: ELINTYS: VERATYS: VERATYS: ELINTYS: EL	REPSICO(RYVNRYURLDDPD) REPSICO(RYVNRYURLDDDD) REPSICO(RYVNRYULD) REPSICO(RYVNRYURLDPD) REPSICO(RYVNRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYURPD) REPSICO(RYVRRYURLPPD) REPSICO(RYVRRYRRY) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYR) REPSICO(RYVRRYRRYRRYR) REPSICO(RYVRRYRRYRRYR) REPSICO(RYVRRYRRYRRYRRYRRYRRYRRYRRYRRYRRYRRYRRYR	VNORT-BREEKT FOS LLONT-BREEKT FOS LLONT-BREEKT FOS LLONT-BREEKT FOS VNORT-BREEKT FOS PEDILLARS LEART DES PEDILLARS LEART DES PEDILLARS DES PEDILLARS DES PEDILLARS DES PEDILLARS DES PEDILLARS DES PEDILLARS DES APPOLLARS DES APPOLLARS DES APPOLLARS DES PEDILLARS DES PED	POAGET FROM POAGET FROM POAGET FROM POAGET POET POAGET POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT PEA
<pre>violacea pfennigii shaposhnikovii mechocystis ap, recarchaeota nechocystis ap, recarchaeota colligenes ap, acidovorans latus ramigera ap, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) cepacia caryophyli (Cl) putida (Cl) oleovorans (Cl) setiones (Cl) mendocina (Cl) putida (Cl) aeruginosa (Cl) aeruginosa (Cl) putida (Cl) putida (Cl) putida (Cl) cleovorans (Cl) putida (Cl) pseudolalgenes (Cl) pseudolalgenes (Cl) pseudolalgenes (Cl) pseudolacalgenes (Cl) pseudolacalgen</pre>	VIOLUM VI	INT PUP OF TRESHLEAM INT PUP OF TRESHLEAM	VUINDIDLAVETMES	RG 205 2 RG 205 F RG 205 F RG 205 F RG 212 C RG 227 S PY 212 C RG 227 S PY 211 B RG 425 D RG 427 B RG 427 B RG 6 367 F RG 6 367 F RG 566 P SG 355 B SG 355 B SG 355 P SG 359 P	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . acidovorans . violaceum . cepacia . ruber . colacoraphis (Cl) . putida (Cl) . sp. 61-3 (Cl) . speduolacilgenes (Cl) . aeruophyli (Cl) . putida NGO (Cl) . putida (Cl) . aeruophyli (Cl) . aeruophyli (Cl) . putida (Cl) . stutzeri (Cl)</pre>	ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTIS: ELINGTIS: UDASTRIF: VERATIS: ELINTIS: EL	KP P3 LTGGKYYNNYULLODPO KP Y2LGGKYYLWNYULLODPO KP Y2LGGKYYLWNYULDRO KP Y2LGGKYYLYNU KP JLTGYGYYLYNU KP JLTGYGYYLYNU KP JLTGYGYYLYNU KP JTNYYGP YYALVORSENNE FP NDULWYYYUNYULGKET FP NDULWYYVYUNYULGKET FP NDULWYYVYUNYULGKET FP NDULWYYVYUNYULGKET FP NDULWYYVYNYULGKET FP NDULWYYVYNYULGKET FP NDULWYYVYNYULGKET FP NDULWYYVYNYULGKET FP NDULWYYVNYULGKET FP NDULWYYNNYULGKET FP NDULWYYNNYULGKET FP NDULWYYNNYULGKET FP NDULWYYNNYULGKET FP NDULWYYNNYULGKET FP NDULWYYNNYULGKET FP NDULWYYNNYULGKET FP NDULWYNNYUNYU FP NDULWYNNYUNYUNYU FP NDULWYNNYUNYUNYU FP NDULWYNNYUNYUNYUNYUNYU FP NDULWYNNYUNYUNYUNYUNYUNYUNYUNYUNYUNYUNYUNYUNY	WINITLABER I TOSS KINTLABER I TOSS KINTLABER I TOSS KINTLABER I TOSS KINTLABER I TOSS VINITLABER I TOSS VINITLABER I TOSS PEPDIL WINSS PEPDIL WISS PEPDIL WISS AFDEL	PEQAGET FPLOFT POLYMETTROFT POLYMETTROFT PEDAGETTROFT PED
<pre>violacea pfennigii shaposhnikovii mechocystis sp. resarchaeota nechocystis sp. resarchaeota icilius sp. cicilius sp. cicilius sp. acidovorans latus ranipera sp. 61-3 (PhbC) violaceum cepacia ruber puber pottafa (C1) pottafa (C1) pottafa (C1) pottafa (C1) pottafa (C1) pottafa (C1) resinovorans (C1) puttafa (C1) resinovorans (C1) puttafa (C1) resinovorans (C2) puttafa (C2) caryophylli (C2) nitorceduceas (C2) pesudoalcaligenes C2 mendocina (C2) resinovorans (C2) resinovorans (C2) resinovorans (C2) resinovorans (C2) resinovorans (C2)</pre>	VIOLUM VI	INT PUP FOR FORLLAAM- INT PUP OF FORLLAAM- INT PUP OF FORLLAAM- INT PUP OF FORLLAAM- MAPUP OF EQUILABING INT PUP STOCK INT PUP STOCK MISPPOFSETGLINGELLO- INTS PUP STOCK MISPPOFSETGLINGELLO- INT PUP STOCK INT PUP STOCK I	VUINDI DLAVDTNG	RG 205 2 RG 205 T RG 227 Sj PY 212 C: RG 327 A RG 425 D RG 425 D RG 422 H RG 422 H RG 425 D RG 422 H RG 5370 T RG 6367 R RG 6367 R RG 6367 R RG 5355 C RG 355 C RG 355 P	<pre>violacea , violacea , pfennigli , shaposhnikovii ymechocystis sp. renarchaeota , megatorium aciilus sp. i.acidovorans . acidovorans . (cl) . paetudoalcoligenes . (cl) . acivophylli (cl) . acidovorans . (cl) . acidovorans</pre>	ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYS: ELINTYS: ELINTYS: ELINTYS: UDASTROM ELINTYS: UDASTROM ELINTYS: USANTYS:	KP F3LTGG(KY/NNY/ULDDP) KP F3LTGG(KY/NNY/ULDDP) KP F3LTGG(KY/UNY/ULDDP) KP F3LTGG(KY/UNY/ULDN) KP F3LTGG(KY/UNY/ULDN) KP F1TNY/GFY/UN/F5 KP T1NY/GFY/UN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYUN/SI/LGGT F3 PNDL/WY/VOJYU/V/V/V/V/V/V/V/V/V/V/V/V/V/V/V/V/V/V/V	VNORT-BREEKT FOS LLONF-JORGENT FOS LLONF-JORGENT FOS LLONF-JORGENT FOS WONF-JAC LLONFLOW PERCIL-YONG PEPCIL-YONG PEPCIL-YONG PEPCIL-YONG APPCIL-YONG APPCIL-YONG APPCIL-YONG APPCIL-YONG APPCIL-YONG PEPCIL-YONG APPCIL-YONG APPCIL-YONG PEFCIL-YONG PE	POAGET FROM POAGET FROM POAGET FROM POAGET POET POAGET POET PEGENTYROFT PEGENTYROFT PEGENTYROFT PEGENTYROFT PEGENTYROFT PEGENT PEGE
<pre>violacea pfennigii shaposhnikovii mechocystis sp. resarchaeota mechocystis sp. resarchaeota scillus sp. cicilus sp. cicilus sp. acidovorans latus ranipera sp. 61-3 (PhbC) violaceum cepacia ruber puber pottofa (C1) pottofa (C2) caryophylli (C2) nitroreducens (C2) pottofa (C2) resinovorans (C2) resinovorans (C2) resinovorans (C2) resinovorans (C2) sp. 61-3 (C2) aeruginos (C2) sp. 61-3 (C2) aeruginos (C2)</pre>	VIOLUM VI	INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INTS PUP STORY AND	VUINDI DLAVDTNG	RG 205 2 RG 205 T RG 227 S; PY 212 C: RG 227 S; PP 211 B RG 425 D RG 425 C RG 425 G RG 422 B RG 425 C RG 5370 T RG 6387 R RG 6387 R RG 6387 R RG 360 P RG 36359 P RG 359 P	<pre>violacea .pfennigii .shaposhnikovii ymechocystis.sp. renarchaeota .megatorium aciilus sp. ladius sp. ladius sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .aciadovorans .aciadovorans .violaceum .violaceum .dicerosphis (C1) .potida (C1) .potida (C1) .potida (C1) .potida (C1) .patudo loligiona (C1) .patudo (C1) .potida (C2) .potida (C2) .poti</pre>	ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYTLS: ELINTYS: ELINTYS: ELINTYS: ELINTYS: VERATYS: VERATYS: USAATYS:	REPSICO(RYVNRYURLDDPD) REPSICO(RYVNRYULDDDD) REPSICO(RYVNRYULDDD) REPSICO(RYVNRYULDPD) REPSICO(RYVNRYULDPD) REPSICO(RYVNRYULDPD) REPSICO(RYVNRYULDPD) REPSICO(RYVNRYURYULDR) REPSICO(RYVNRYURYULDR) REPSICO(RYVNRYURYURYURYURYURYURYURYURYURYURYURYURYUR	VNORT-BREEKT FOS KLONF-JREEKT FOS KLONF-JREEKT FOS KLONF-JREEKT FOS VNORT-BREEKT FOS PERCIL: VNORT-BREEKT FOS PERCIL: VNORT-BREEKT FOS PERCIL: VNORT-BREEKT FOS PERCIL: VNORT-BREEKT APPCL: VNORT-BREEKT PERCIL: VNORT-BREEKT VNO	POAGET FROM POAGET FROM POAGET FROM POAGET POAGET POAGET POAGET PEGENT POEN PEGENT POEN PEGENT POEN PEGENT POEN PEGENT PEGEN
<pre>violacea , violacea , pfennigii , shaposhnikovii , shaposhnikovii , shaposhnikovii , negaterium tcilius sp acidovcans . acidovc</pre>	VIOLUM VI	INT PUP POPERALLAAM INT PUP P	VQINVDILAVITRO INI VQINVDILAVITRO INI VQINVDULAVITRO INI VQINVDULAVITRO INI VARIDI DILVITRO INI ARIBUI DIKVLAYIRI INI ARIBUI DIKVLAYIRI INI DEVYRLAKAVITRO INI EXVIRTROMATI GENAPARA ESVVIRTROMATI GENAPARA ES	RG 205 T RG 212 S PY 212 G RG 227 S PY 211 G RG 227 S RG 346 A RG 425 D RG 422 B RG 627 R RG 636 P RG 366 P RG 366 P RG 366 P RG 367 B RG 367 B RG 355 B RG 355 P RG 355 P <td><pre>violacea .violacea .pfennigii .shaposhnikovii ymechocysti sp. renarchaeota .edilus sp. lealigenes sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .cepacia .ruber .cepacia .ruber .cepacia .ruber .cepacia .caryophyli(cl) .setudorins (cl) .setudorins (cl) .setudorins (cl) .aerubyli(cl) .setudorins (cl) .aerubyli(cl) .aerubyli(cl) .setudorins (cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aeruphyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .setudorins (cl) .setudorins (cl) .se</pre></td> <td>ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTSE ELINYTSE ELINYTSE ELINYTSE OLASTREE UDASTREE ELINYTSE ELINY</td> <td>KR PSILOGIAYUNNYLLALDED KR PSILOGIAYUNNYLLALDED KR YQLAKOKYLLAWIZYULADD KR YQLAKOKYLIAWIZYULADD KR YQLAKOKYLAN PSILOS KR PSILOGIAYUNYLUAR SIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN R PHOLINGYUNGYLLAKET PRODUNGYUNGYLLAKET</td> <td>VNNITLABLER (TOS) KLINTLABLER (TOS) KLINTLABLER (TOS) KLINTLABLER (TOS) VNNITLABLER (TOS) VNNITLABLER (TOS) PFPOLI (VNNISS) PFPOLI (VNSISS) PFPOLI (VNSISS) PFPOLI (VNSISS) AFPOLI (VNSISS) PFPOLI (VNS</td> <td>POLYAGET FROM THE CONSIST FROM THE PERSON FROM THE PERSON</td>	<pre>violacea .violacea .pfennigii .shaposhnikovii ymechocysti sp. renarchaeota .edilus sp. lealigenes sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .cepacia .ruber .cepacia .ruber .cepacia .ruber .cepacia .caryophyli(cl) .setudorins (cl) .setudorins (cl) .setudorins (cl) .aerubyli(cl) .setudorins (cl) .aerubyli(cl) .aerubyli(cl) .setudorins (cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aeruphyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .setudorins (cl) .setudorins (cl) .se</pre>	ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTLES ELINYTSE ELINYTSE ELINYTSE ELINYTSE OLASTREE UDASTREE ELINYTSE ELINY	KR PSILOGIAYUNNYLLALDED KR PSILOGIAYUNNYLLALDED KR YQLAKOKYLLAWIZYULADD KR YQLAKOKYLIAWIZYULADD KR YQLAKOKYLAN PSILOS KR PSILOGIAYUNYLUAR SIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN R PHOLINGYUNGYLLAKET PRODUNGYUNGYLLAKET	VNNITLABLER (TOS) KLINTLABLER (TOS) KLINTLABLER (TOS) KLINTLABLER (TOS) VNNITLABLER (TOS) VNNITLABLER (TOS) PFPOLI (VNNISS) PFPOLI (VNSISS) PFPOLI (VNSISS) PFPOLI (VNSISS) AFPOLI (VNSISS) PFPOLI (VNS	POLYAGET FROM THE CONSIST FROM THE PERSON
<pre>violacea pifennigii shaposhnikovii nechocysti sp. recarchaeota</pre>	VIOLUM VI	INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INT PUP OF TORNILESAM- INTS PUP OF STGLING PLO- INTS PUP OF STGLING PLO- INTS PUP OF STGLING PLO- INT PUP OF STGLING PLO-	VUINDI DLAVDYNG	RG 205 2 7 RG 205 F 7 RG 227 S3 7 RG 227 S3 7 RG 307 F 7 RG 425 D N RG 422 H N RG 425 D N RG 422 H N RG 5370 F N RG 360 P N SG 355 C N RG 360 P N SG 355 C N RG 359 P N SG 359 P N RG 359 P N SG 359 P N SG 359 P N SG 359 P N SG 359 P N	<pre>violacea .pfennigii .shaposhnikovii ymechocystisgp. renarchaeota .megatorium aciilus sp. ladius sp. ladius sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .caligora .violaceum .violaceum .caligora .diacoraphis (Cl) .putida (Cl</pre>	ELINTYILSE ELINTYILSE ELINTYILSE ELINTYILSE ELINTYILSE ELINTYILSE ELINTYISE ELINTYISE ELINTYISE ELINTYISE ELINTYISE UGLASTYSE	REPSICO(RY)NNYULLOBPI NEPSICO(RY)NNYULLOBD REPSICO(RY)NNYULLOBD REPSICO(RY)NNYULLOBD REPSICO(RY)NNYULLOBD REPSICO(RY)NNYULLOBD RETINYYGPYYALVDBESKIR RETINYYGPYYALVDBESKIR RETINYYGPYYALVDBESKIR RETINYYGPYYALVDBESKIR RETINYGPYYALVDBESKIR REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYVNYULLOBT REPSICUNWYNNYULDU	NINITLABERGIT DIS KLONT LABERGIT DIS KLONT LABERGIT DIS KLONT LABERGIT DIS KLONT LABERGIT DIS PERCIL XI SI AND	POAGET FROM POAGET FROM POAGET FROM POAGET POEN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET POWN PIAGET
<pre>violacea pressure violacea violace</pre>	VIOLUM VI	INT PUP POPERALLAAM INT PUP POPERALLAALAAM INT PUP POPERALLAAM INT PU	VQINVDIDLAVETMS INT VQINVDULAVETMS INT VQINVDULAVETMS INT VQINVDULAVETMS INT VQINVDULAVETMS INT VGINVDULAVETMS INT INTERNOVET INT INT INTERNOVET INT INT INT INT INT INT INT INT INT INT	RG 205 T RG 227 S PY 212 C SG 361 A KG 425 D ARG 422 B RG 6 367 R RG 6 367 R RG 6 367 R RG 6 367 B SG 355 B SG 355 P SG 355 P SG 355 P SG 355 P SG 359 P	<pre>violacea .violacea .pfennigii .shaposhnikovii ymechocysti sp. renarchaeota .edilus sp. lealigenes sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .cepacia .ruber .cepacia .ruber .cepacia .ruber .cepacia .caryophyli(cl) .setudorins (cl) .setudorins (cl) .setudorins (cl) .aerubyli(cl) .setudorins (cl) .aerubyli(cl) .aerubyli(cl) .setudorins (cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aeruphyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .aerubyli(cl) .setudorins (cl) .setudorins (cl) .se</pre>	ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATISE ELINATISE ELINATISE ELINATISE ELINATISE VERANTISE VERANTISE ELINATISE	KR PSILOGIAYUNNYLLALDED KR PSILOGIAYUNNYLLALDED KR YQLAKOKYLLAWIZYULADD KR YQLAKOKYLIAWIZYULADD KR YQLAKOKYLAN PSILOS KR PSILOGIAYUNYLUAR SIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN KR TINYGPYULAUNSELSIN R PHOLINGYUNGYLLAKET PRODUNGYUNGYLLAKET	MONITLABLE INTERNET TO SE UNITLABLE INTO SECOND SE	POJAGET FROM T POJAGET FROM T POJAGET FROM T POJAGET STROFT POJAGET STROFT POJAGE
<pre>violacea pfennigii shaposhnikovii mechocystis ap, recarchaeota negaterium tolilus sp, acidovorans latus ramigera ap, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) sp, 61-3 (PhbC) violaceum cepacia ruber chlororaphis (Cl) sp. 61-3 (Cl) caryophyli (Cl) putida (Cl) oleovorans (Cl) setuicaigenes (Cl) setuicaigenes (Cl) putida (Cl) setuicaigenes (Cl) putida (Cl) setuicaigenes (Cl) putida (Cl) putida (Cl) setuicaigenes (Cl) putida (Cl) setuicaigenes (Cl) putida (Cl) putida (Cl) setuicaigenes (Cl) putida (Cl) putida (Cl) setuicaigenes (Cl) pseudolcaigenes (Cl) pseudolcaigenes (Cl) rutororubcens (Cl) rutororubcens (Cl) rutororubcens (Cl) rutororubcens (Cl) rutororubcens (Cl) setuicaigenes (Cl) setuicai</pre>	VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT RELASVIJ RELASVIJ RELASVIJ RELASVIJ RELASVIJ RELASVIJ RELASVIJ RELASVIJ VIOLUT RELASVIJ RELASVIJ RELASVIJ VIOLUT RELASVIJ RELASVIJ RELASVIJ VIOLUT RELASVIJ RELASVIJ RELASVIJ RELASVIJ RELASVIJ VIOLUT	INT PUP OF TORMLEAM INT PUP O	VUINDIDLAVETRO	RC 205 T RC 205 F RC 205 F RC 205 F RC 205 F RC 212 C RC 212 S RC 227 S PY 211 G RC 425 D RC 427 B RC 427 B RC 427 B RC 427 B RC 537 F RC 427 B RC 537 F RC 366 P SC 355 B SC 355 B SC 355 P SC 355 P SC 359 P <td><pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . cepacia . ruber . violaceum . cepacia . ruber . chloeroraphis (C1) . optida (C1) . othororaphis (C1) . aerophyli(C1) . mendocina (C1) . aerodovina (C1) . aerodovinas (C1) . aerodovinas (C1) . aerodovinas (C1) . aerodovinas (C1) . aerodovorans (C2) . oleovorans (C2) . putida (C2) . caryophyli(C2) . aerodovans (C2) . putida (C2) . stutzeri (C2) . caryophyli(C2) . aerodovans (C2) . putida (C2) . caryophyli(C2) . aerodovans (C2) . caryophyli(C2) . caryophyli(C2) . aerodovans (C2) . chlororaphis (C2) . aeroginosa (C2) . aerodovans (C2) . aerodovans (C2) . aerodovans (C2) . aerodovans (C2) . puctata</pre></td> <td>ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTIS: ELINGTIS: ELINGTIS: VERATIS: ELINGTIS:</td> <td>KP P3 LTGG(KYVNNYLLGDP) KP P3 LTGG(KYVNNYLLGDP) KP P3 LTGG(KYVNNYLLGDP) KP P3 LTGG(KYVNYLLGP) KP P1 LTGG(KYVNYLLGP) KP P1 LTG(KYVNYLUS) KP ITNYGP YVALVDP3 ENGEN KP ITNYGP YVALVDP3 ENGEN KP ITNYGP YVALVDP3 ENGEN KP ITNYGP YVALVDP3 ENGEN FP NDLVNYYVVGYLLGKT FP NDLVNYYVVGYLLGKT FP NDLVNYYVVTYLLGKT FP NDLVNYYVVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYVNYLLGKT FP NDLVNYVNYLLGKT FF NDLVNYVNYLGYLGKT FF NDLVNYVNYLGT FF NDLVNYVNYLGYLGKT FF NDLVNYVNYLGT FF NDLVNYVNYLGT FF NDLVNYVNYLGT FF ND</td> <td>MONITLABLE INTERNET TO SE UNITLABLE INTO SECOND SE</td> <td>PEQAGET FPLOFT POLYMETTROFT POLYMETTROFT PEQAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGET PEDAGET PEDAGET PEDAGET PEDEAGET PEDEAGET PEDEAGET PEDEAGETTRO PEDAGET PEDAGE</td>	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . cepacia . ruber . violaceum . cepacia . ruber . chloeroraphis (C1) . optida (C1) . othororaphis (C1) . aerophyli(C1) . mendocina (C1) . aerodovina (C1) . aerodovinas (C1) . aerodovinas (C1) . aerodovinas (C1) . aerodovinas (C1) . aerodovorans (C2) . oleovorans (C2) . putida (C2) . caryophyli(C2) . aerodovans (C2) . putida (C2) . stutzeri (C2) . caryophyli(C2) . aerodovans (C2) . putida (C2) . caryophyli(C2) . aerodovans (C2) . caryophyli(C2) . caryophyli(C2) . aerodovans (C2) . chlororaphis (C2) . aeroginosa (C2) . aerodovans (C2) . aerodovans (C2) . aerodovans (C2) . aerodovans (C2) . puctata</pre>	ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTIS: ELINGTIS: ELINGTIS: VERATIS: ELINGTIS:	KP P3 LTGG(KYVNNYLLGDP) KP P3 LTGG(KYVNNYLLGDP) KP P3 LTGG(KYVNNYLLGDP) KP P3 LTGG(KYVNYLLGP) KP P1 LTGG(KYVNYLLGP) KP P1 LTG(KYVNYLUS) KP ITNYGP YVALVDP3 ENGEN KP ITNYGP YVALVDP3 ENGEN KP ITNYGP YVALVDP3 ENGEN KP ITNYGP YVALVDP3 ENGEN FP NDLVNYYVVGYLLGKT FP NDLVNYYVVGYLLGKT FP NDLVNYYVVTYLLGKT FP NDLVNYYVVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYYVNYLLGKT FP NDLVNYVNYLLGKT FP NDLVNYVNYLLGKT FF NDLVNYVNYLGYLGKT FF NDLVNYVNYLGT FF NDLVNYVNYLGYLGKT FF NDLVNYVNYLGT FF NDLVNYVNYLGT FF NDLVNYVNYLGT FF ND	MONITLABLE INTERNET TO SE UNITLABLE INTO SECOND SE	PEQAGET FPLOFT POLYMETTROFT POLYMETTROFT PEQAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGET PEDAGET PEDAGET PEDAGET PEDEAGET PEDEAGET PEDEAGET PEDEAGETTRO PEDAGET PEDAGE
<pre>violacea pfennigii shaposhnikovii rechecystis ap. rechecystis ap. rechecystis ap. rechecystis ap. rechecystis ap. acidovorans latus ranigera sp. 61-3 (PhC) violaceum cepacia ruber chlorcraphis (Cl) colecvorans (Cl) putida (Cl) olecvorans (Cl) resinovarans (Cl) resinovarans (Cl) resinovarans (Cl) putida (Cl) putida (Cl) putida (Cl) putida (Cl) resinovarans (Cl) resinovarans (Cl) resinovarans (Cl) putida (Cl) clevorans (Cl) resinovarans (Cl) resinovarans (Cl) resinovarans (Cl) resinovarans (Cl) setutiat (Cl) resinovarans (Cl) rubripertinctus chloracepticus intecbacter ap. punctata hydrophila</pre>	VIOLUM VI	INT PUP OF TORMLEAM INT PUP OF TORMLEAM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INT PUP STOCKNEY LINM INT LUP STOCKNEY LINM INT LU	VQINDI DLAVDYBS	RG 205 2 7 RG 205 7 T RG 205 8 7 RG 205 8 7 RG 205 8 7 RG 227 35 7 RG 227 35 7 RG 227 10 8 NK0 425 0 8 RG 422 8 8 RG 422 8 8 RG 425 0 8 RG 5307 2 8 RG 535 C 8 RG 535 7 8 RG 535 7 8 RG 368 8 9 RG 359 7 8 RG 359 7 7	<pre>violacea .pfennigii .shaposhnikovii ymechocystis.gp. renarchaeota .megatorium aciilus sp. ladius sp. ladius sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .calicopa .acidovorans .calicopa .caposhis.(Cl) .sp. 61-3 (Cl) .sp. 61-3 (Cl) .sp. 61-3 (Cl) .aciyophyli(Cl) .putida (Cl) .sectorial disputes .calicoparas (Cl) .putida (Cl)</pre>	ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELIDITIGICS OLASTIFIC VERATIFIC ELINTIFIC VERATIFIC ELINTIFIC ELINTIFIC VERATIFIC ELIN	REPELOGIATIONELLOPPI REPELOGIATIONELLOPPI REPLOGIATIONEVILLOPPI REPLOGIATIONEVILLOPPI REPLOGIATIONEVILLOPPI REPLOGIATIONEVILLOPPI REPLOGIATIONEVILLOPPI REPLOCENTIALISSI REPLOCENTIALISSI REPLOCHMENTIALISSE REPLOCHMENTALISSE REPLO	VNORT-BREEKT POS LLONT-BREEKT POS LLONT-BREEKT POS LLONT-BREEKT POS VNORT-BREEKT POS PPOLLANS AND	POAGET FRUET POAGET FRUET POAGET FRUET POAGET FRUET POAGET POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT POET PEAGENT P
<pre>violacea pfennigii shaposhnikovii mechocystis ap, recarchaeota . esqaterium tcillus sp, lealigenes sp. acidovcans . acidovcans . a</pre>	VVICUM VV	INT PUP OF TREAL LAW INT PUP OF TREAL LAW	VQINVDIDLAVDTRG	RC 205 2 RC 205 E RC 205 E RC 205 E RC 205 E RC 212 C RC 227 S PY 212 C RC 425 D MKG 425 D MKG 425 D RC 427 R RC 6 277 Z RC 6 277 Z RC 6 367 R RC 6 375 B SG 355 C SG 355 R SG 355 P SG 359 P <td><pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . cepacia . violaceum . cepacia . ruber . chlocoraphis (C1) . optida (C1) . putida (C1) . sp. 61-3 (C1) . devorans (C1) . speudoalcaligenes (C1) . aeruphylid (C1) . aeruphylid (C1) . aeruphylid (C1) . aeruphylid (C2) . aeruphylid (C2) . aeruphylid (C2) . putida SUG . caryophylid (C2) . putida (C2) . putida (C2) . putida (C2) . putida (C2) . putida (C2) . caryophylid (C2) . putida (C2) . speudoalcaligenes (C2) . speudoalcaligenes (C2) . chlororaphis (C2) . aeruginosa (C2) . aeruginosa (C2) . aerupinosa (C2) . ae</pre></td> <td>ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATISE ELINATISE ELINATISE ELINATISE ELINATISE UDASTISE VERANTISE ELINATISE E</td> <td>REPSICOGNYUNYULLADEP REPSICOGNYUNYULLADEP REPSICOGNYUNYULLADEP REPLOLOGNYUNYULLADEP REPLOLOGNYUNYULADEP REPLOLOGNYUNYULADEP REPNDUNYUNYUNYUNYUNYU REPNDUNYUNYUNYUNYU REPNDUNYUNYUNYUNYU REPLOLOGNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU REPLOIMAYYUNYU RANDUNYUNYU RANDUNYUNYU REPLOIMAYYUNYU REPLOI</td> <td>MONITLANGEN I TOSS MONTLANGEN I TOSS LLINTLANGEN I TOSS LLINTLANGEN I TOSS MONTLANGEN I TOSS MONTLANGEN I TOSS PEPGLI MONTS PEPGLI MONTS PEPGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS PEPGLI MONTS PEPGLI MONTS PEPGLI MONTS PEPGLI MONTS PEFGLI MONT</td> <td>PEQAGET FRUET POLYMETTROFI POLYMETTROFI PEQAGETTROFI PEDAGETTROFI PEDAGETTROFI PEDAGETROFI PEDAGETROFI INTLOOPTONIC</td>	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . cepacia . violaceum . cepacia . ruber . chlocoraphis (C1) . optida (C1) . putida (C1) . sp. 61-3 (C1) . devorans (C1) . speudoalcaligenes (C1) . aeruphylid (C1) . aeruphylid (C1) . aeruphylid (C1) . aeruphylid (C2) . aeruphylid (C2) . aeruphylid (C2) . putida SUG . caryophylid (C2) . putida (C2) . putida (C2) . putida (C2) . putida (C2) . putida (C2) . caryophylid (C2) . putida (C2) . speudoalcaligenes (C2) . speudoalcaligenes (C2) . chlororaphis (C2) . aeruginosa (C2) . aeruginosa (C2) . aerupinosa (C2) . ae</pre>	ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATISE ELINATISE ELINATISE ELINATISE ELINATISE UDASTISE VERANTISE ELINATISE E	REPSICOGNYUNYULLADEP REPSICOGNYUNYULLADEP REPSICOGNYUNYULLADEP REPLOLOGNYUNYULLADEP REPLOLOGNYUNYULADEP REPLOLOGNYUNYULADEP REPNDUNYUNYUNYUNYUNYU REPNDUNYUNYUNYUNYU REPNDUNYUNYUNYUNYU REPLOLOGNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU REPLOIMAYYUNYU RANDUNYUNYU RANDUNYUNYU REPLOIMAYYUNYU REPLOI	MONITLANGEN I TOSS MONTLANGEN I TOSS LLINTLANGEN I TOSS LLINTLANGEN I TOSS MONTLANGEN I TOSS MONTLANGEN I TOSS PEPGLI MONTS PEPGLI MONTS PEPGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS AFFGLI MONTS PEPGLI MONTS PEPGLI MONTS PEPGLI MONTS PEPGLI MONTS PEFGLI MONT	PEQAGET FRUET POLYMETTROFI POLYMETTROFI PEQAGETTROFI PEDAGETTROFI PEDAGETTROFI PEDAGETROFI PEDAGETROFI INTLOOPTONIC
<pre>violacea , violacea , pfennigii , shaposhnikovii , shaposhnikovii , shaposhnikovii , negaterium tcilius sp acidovcans . acidovc</pre>	VIOLUM VI	INT PUP OF TEGNILESAM- INT PUP OF TEGNILESAM- INT PUP OF TEGNILESAM- INT PUP OF TEGNILESAM- INT PUP OF TEGNILESAM- MISPPOSETGLINGELDE- INTS PUP SETGLINGELDE- INTS PUP SETGLINGELDE- INT PUP SETGLINGENDE- SETTLID FOR SETVERDAME FETTLID FOR SETVERD	VUINDI DLAVDING	RG 205 2 7 RG 205 7 T RG 205 8 7 RG 205 8 7 RG 205 8 7 RG 212 C: 7 RG 227 35 7 RG 227 10 8 NK0 425 D 8 RG 422 8 8 RG 422 8 8 RG 5370 2 8 RG 5367 8 8 RG 5359 7 8 RG 355 7 8 RG 355 7 8 RG 355 7 8 RG 359 7 8	<pre>violacea .pfennigii .shaposhnikovii ymechocystis.gp. renarchaeota .megatorium aciilus sp. ladius sp. ladius sp. .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .acidovorans .calicopa .calicopa .calicopa .caposhis.(Cl) .putida (Cl) .putida (Cl)</pre>	ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTILS: ELINGTIS: ELINGTIS: ELINGTIS: VERNITS: ELINGTIS:	REPSICOGNYUNYULLADEP REPSICOGNYUNYULLADEP REPSICOGNYUNYULLADEP REPLOLOGNYUNYULLADEP REPLOLOGNYUNYULADEP REPLOLOGNYUNYULADEP REPNDUNYUNYUNYUNYUNYU REPNDUNYUNYUNYUNYUNYU REPNDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RANDUNYUNYU RAND	MANIFLANDEN I TOSS MANNELANDEN I TOSS LLANFLINDEN I TOSS LLANFLINDEN I TOSS LLANFLINDEN I TOSS MANNELANDEN PEPOLI AND AND PEPOLI AND AND PEPOLI AND	PEQAGET FPLOFT POLYMETTROFT POLYMETTROFT PEQAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT PEDAGETTROFT INTLOOPYTANTLE INTLOOPYTANTLE INTLOOPYTANTLE INTLOOPYTANTLE INTLOOPYTANTLE INTLOOPYTANTLE INTLOOPYTANTLE PEDAGETTRO INTLOOPYTANTLE INTLOOPYTANTSIL
<pre>violacea pfennigii shaposhnikovii nechocystis ap. renarchaeota . megaterium tcillus sp. icaligenes sp. acidovorans latus urkholderia sp. eutropha ramigera sp. 61-3 (PhC) violaceum cepacia ruber chlorcraphis (Cl) caryophylli (Cl) putida (Cl) oleovorans (Cl) estinovarias (Cl) estinovarias (Cl) putida (Cl) resinovarias (Cl) putida (Cl) interveducens (C2) putida (Cl) resinovarias (C2) ribroreducens (C2) putida (Cl) resinovarias (C2) rubrigertinctus chlorarepics (C2) putida (Cl) resinovarias (C2) rubrigertinctus chlorarepics parahaemolyticus parahaemolyticus edilicii tumefaciens caulindams</pre>	VVRLVY VVRLVX VVRVX VVRLVX VVRLVX VVRLVX VVRLVX VVRLVX VVRLVX VVRLVX VVRLVX VVR	INT PUP OF TORMLEAM INT PUP OF TORMLEAM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INT PUP STOCKNEY LINM INT LUP STOCKNEY LINM INT LU	VUNIVDIDAVDYBS	RG 205 2 7 RG 205 7 T RG 205 8 7 RG 205 8 7 RG 205 8 7 RG 212 C: 7 RG 227 35 7 RG 227 35 7 RG 425 D A NK0 425 D A RG 422 B 8 RG 5370 2 7 RG 5367 R 7 RG 5359 P 8 RG 355 C 7 RG 355 P 8 RG 355 P 8 RG 359 P 9 RG 359 P 8 RG 359 P 9 RG 359 P 9 RG 350 P 9 RG 350 P 9 RG 350 P 9 RG 350 P 9	<pre>violacea , violacea , pfennigii , shaposhnikovii ymechocysti sp. renarchaeota . megaterium aciilus sp. iacidovorans . acidovorans . acidiacid . acidovorans . acidiacid . acidiac</pre>	ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTISE ELINGTISE ELINGTISE ELINGTISE ELINGTISE VERATISE VERATISE VERATISE ELINGTISE ELI	REPSICO(NY)NNYLLODED REPSICO(NYLNNYLLODED REPSICO(NYLNNYLLODED REPSICO(NYLNYNL) REPSICO(NYLNYNL) REPSICO(NYLN) REPSICO(NYLN) REPSICO(NYLN) REPSICO(NYLN) REPSICO(NYLNYL	NINITLABERGIT DIS NINITLABERGIT DIS LLONTLABERGIT DIS LLONTLABERGIT DIS LLONTLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT PERCENTRA PERCENTRA APPOLLY NISSIS APPOLLY NISSIS APPO	POAGET FROM POAGET FROM POAGET FROM POAGET FROM PERSONAL AND AND AND PERSONAL AND
<pre>violacea , violacea , pfennigii , shaposhnikovii , shaposhnikovii , shaposhnikovii , negaterium tcilius sp acidovcans . acidovc</pre>	VIOLUM VI	INT PUP IFY FORLLAAM INT PUP IFY IFY INT INT IFY IFY IFY IFY IFY INT IFY IFY IFY IFY IFY IFY INT IFY IFY IFY IFY IFY IFY INT IFY	VUNNDIDLAVETRES HI VUNNDIDLAVETRES HI VUNNDILAVETRES HI VUNNDIDLAVETRES HI VERNDIDLAVETRES HI HARDEDIRVLAVIES HARDEDIR HARDEDIRVLAVIES HARDEDIR HARDEDIR HARDEDIRVLAVIES HARDEDIR HARDEDIRVLAVIES HARDEDIR HARDEDIR HARDEDIRVLAVIES HARDEDIR HARDEDIRVLAVIES HARDEDIR	RC 205 T RC 212 S RC 212 S RC 227 S RC 425 D RC 425 D RC 422 H RC 422 R RC 537 T RC 6360 P RC 366 P RC 366 P RC 366 P RC 367 T RC 367 T RC 355 B RC 355 P RC 355 P <td><pre>violacea , violacea , pfennigii , shaposhnikovii ynechocysti sp. renarchaeota , megaterium aciilus sp. latus urkholderia sp. , acidovorans , acidinodans , acidinodan , acidinodan , acidinodan , acidinodan ,</pre></td> <td>ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATISE ELINATISE ELINATISE ELINATISE UDASTISE VERANTISE VERANTISE ELINATISE</td> <td>RE PSILOGIATYUNTYULURE REPSILOGIATYUNTYULURE REPSILOGIATYUNTYULURE REPLOLOGIATYUNTYULURE REPLOLOGIATYUNTYUNTYULURE REPINTYUREYYUNTYUNTYU REPINTYUREYYUNTYUNTYU REPINTYUREYYUNTYU REPINTYUREYYUNTYU REPINTYUREYYUNTYU REPUNTYUNTYUNTYU REPUNTYUNTYUNTYU RANDUNTYUNTYUNTYU RANDUNTYUNTYU REPUNTYU REPUNTYUNTYU REPUNTYU R</td> <td>MONITLANGEN I FOS MONITLANGEN I FOS LLINTI-LINGEN I FOS LLINTI-LINGEN I FOS MONITALES I FOS PERSIANA I FOS PERS</td> <td>POJAGET FROM T POJAGET FROM T POJAGET FROM T POJAGET STOPPIN PEDAGET FROM T PEDAGET STOPPIN PEDAGET STOPPIN PEDAGET STOPPIN PEDEAGET STOPPIN PEDAGET STOPPIN</td>	<pre>violacea , violacea , pfennigii , shaposhnikovii ynechocysti sp. renarchaeota , megaterium aciilus sp. latus urkholderia sp. , acidovorans , acidinodans , acidinodan , acidinodan , acidinodan , acidinodan ,</pre>	ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATILSE ELINATISE ELINATISE ELINATISE ELINATISE UDASTISE VERANTISE VERANTISE ELINATISE	RE PSILOGIATYUNTYULURE REPSILOGIATYUNTYULURE REPSILOGIATYUNTYULURE REPLOLOGIATYUNTYULURE REPLOLOGIATYUNTYUNTYULURE REPINTYUREYYUNTYUNTYU REPINTYUREYYUNTYUNTYU REPINTYUREYYUNTYU REPINTYUREYYUNTYU REPINTYUREYYUNTYU REPUNTYUNTYUNTYU REPUNTYUNTYUNTYU RANDUNTYUNTYUNTYU RANDUNTYUNTYU REPUNTYU REPUNTYUNTYU REPUNTYU R	MONITLANGEN I FOS MONITLANGEN I FOS LLINTI-LINGEN I FOS LLINTI-LINGEN I FOS MONITALES I FOS PERSIANA I FOS PERS	POJAGET FROM T POJAGET FROM T POJAGET FROM T POJAGET STOPPIN PEDAGET FROM T PEDAGET STOPPIN PEDAGET STOPPIN PEDAGET STOPPIN PEDEAGET STOPPIN PEDAGET STOPPIN
<pre>violacea , violacea , pfennigii , shaposhnikovii , shaposhnikovii , shaposhnikovii , seqaterium terlium sp,</pre>	VVRLUY VV	INT PUP OF TORMLEAM INT PUP OF TORMLEAM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INTS PUP STOCKNEY LINM INT PUP STOCKNEY LINM INT LUP STOCKNEY LINM INT LU	VUNIVDIDAVDYBS	RC 205 2 RC 205 F RC 212 C RC 227 S; PP 211 B RW 425 B RG 425 F RG 425 F RG 422 B RG 425 F RG 425 F RG 5307 F RG 535 C RG 535 F RG 535 F RG 360 P RG 355 F RG 355 F RG 355 F RG 359 P RG 350 P RG 350 P RG 350 P </td <td><pre>violacea , violacea , pfennigii , shaposhnikovii ymechocysti sp. renarchaeota . megaterium aciilus sp. iacidovorans . acidovorans . acido</pre></td> <td>ELINNTILSE ELINNTILSE ELINNTILSE ELINNTILSE ELINNTILSE ELINNTILSE ELINNTISE ELINNTISE ELINNTISE ELINNTISE ELINNTISE QUASTISE VERANTISE VERANTISE RELENTISE ELENNTISE E</td> <td>KP F5LTQG/KYVNNYLLGDPD KP F5LTQG/KYVNNYLLGDPD KP F5LTQG/KYVNNYLLGDPD KP F5LTQG/KYVNYLLGND KP F5LTQG/KYVNYLLGND KP F1TNYGFYVALVDP5ENSE KP F1TNYGFYVALVDP5ENSE KP F1TNYGFYVALVDP5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVANYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVNNYLLGNFF F8 NDLVMYVNNYLGNFF F8 NDLVMYVNNYLGNFF</td> <td>NINITLABLE AT DESIGN TO SELECTION OF A DESIGN AND A DESIG</td> <td>POCAGET FROM THE CON- CONAGET FROM THE CONAGET FROM THE CONAGET FROM THE CONAGET FROM THE CONAGET FROM THE PERSON THE CONAGET FROM THE PERSON THE CONAGET FROM THE PERSON TH</td>	<pre>violacea , violacea , pfennigii , shaposhnikovii ymechocysti sp. renarchaeota . megaterium aciilus sp. iacidovorans . acidovorans . acido</pre>	ELINNTILSE ELINNTILSE ELINNTILSE ELINNTILSE ELINNTILSE ELINNTILSE ELINNTISE ELINNTISE ELINNTISE ELINNTISE ELINNTISE QUASTISE VERANTISE VERANTISE RELENTISE ELENNTISE E	KP F5LTQG/KYVNNYLLGDPD KP F5LTQG/KYVNNYLLGDPD KP F5LTQG/KYVNNYLLGDPD KP F5LTQG/KYVNYLLGND KP F5LTQG/KYVNYLLGND KP F1TNYGFYVALVDP5ENSE KP F1TNYGFYVALVDP5ENSE KP F1TNYGFYVALVDP5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVALVDF5ENSE KP F1TNYGFYVANYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVVNYLLGNFF F8 NDLVMYVNNYLLGNFF F8 NDLVMYVNNYLGNFF F8 NDLVMYVNNYLGNFF	NINITLABLE AT DESIGN TO SELECTION OF A DESIGN AND A DESIG	POCAGET FROM THE CON- CONAGET FROM THE CONAGET FROM THE CONAGET FROM THE CONAGET FROM THE CONAGET FROM THE PERSON THE CONAGET FROM THE PERSON THE CONAGET FROM THE PERSON TH
<pre>violacea , violacea , pfennigii , shaposhnikovii prenarchaeota , shaposhnikovii renarchaeota , snegaterium relius sp, . acidovorans . aci</pre>	VVRLUT VVRLUT VVRLUT VVRLUT VVRLUT VVRLUT VVRLUT VVRLUT VVRLUT VRL	INT PUP FOR FORLLAAM- INT PUP FOR FORLLAAM- LITTLE FOR FORLLAAM- LITTLE FOR FORLLAAM- LITTLE FOR FORLLAAM- LITTLE FOR FORLLAAM- LITTLE FOR FORLLAAM- LITTLE FORLLAAM- LINE FORLLAAM- THILD FORLLAAM- FITLLOFFORLIGUE VERLAAM- LINE FORLLAAM- LINE FORLLAAM- THILD FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- THILD FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- LINE FORLLAAM- FITLLOFFORLING LINE FORLLAAM- FITLLOFFOR FORLAAM- FITLLOFFORLING LINE FORLLAAM-	-VUNIVDIDAVDYBS-HI -VUNIVDULAVDYBS-HI -VUNIVDULAVDYBS-HI -VUNIVDULAVDYSS-HI -VUNIVDULAVDYSS-HI -VUNIVDULAVDYSS-HI -NENDDIDKVLSYBS-HI -NENDDIDKVLSYBS-HI -RAVVLBRMMCH-GGIM ESVYNFREMMEN EAVYLFREMMENT CENAPORGSLI -RAVVLBRUTICENAPORGSLI -R	RG 205 2 7 RG 205 7 T RG 205 8 7 RG 205 8 7 RG 205 8 7 RG 205 8 7 RG 227 35 7 RG 227 10 8 NG 425 0 8 RG 422 8 8 RG 422 8 8 RG 5307 2 8 RG 5307 8 8 RG 535 6 6 SG 355 7 8 SG 355 7 8 SG 355 7 8 SG 355 7 8 SG 359 7 8	<pre>violacea , violacea , pfennigii , shaposhnikovii ymechocysti sp. renarchaeota . megaterium aciilus sp. i.acidovorans . acidovorans . acid</pre>	ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTILSE ELINGTISE ELINGTISE ELINGTISE ELINGTISE UDASTISE ELINGTISE	REPSICO(RY)NNYUKULOBE) REPSICO(RY)NNYUKULOBE) REPSICO(RY)NNYUKULOBE) REPSICO(RY)NNYUKUROBE) REPSICO(RY)NNYUKUROBE) REINNYUYNYUKUROBE) REINNYUNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE) REINNYUNYUKUROBE)	NINITLABERGIT DIS NINITLABERGIT DIS LLONTLABERGIT DIS LLONTLABERGIT DIS LLONTLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT PERCILIVASION PERCILIVASION APPOLLIVASION APPOLLIVASION APPOLLIVASION APPOLLIVASION APPOLLIVASION APPOLLIVASION APPOLLIVASION PERCILIV	POAGET FROM POAGET FROM POAGET FROM POAGET FROM PIAGET FROM PIAGET PIAGET FROM PIAGET PIA
 violacea pfennigii shaposhnikovii phenogati sp. ceartonaeota negaterium renarchaeota negaterium relius sp. acidovcans latus runigera eutropha ramigera sp. 61-3 (PhbC) violaceum copacia ruber chlororaphis (C1) sp. 61-3 (C1) caryophyli (C1) petdoalcalgenes (C1) spedicalalgenes (C1) spedicalalgenes (C1) spedicalalgenes (C2) putida WC1 (C2) oleovorans (C1) putida (C2) spetidoalcalgenes (C2) putida UC2) spetidoalcalgenes (C2) putida UC2) sturreri (C2) resinovans (C2) putida UC2) stureri (C2) resinovans (C2) rubrum HA rubrum HA rubrum ATCC25903 prowaski (PhbC2) 	VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT VIOLUT RELASSIS REPAID VIOLUT RELASSIS REPAID VIOLUT RELASSIS REPAID VIOLUT RELASSIS REPAID VIOLUT RELASSIS REPAID VIOLUT RELASSIS REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT REPAID VIOLUT VIOL	IMT PUP PT FORLLAAM IMT PUP STORLAAM IMT PUP STORLAAM INT PUP STORLAA	VUNNDIDLAVETRØS	RC 205 T RC 212 C RC 212 C RC 227 S PP 211 B RC 425 B RC 422 B RC 422 B RC 6 370 T RC 6 370 T RC 56 S55 RC 366 P RC 366 P RC 367 R RC 365 B RC 355 B RC 355 P	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . cepacia . ruber . violaceum . cepacia . ruber . chlocoraphis (C1) . ap. 61-3 (C1) . othororaphis (C1) . putida (C1) . othororaphis (C1) . devorans (C1) . devorans (C1) . devorans (C1) . devorans (C1) . putida (C1) . devorans (C1) . putida (C2) . othororaphis (C2) . othororaphis (C2) . putida (C2) . caryophylli (C2) . devorans (C2) . putida (C2) . devorans (C2) . putida (C2) . sp. 61-3 (C2) . devorans (C2) . putida (C2) . caryophylli (C2) . devorans (C2) . devorans (C2) . chlororaphis (C2) . devorans (C2)</pre>	ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATILES ELINATISE ELINATISE ELINATISE ELINATISE UDASTRIE ELINATISE	KP F3 LTGG(KYVNNYLLGDP) KP F3 LTGG(KYVNNYLLGDP) KP F3 LTGG(KYVNNYLLGDP) KP F3 LTGG(KYVNYLLGDP) KP F3 LTGG(KYVNYLLGDP) KP F1 TNY (F4 FYVLOP5 ENGL) KP F1 TYF (F4 FYVLOP5 ENGL) KP F1 TYV	MUNIT.REGEN 11035 MUNIT.REGEN 11035 LLINTLREGEN 11035 LLINTLREGEN 11035 MUNIT.REGEN 11035 MUNIT.REGEN 11035 PEPDLI 11035 PEPDLI 11035 PEPDLI 11035 PEPDLI 11035 MUNIT.REGEN 11	POAGET FROPT IN POAGET FROPT IN POAGET FROPT IN POAGET TROPIN PT JASET TROWN PT J
<pre>violacea , violacea , pfennigii , shaposhnikovii , shaposhnikovii , shaposhnikovii , shaposhnikovii , seqaterium terlilus sp, . acidovorans . (Cl) sp. acidovorans . (Cl) setuficacili, (Cl) putida (Cl) resinovorans . (Cl) . resinovorans</pre>	VVRLUT VV	INT PUP FOR FORLLAAM INT PUP FORLIAM INT PUP FOR FOR FORLIAM INT PUP FOR FOR FORLIAM INT PUP FOR FOR FORLIAM INT PUP FOR FORLIAM INT PUP FOR FORLIAM INT PUP FOR FORLIAM INT PUP FOR FORLIAM INT P	-VUNIVDIDAVDYBS-HI -VUNIVDULAVDYBS-HI -VUNIVDULAVDYBS-HI -VUNIVDULAVDYSS-HI -VUNIVDULAVDYSS-HI -VUNIVDULAVDYSS-HI -NENDDIDKVLSYBS-HI -ARHODIDKVLSYBS-HI -RAVVLRBNDYTG-HI -RAVVLRBNDKRVDTFO-HI -ESVVHFREMMCH-GGIM -ESVILESAMMRSSG -GGUM -ESVILESAMMRSSG -GGUM -ESVHFREMMCH-GGIM -ESVHFREMMCH-GGIM -ESVHFREMMCH-GGIM -ESVHFREMMCH-GGIM -ESVHFREMMCH-GGIM -ESVHFREMMCH-GGIM -ESV	RC 205 2 7 RC 205 7 T RC 205 8 7 RC 205 8 7 RC 205 8 7 RC 205 8 7 RC 227 35 7 RC 425 0 8 RC 422 8 7 RC 425 0 8 RC 422 8 7 RC 5370 2 7 RC 5359 7 8 RC 5359 7 8 RC 355 8 5 RC 355 8 5 RC 355 7 8 RC 355 8 7 RC 355 8 7 RC 355 7 8 RC 355 8 7 RC 355 8 7 RC 355 7 8 RC 355 7 8 RC 355 7 8	<pre>violacea , violacea , pfennigii , shaposhnikovii ynechocysti sp. renarchaeota . megaterium aciilus sp. i.acidovorans . acidovorans . acid</pre>	ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS OLASTIFICS ELINTIFICS	REPSICO(RY)NNYUKULDEPI REPSICO(RY)NNYUKULDEPI REPSICO(RY)NNYUKULDEPI REPSICO(RY)NNYUKUPPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPIDESI REPSICO(RY)NNYUKUPPIDESI REPSICO(RY)NYUKUPPIDESI REPSICO(RY)	NINITLABERGITED	PEDAGET FROM PEDAGET FROM PEDAGET PEDAGET FROM PEDAGET PEDAGET FROM PEDAGET P
. stutseri (Cl) . stutseri (Cl) . seruginosa (Cl) . seruginosa (Cl) . oleovorans (Cl) . oleovorans (Cl) . oleovorans (Cl) . putida (Cl) . putida (Cl) . putida (Cl) . stutieri (Cl)	VVRILY VV	INT PUP FOR FORLLAAM INT PUP FOR FORLAAM INT PUP FOR FORLAAM	VUNNDIDLAVETRØS	RC 205 2 7 RC 205 7 T RC 205 8 7 RC 205 8 7 RC 205 8 7 RC 205 8 7 RC 227 35 7 RC 425 0 8 RC 422 8 8 RC 422 8 8 RC 422 8 8 RC 422 8 8 RC 537 7 7 RC 535 8 6 SC 355 8 6 SC 355 8 5 SC 355 8 5 SC 359 7 8 SC 359 7 8 SC 359 7 8 SC 359 7 7 SC 359 7 7 SC 359 7 8 SC 359 7 7 SC 359 7 7 <	<pre>violacea . violacea . pfennigii . shaposhnikovii ymechocysti sp. renarchaeota . megaterium actilus sp. latus urkholderia sp. . acidovorans . cepacia . ruber . violaceum . cepacia . ruber . chlocoraphis (C1) . ap. 61-3 (C1) . othororaphis (C1) . putida (C1) . othororaphis (C1) . devorans (C1) . devorans (C1) . devorans (C1) . devorans (C1) . putida (C1) . devorans (C1) . putida (C2) . othororaphis (C2) . othororaphis (C2) . putida (C2) . caryophylli (C2) . devorans (C2) . putida (C2) . devorans (C2) . putida (C2) . sp. 61-3 (C2) . devorans (C2) . putida (C2) . caryophylli (C2) . devorans (C2) . devorans (C2) . chlororaphis (C2) . devorans (C2)</pre>	ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS ELINTIFICS COLASTIFIC VERATIFIC ELINTIFICS E	KP F3 LTGG(KYVNNYLLGDP) KP F3 LTGG(KYVNNYLLGDP) KP F3 LTGG(KYVNNYLLGDP) KP F3 LTGG(KYVNYLLGDP) KP F3 LTGG(KYVNYLLGDP) KP F1 TNY (F4 FYVN) KP F1 TYVN) KP F1 TYVNNY KP F1 TYVN) KP F1 TYVN) KP F1 TYVN) KP F1 TYVN) KP F1 TYVNY KP F1	NINITLABERGIT DIS NINITLABERGIT DIS LLINTLABERGIT DIS LLINTLABERGIT DIS LLINTLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT DIS NINITLABERGIT PFPOLLY NISSI APPOLLY NISSI	PEQAGET FROM POLYANT AND

Figure 2 For legend see facing page

A. vinosum	FYQNNGFLN-GGVVLGGQEVDLKDITCPVLNIFALQ			A. vinosum		IGIYVSGKAQKEVTPAI	
T. violacea	FYQENGFIN-GGVELGGEEIDLENVDCPVLNIYALC			T. violacea			
T. pfennigii	FYGRNGFIN-GOVLIGDQEVDLRNIRCPVLNIYPMC			T. přennigii		IGIYVSGKAQEGVTPAI	
E. shaposhnikovii	FYQNNKLMN-GGLQIGEYEV5LANVTHFVLNIFAEC			E. shaposhnikovii		IGIYV3GKAQKIVPPAJ	
Synechocystis sp.	FYQQNKLIK-GEVMIGDRLVDLHNLTMPILNLYAER			Synechocystis sp.		IGNYVSGKVQRDLPPA1	
Crenarchaeota	IYQQNLFAK-N9MIVGENKINLSHIKVPVLNVVAEI			Crenarchaeota		VGLIASNFSQNNVLPKI	
B. megaterium	FYQQNKLIN-GELEVRGRHVDLKNIKANILNIAASS			B. megaterium		VSVVFGPKAVKETYPS1	
Bacillun sp.	FYONNELVK-GELVIRGOKVDLANIKANVLNISGKP			Bacillus sp.		MSIVYGGTAVKQTYPTI	
Alcaligenes sp.	FYLENNLVKPGKLTVCGEKLDLGNLOLPVYTYGSRE			Alcaligenes sp.		IAGVINPP-AKGKRSHWTRADGKPPGTLDQWLEGATEHPGSWWTDV	
D. acidovorans	LYLENRLAQPGALTVCGERIDMHQLRLPAYIYGSRE			D. acidovorans		LAGVINPP-AKKKRSYWLREDGQLPATLKEWQAGADEYPGSWWADV	
A. latus	TYLENKLRVPGALVICGERVDLSRIEAPVYFYGSRE			A. latus		IAGVINPP-QKXKRSYWINEQLDGDFNQWLEGSTEHPGSWWTDV	
Burkholderia sp.	TYLENRLREPGALTVCGEAVDLSRIDVPTFIYGSRE			Burkholderia sp.		LAGVINPP-AKKKRSFWVN-DNDLPDAADDWFAGAAEQPGSWPTV	
R. eutropha	TYLQNELKVPGKLTVCGVPVDLASIDVPTYIYGSRE			R. eutropha		IAGVINPP-AKNKRSHWIN-DALPESPQQWLAGAIEHHGSWWPDV	
2. ramigera	TYLENSLKVPGKLTVAGEKIDLGLIDAPAFIYGSRU			 ramigera 		IAGVINSV-AKNKRTYWINDGGAADAQAWFDGAQEVPGSWWPQV	
P. sp. 61-3 (PhbC)	TYLQNDLK-SGELECCONKLDLRAIDAFAYILATHD			P. sp. 61-3 (PhbC)		LAGVINPP-AKQKRHYWTNNRVTKNPETWFKNAEQHPGSWWNDV	
C. violaceum	FYMNNALVRPGAITLCGVPIDIAKIDVPVYMFAARD			C. violaceum		IAGSINPV-TKDKENYWANDTLPLHAEEWLESAESRPGSWWKDV	
B. cepacia	QLFLNNDLASSRYQVNGRPVSIHNIRVPMFVVGTEF			B. cepacia		NAGIVSEP-GHPHRQFRIRETTADDLRVSPDEWTAAATQQEGSWWPVV	
R. ruber	SLYGRNELAEGLYVLDGQPLNLHDIACDTYVVGAIN			R. ruber		VAGAVNPP-GKRVWFKAVGAPDAESGTPLPADPQVWDEAATRYEHSWWEDV	
P. chlororaphis (C1)	MFRNSPLIRPNALEVCGTPIDLRQVTADIFSLAGTN			P. chlororaphis (C1)		IQSILNPP-GNPKSRYMTSEEMPPSADDWQENSTHHTDSWWLHV	
P. sp. 61-3 (C1)	MFKNNPLVBANALEVSGTPIDLKQVTADIYSLAGTN			P. sp. 61-3 (C1)		IQSILNPP-GNPKSRYNTSTDMPATANEWQENSTKHTDSWWLHV	
B. caryophylli (C1)	NFWNNPLTRADALEVCGTPIDLKKVTSDIYNVAGTA			B. caryophylli (C1)		IQ\$ILNPP-GNPKARYLTGGELLSQASEWQENAIKHPDSWWLHV	
P. putida (Cl)	MFKSNPLIRPDALEVCGTPINLKNVQCDIFSVAGTA			P. putida (C1)		IQSILNPP-GNPKARFMTGADRPGDPLAWQENATHHADSWWLHW	
P. oleovorans (Cl)	NFKSNPLTRPDALEVCGTPIDLRQVKCDIYSLAGTS			P. oleovorans (Cl) P. mendocina (Cl)		IQSILNPP-GNPKARFMTGADRFGDPVAWQENATHHADSWWLHV	
P. mendocina (C1)	MFQTNPLTRPGALEVCGTPIDLKQVTCDFFVVAGT7			P. pseudoalcaligenes Cl		IQSILNPP-GNPKARYMINSEMPLDPKANQESSIKHADSWWLHV	
P. stutzeri (CI)	MFRTNPLTRPGALEVCGTPIDLKQVTCDFFVVAGTT			P. stutzeri (Cl)		IQSILNPP-GNPKARYMINSAMPLDPKANQESSINHADSWILH9 IQSILNPP-GNPKARYMINSEMPADPKANQESSINHADSWILH9	
P. resinovorans (C1)	MFGTNPLTRPGALEVCGTPIDLKQVTCDFFCVAGT MFKTNALTRPNALEVCGTPIDLKQVTSDFFCLAGT			P. resinovorans (C1)		LOSILAPP-GAPKARFSTGSEMPKDPKAWLENATKHADSWWLH	
P. aeruginosa (CI)	LFKSNPLNRPGALEVSGTFIDLKQVTCDFYCVAGLA			P. aeruginosa (Cl)		IQSILNPP-GNPKARFMINPELPAEPKOWLEGAGICHADSWILH	
P. putida BM01 (C2)	FFKLNPLTHPAGLEVCGTPIDLOKVDLDSFTVAGSN	HITPWDAVYRSALLLGGDR		P. putida BM01 (C2)		IQSIINPP-GNPKAYYLANPKLSSDPRAWFHDAKRSEGSWWPLA	
P. oleovorans (C2)	FFKLNPLTHFAGLEVCGTFIDLQKVELDSFTVAGS			P. oleovorans (C2)		IQSIINPP-GNPKAYYLANPKLSSDPRAWLHDAKRSEGSWNPL	
P. putida (C2)	FFKHNPLTHPAGLEVCGTFIDLKQVDLDSFTVAGSN	HITFWDAVYRSALLLGGDR		P. putida (C2)		IQ511NPP-GNPKAYYLENPKLSGDPPAWEYEAKPSDGSWWPLW	
B. caryophylli (C2)	FYEHNPLAHAAGLEVCGTPIDLOKVTLDSFTVAGDN	HITPWDAVYRSTLLLGGER		B. caryophylli (C2)		IQSIINPP-GNPKANFMENPKLSSDPRANFYDAKQVDGSWNPT	
P. nitroreducens (C2)	FFKHNPLSRNOGLEVCGTPVDLTKVNVDSFSVAGIN			P. nitroreducens (C2)		IQSILNPP-GNPKANYYENTKLTSDPRAWYHDATHQQGSWWPOW	
	FFIOINPLSRNGGLEVCGTPVDLTKVNVDSFSVAGIN					IQSILNPP-GNPKANYYENTKLTSDPRAWYHDATHOOGSWWPO	
P. mendocina (C2)	FFKHNPLSRAGGLEVCGTPVDLAKVNVDTFSVAGIN			P. mendocina (C2)		IQSILNPP-GNPKANYYENTKLTSDPRAWYHDACHQQGSWWPOW	
P. stutseri (C2)	FFKHNPLSRAGGLEVCGTPVDLSKVAVDSF5VAGIN	HITPWDAVYRSALLLGGER		P. stutzeri (C2)		IQSILNPP-GNPKANYYENGKLTSDPRAWYEDARHVQGSWWPQ	
P. resinovorans (C2)	FFRHNPLTRSGGLEICGTPIDLQKVTVDSFSVAGIN			P. resinovorans (C2)		IQSILNPP-GNPKANFYENGKLSSDPRAWYYDAXHVQGSWWQQV	
P. chlororaphis (C2)	FFKHNPMSHPGGLEVCGTPIDLQKVTVDSFSVAGIN			P. chlororaphis (C2)		VQSILNPP-SNPKANYVENGKLSSDPRAWYYDAMHVDGSWWTQ9	
P. sp. 61-3 (C2)	FFEHNPLTHPOGLEVCGTPIDLOKVNVDSFSVAGIN			P. sp. 61-3 (C2)		IQSILNPP-SNPKSNYIENPKLSGDPRAWYYDGTHVEGSWWPRW	
P. aeruginosa (C2)	LFKHNPLTRPGALEVSGTAVDLGKVAIDSFHVAGIT			P. aeruginosa (C2)		IQSILNPP-GNPKACYPENDKLSSDPRAWYYDAXREEGSWWPVV	
G. rubripertinctus	IFRONVLVEPGRLAVLGTPVDLKSITVPTFVSGA1A			G. rubripertinctus		LASLVNPP-GNPKAHYWTGGTPGPDPDAWLENAERQQGSWWQAM	
V. cholerae	LYLENKLVODKGVKVGGVWIDLDKIKVPSYFISTKE			V. cholerae		IAGIVNHP-DKRKYGYWVNDTLDDSAEDWLETACHREGSWWVHW	
V. parahaemolyticus	LYLENKLVODRGVKIGGVWIDLNKIK1PSYFVSTKR			V. parahaemolyticus		LAGIVNHP-AKNKYGYWLNDNLDDSADEWFNNANHQEGSWWTHV	
Acinetobacter sp.	LYLNNELISPNAVKVNGVGLNLSRVKTPSFFIATOR	HIALWDTCFRGADYLGGES		Acinetobacter sp.		VAGIVNPP-SRNKYGCYTNAAKFENTKOWLDGAEYHPESWWLR9	
A, punctata	LYLENQLVKG-ELKIENTRIDLGKVKTPVLLVSAVI			A. punctata		LAGIINPP-AANKYGFWHNGAEAESPESWLAGATHQGGSWWPEN	
A. hydrophila	LYLENGLVKG-ELKIRNTRTDLGKVKTPVLLVSAVI			A. hydrophila		LAGIINPP-VANKYGFWHNGABADSPESWLAGATHQSGSWWPEN	
R. etli	CYLENALTO-NENTLDGKRISLEDVEIPIYNLATRI	HIAPAKSVFLGSRFFGGKV	547	R. etli		IAGVVNPP-DKRKYQFWTGGPAKGEYETWLEQASETPGSWWPHW	
S. meliloti	CYLENRLSK-GEMVLAGRRVSLGDVKIPIYNLATKE	HIAPAKSVFLGSSSFGGRV		S. meliloti		IAGVVNPP-ARSKYQYWTGGAPKGDIETWMGKAKETAGSWWPHW	
A. tumefaciens	CYLENNLAR-GLMRVAGKRINLGDITIPVYDLATRI	HIAPAKSVFTGAALFG+GTV	550	A. tumefaciens	EFVLGASG	LAGVINPP-QLEKYQYWTGPSPSGDFEAWQAAATAHKGSWMMM	4 602
A. caulinodans	CYLONNIAK-GLARIAGVKIDMGKVTIPVYSLATRE	HIAPPESAYIGAGLLGGPV	494	A. caulinodans	REVLAGE	LAGVVNPP-VKHKYQYWTGGPTGGDYDVWLKGAQEHKGSWWPDW	1 546
M. extorquens	CYLNWTLAK-GOMVLGNVRLDLEKVKVPVFNLATRS			M. extorquens	DYVLAGSG	IAGVVAPPGPKAKYGFRTGGPARGRFEDWVAAATEHPGSWWPYW	1 568
C. crescentus	FYEDNALTT-GKLSLGGERLDLSKVKIPIYVQSSKI	HIAPYRSVYRGARAFGGPV		C. crescentus	TETHAGSG	IAGVINHP-DARKYCHWINSELPADVSEWIAGANEHPGSWWPHW	# 633
A. rubrum HA	MYRENVLKDPGGVTLLGVPIDLRRNRTPSYFVSARE		509	R. rubrum HA		IAGVVNPP-SANKYCFWINIKKAKDAESWLEKASQTDGSWWIDW	
R. rubrum ATCC25903	MYQKNKLVQPGGLTVLGHALDLRRIRTPVYLLSARI	HIAPWTSTFKATGLYGGPL		R. rubrum ATCC25903		LAGVINPP-AKARYGYWINADTSLEAESWLEGATPHGGSWWPD9	
R. prowazekii (PhbC2)	TYCNNLLKESNALEVLGTKIDLGNVDCNSFFLAAKE	HITTWRSIYDGVKLLNGRK		R. prowazekii (PhbC2)		VAGVVNHP-DNAKYNYRLNYDLSLSSNEWFMQATEYKGSWWNYW	
P. denitrificans	LCOOMRFVK-EGPDLMGHRLHVGDVTVPLCAIACET	HIAPWKDSWRGIAQMGSRDK		P. denitrificans		LAGIVNPP-SKXKYGHYTSDAGPGQGEQNWLDKASHHEGSWWGR9	
R. capsulatus	LCQADAFTT-EGFELMGERLHVSGVKVFLCAIACET			R. capsulatus		LAGIVNPP-SKDKYGHYTSAAPIAD-HQVWXAQATYTKGSWWPRM	
R. sphaeroides	LCQQDRLAG-GTPPVLGSPVGLKDVTLPVCAIACET			R. sphaeroides		VAGIVNPP-SRNKYGHYTNEGPLDT-PAAFREGAEFHAGSWWPRW	
R. prowazekii (PhbCl)	ILYENMFIN-LEWKINNFIIDPSLIDCSVYIVSAEN	QIVFKSSILTLOKLLONS	312	R. prowazekii (PhbCl)	KLIEVKOG	ISYLINDKI	. 330

Figure 2 Multiple alignment of 59 polyester synthases

Only regions containing conserved residues are presented. Amino acid residues highlighted in yellow were found to be conserved among all synthases. The conserved tryptophan residue has been considered to be involved in protein—protein interaction. Conserved residues involved in catalysis are highlighted with a blue background. The conserved histidine located directly after the catalytic aspartate was found to by the major base catalyst in class II synthases. However, this histidine is not present in *Rickettsia prowazekii* PhbC1. The red bar indicates the position of the putative lipase box. Full species names are provided in Figure 4.

Paracoccus denitrificans, possess other genes adjacent to the PHA synthase like *phaP* (encoding phasin) and *phaR* (encoding regulator protein) related to PHA biosynthesis. Among the β -proteobacteria PHA-accumulating bacteria, such as *R. eutropha*, *Burkholderia sp., Alcaligenes latus* and *Delftia acidovorans* [33,44–46], an operonic organization exists of PHA biosynthesis genes, which are related to the short-chain-length PHA biosynthesis (class I PHA synthase gene).

All pseudomonads, which accumulate medium-chain-length PHAs resembling elastomers, possess two different *phaC* genes encoding class II synthases which are separated by the structural gene *phaZ* encoding a intracellular PHA depolymerase. In addition, downstream of the synthase gene arrangement, the *phaD* gene (encoding a structural protein with unknown function) is collinearily located, followed by the genes *phaI* and *phaF*, which are transcribed in the opposite direction (Figure 5). The latter genes encode structural and regulatory proteins.

In all bacteria possessing a class III PHA synthase, *phaC* and *phaE* are directly linked in their genomes and most probably constitute a single operon. In *A. vinosum, phaA* and *phaB* are located on the opposite strand in a gene cluster related to PHA metabolism. The organization of the genes is most probably similar, if not identical, in *Thiocystis violacea* and *T. pfennigii*, whereas in *Synechocystis* sp. PCC 6803, further *pha* genes definitely do not map close to the *phaEC* locus (Figure 5). The class IV synthase genes are found in bacteria belonging to

the genus *Bacillus* and comprise *phaR* and *phaC*, which are separated by *phaB* [27,36] (Figure 5).

STRUCTURAL FEATURES OF POLYESTER SYNTHASES

Unfortunately the tertiary structure of PHA synthases has not yet been resolved by X-ray diffraction analysis. Evidence for secondary structure composition has been obtained by predictions considering the multiple alignment of synthases. Accordingly, PHA synthases are mainly composed of variable-loop (49.7%) and α -helical (39.9%) secondary structures, whereas only 10.4% are predicted to be β -sheet secondary structures [47]. Experimental evidence that the synthase from *P. aeruginosa* shows the following secondary structure composition was obtained by CD spectroscopy: 10% α -helix, 50% β -sheet and 40% random coil [48]. Thus, PHA synthases correspond to the mixed class of proteins with respect to secondary structure prediction.

In vitro PHA synthases exist as an equilibrium of monomeric and dimeric forms, whereas dimerization is significantly induced in the presence of substrate or trimeric CoA analogues (3hydroxybutyryl)₃-CoA, respectively [48,49]. In addition, a reduction in enzymic lag phase is observed, and the specific activity increased, in the presence of trimeric analogues [35,49]. This indicates that the dimeric form is substantially more active

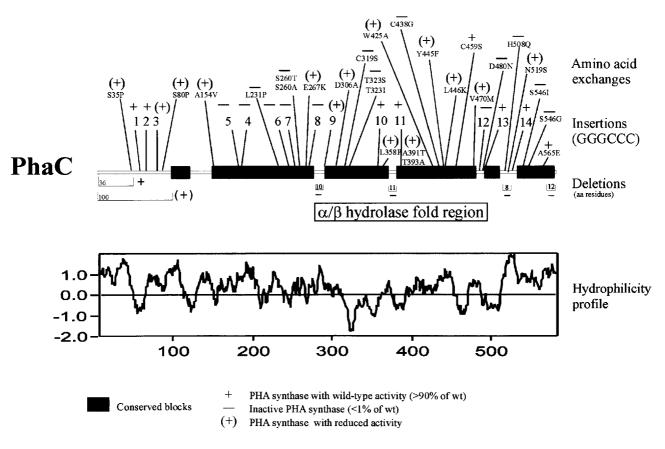


Figure 3 Primary structure analysis of the PHA synthase from R. eutropha and various site-specific mutants (modified according to [3])

The insertion of *Smal* restriction sites was performed by Kalousek et al. [61]. The site-specific deletions were achieved by Rehm et al. [4]. The following site-specific mutations were done: C319S, C459S [62]; S260A, S260T, S546I [63]; E267K, T323S, T323I, C438G, Y445F, L446K [4]; W425A, D480N, H508Q [64]. The PCR-mediated random mutagenesis was performed by Taguchi et al. [65] resulting in: S35P, S80P, A154V, L231P, D306A, L358P, A391T/T393A, V470M, N519S, S546G, A565E. The hydrophobicity was calculated using an amino acid window size of 17 according to Kyte and Doolittle [34].

than the monomeric form in the absence of the putative primer. Since radiolabelled trimeric CoA analogues were found to be covalently bound to the PHA synthase of *R. eutropha*, the radiolabel must only reside in the dimeric form as indicated by size-exclusion chromatography [49].

Gold-labelled anti-PHA antibodies were used for immunoelectron microscopy studies of granules isolated from *A. vinosum*, which clearly indicated the presence of PHA-synthase complexes at the surface of the PHA granule [50,51]. This homogeneous population of particles measuring 11.2–12.8 nm in diameter, and data derived from gel filtration chromatography, indicated that this synthase might be composed of ten subunits [50,51]. These results suggest that the active synthase consists of two subunits (*in vitro*) and that the PHA synthase associated with the PHAgranule surface might be composed of ten subunits (*in vivo*) in *A. vinosum*. Size-exclusion chromatography indicates that the PHAgranule-associated PHA synthase of *A. vinosum* might form a dodecamer and, considering that the PHA synthase is composed of the two subunits PhaC and PhaE, the PHA synthase might form a hexameric protein complex [52].

THREADING OF POLYESTER SYNTHASES, TOPOLOGICAL MODELS

The multiple alignment of the primary structures of PHA synthases showed the presence of six conserved blocks and eight conserved amino acid residues [1]. Moreover, all PHA synthases

seem to contain a lipase box (GX[S/C]XG) in which the essential active site serine of lipase is replaced with a cysteine in the PHA synthase (Figure 2). The conserved-domain-homology search strongly suggested that PHA synthases contain the α/β -hydrolase domain at the C-terminal region (Figure 3).

A BLAST sequence-homology search with the class III A. vinosum PHA synthase (PhaC) showed identity with lipases, particularly to the lipase from Burkholderia cepacia, and the putative active site Cys-149 aligned with the active site serine of the lipase [52]. A ClustalW alignment of three lipases and the three class III PHA synthases from A. vinosum, Thiocystis violacea and Synechocystis sp. PCC6803 showed an overall significant homology implementing an alignment of the active site nucleophile Ser-87 within the lipase box of the lipase with the modified lipase box of the PHA synthase where the key serine is replaced by a cysteine (Cys-149 from A. vinosum PHA synthase). This ClustalW alignment provided an approx. 19% identity of the A. vinosum PHA synthase with the B. cepacia lipase using the insertion of several gaps. Since the protein structure of the B. cepacia lipase has been crystallographically resolved, the multiple alignment was used as input for the SWISS-MODEL protein threading algorithm [53]. An excellent structural model was obtained between residues 131-175 comprising the lipase box and the α/β -hydrolase domain [52] (Figure 6). The application of further threading algorithms such as SAM-T98 [54], 3D-PSSM [55] and the UCLA Foldserver [56], and using the entire PHA synthase sequence, resulted in a comparable structural model which

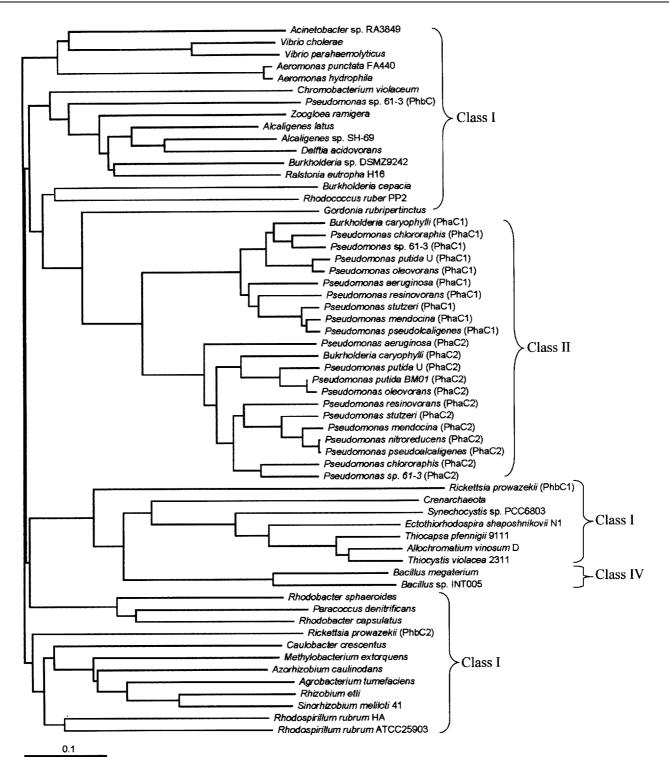
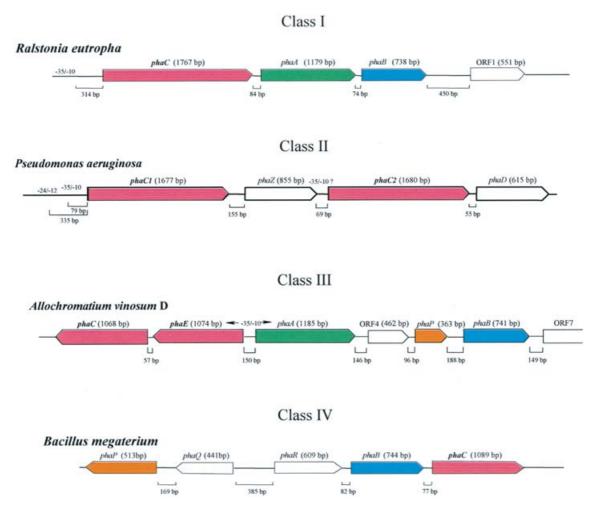


Figure 4 Phylogenetic tree of 59 PHA synthases

The branching order and distance scores were calculated by the program TREE as described by Feng and Doolittle [156]. The bar indicates the distance corresponding to 1 amino acid change per 10 amino acid positions.

revealed that the conserved residues His-331, Asp-302 and His-303 are adjacent to the core structure [52]. Interestingly the residue Cys-130, which has been previously identified as playing an important role in covalent catalysis [35], is not adjacent to the core structure and is, therefore, no longer considered to function in covalent catalysis. However, Cys-149 resides at the conserved nucleophile elbow and replacement of this residue strongly impairs enzyme activity [52].

A similar approach was conducted to build a structural model of the class II PHA synthase, PhaC1, from *P. aeruginosa*, which also showed significant identity with enzymes related to the superfamily of α/β -hydrolases [5]. The conserved-domain-homology





PhaC/C1/C2, gene encoding PHA synthase; *phaE*, gene encoding subunit of PHA synthase; *phaA*, gene encoding β-ketothiolase; *phaB*, gene encoding acetoacetyl-CoA reductase; *phaR*, gene encoding regulator protein; ORF, open reading frame with unknown function; *phaZ*, gene encoding PHA depolymerase; *phaD*, open reading frames with unknown function.

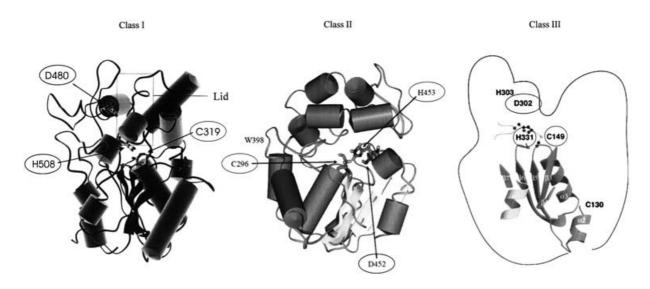


Figure 6 Threading models of class I [4], class II [5] and class III [52] PHA synthases

Catalytic triad residues (cysteine-aspartate-histidine) are circled.

search strongly suggested that PhaC1 contains the α/β -hydrolase domain. The conserved domain alignment revealed that the region of amino acid residues 249-492 exerted 30 % similarity and 17 % identity with the conserved α/β -hydrolase domain. The conserved and proposed catalytic residues of the PhaC1 aligned with amino acid residues constituting the catalytic triad in enzymes belonging to α/β -hydrolases (Figure 6). A 3D-PSSM similarity search [55] resulted in an alignment showing approx. 55% similarity of PhaC1 with 1ek1, the epoxide hydrolase from mouse belonging to the α/β -hydrolase superfamily. This alignment, in combination with the conserved domain alignment, was used to generate a threading model of PhaC1 (Figure 6; [5]). The N-terminal region (1-184 amino acid residues) and a further five regions (234-239, 302-306, 402-407, 434-443, and 455-459) were deleted in PhaC1 used for the protein model. Deletions were introduced because no identity of these regions with structurally conserved regions was found and the loop search against a loop-fold library failed using HOMOLOGY (software package; Accelrys Inc., Cambridge, U.K.). Moreover, deletions were exclusively located in highly variable regions according to the multiple alignment of PHA synthases [1,4]. A threading model of PhaC1 was finally developed using software packages HOMOLOGY (Accelrys Inc.) and DISCOVER (Accelrys Inc.) (Figure 6). Energy minimization was performed employing the consistent-valence force field (CVFF) implemented in DISCOVER. The stereochemistry of the model structure was evaluated with the program PROCHECK [57] and the residue environment was analysed with the VERIFY_3D program that implements the algorithm of Lüthy et al. [58]. The resulting model suggests that PhaC1 is a member of the protein family possessing an α/β -hydrolase fold. Additional submission of the PhaC1 sequence to three other algorithms that search structural databases (SAM-T02 [54], 3D-PSSM [59], and the UCLA Foldserver [56]) also resulted in fits to other enzymes belonging to the α/β -hydrolase-fold family with high confidence levels (results not shown). Inspection of the protein model of PhaC1 showed that the active site Cys-296, the conserved His-480 and the Asp-452, presumably forming a catalytic triad, are adjacent to the core structure (Figure 6). These residues are conserved in all PHA synthases and are proposed to be required for catalytic activity [20]. The active site Cys-296 was located at the nucleophile elbow, a sharp γ -turn containing the nucleophilic residue, positioned between a β -strand and an α -helix, which is one of the most conserved features of the α/β -hydrolase enzymes.

Recently a model was also generated for the class I PHA synthase from R. eutropha [4]. A PSI-BLAST search, in combination with a conserved-domain alignment, showed approx. 18% similarity of this synthase with the lipase from Burkholderia glumae (Figure 2). This alignment was used to generate a threading model (residues 230–547) including three deletions (286–289, 354–371, and 435-436) (Figure 6). Moreover, the alignment was further improved by matching the catalytic His-508 of this synthase with catalytic His-285 of the B. glumae lipase [60]. Deletions were introduced because no identity of these regions with structurally conserved regions was found, and the loop search against a loopfold library failed (HOMOLOGY Accelrys Inc.). However, deletions were exclusively located in highly variable regions according to the multiple alignment of PHA synthases [3]. A threading model of this class I synthase was finally developed (Figure 6) using software packages HOMOLOGY (Accelrys Inc.) and DISCOVER (Accelrys Inc.). Energy minimization was performed using the CVFF implemented in DISCOVER. The stereochemistry of the model structure was evaluated with the program PROCHECK [57] and the residue environment was analysed with the VERIFY_3D program that implements the algorithm of Lüthy

et al. [58]. The resulting model suggests that PhaC from R. *eutropha* is a member of the protein family possessing an α/β hydrolase fold comparable with prokaryotic lipases. Inspection of the protein model of R. eutropha synthase showed that the active site Cys-319, the conserved His-508 and the Asp-480, presumably forming a catalytic triad, are adjacent to the core structure (Figure 6). These residues are conserved in all PHA synthases and are required for catalytic activity [20]. The active site Cys-319 was located at the nucleophile elbow, a sharp γ -turn containing the nucleophilic residue, positioned between a β -strand and an α -helix, which is one of the most conserved features of the α/β hydrolase enzymes. This enzyme has been studied in detail and most of the mutagenesis approaches have been performed with this class I PHA synthase [4,33,61-65]. These mutations are summarized in Figure 3. These data indicated that the highly variable N-terminus (the first 100 amino acid residues), which could be mutated by insertion of Smal restriction sites as well as by a deletion of the entire first 100 N-terminal amino acid residues, without inactivation of the enzyme, is not essential for the enzymic activity of the PHA synthase. In contrast, two deletions at the C-terminus (5 and 12 amino acid residues) did abolish the PHAsynthase activity, which suggested that the C-terminus, although not present in the class III PHA synthase but rather conserved among class I and II PHA synthases, is essential for enzymic activity [4]. As indicated above, the C-terminus of synthases appears to be hydrophobic, suggesting that this region interacts with the hydrophobic core of PHA granules. Further deletions between the conserved blocks 2 and 3 as well as blocks 3 and 4, respectively, were not tolerated by the PHA synthase, leading to an inactive enzyme [4] (Figure 3). However, SmaI restrictionsite insertions between the conserved blocks 2/3, 3/4 and 5/6, respectively, were permissive mutations, suggesting that these regions are not adjacent to the core structure and thus surfaceexposed [61]. Five SmaI restriction site insertions in the second conserved block were not tolerated by the PHA synthase and caused inactivation, indicating that this region might be structurally relevant and unlikely to be surface-exposed. Various site-specific mutations were introduced based on the multiple amino-acid-sequence alignment as well as with the aid of the topological models [4,62-64].

Fusion proteins composed of the N-terminal part of the class II PHA synthase from P. aeruginosa and the C-terminal part of the class I PHA synthase from R. eutropha indicated that fusion points located in the α/β -hydrolase fold region are not tolerated [4]. Furthermore these fusion points were located in predicted and structurally conserved α -helical regions. However, a fusion point at position 289, relative to the amino acid sequence of the R. eutropha PHA synthase and located at a variable surface-exposed loop in the protein model, resulted in a hybrid PHA synthase, which exhibited in vitro enzyme activity, but no detectable in vivo activity. These results suggest that the first 288 amino acid residues of R. eutropha PHA synthase can be replaced by the N-terminus of a class II PHA synthase and provide evidence for the importance of the α/β -hydrolase fold region. Since this fusion protein showed only 13% of wild-type in vitro activity, this enzyme activity might be insufficient to mediate detectable accumulation of PHA in recombinant E. coli.

CATALYTIC MECHANISM

Catalytic residues

Site-specific mutagenesis studies of the class I PHA synthase from *R. eutropha* provided evidence that the conserved residues

Cys-319, Asp-480 and His-508 are directly involved in covalent catalysis [62,64] (Figure 2). The highly conserved Trp-425 was replaced by alanine, which reduced *in vivo* activity to 19% and *in vitro* activity to 0.003% of wild-type activity. This Trp-425 has been postulated to play an important role in protein-protein interaction, i.e. in the dimerization of the PhaC subunit, by generating a hydrophobic surface [64].

Mutational analysis of residues Cys-130, Cys-149, His-303, His-331, Asp-302 of PhaC from *A. vinosum* clearly indicated that the residues Cys-149, His-331 and Asp-302 are involved in covalent catalysis. Replacement of these residues did almost abolish enzymic activity [52].

Accordingly, the conserved catalytic triad residues of class II PHA synthase from P. aeruginosa were replaced [5]. Interestingly, replacement of the putative general base catalyst His-480 did strongly impair enzyme activity whereas, as expected, replacement of conserved cysteine and aspartic acid did abolish enzyme activity. Consistent with the class II synthase threading model, a conserved and adjacent His-453 was identified residing in the core structure close to the catalytic nucleophile, and replacement of this histidine had strong impact on enzyme activity. Thus the two histidines might functionally replace each other. However, Asp-452 was found to be essential for PHA synthase activity. In contrast to class I and III PHA synthases, the replacement of the class II synthase catalytic Cys-296 by serine resulted in a still highly active enzyme [5]. The conserved Trp-398, which might constitute the hydrophobic surface for PHA synthase dimerization, was replaced by phenylalanine or alanine. These replacements caused inactivation of the enzyme, indicating an essential role of this residue, presumably in protein dimerization, as postulated for class I synthases [5,64] Overall the class II enzymes from pseudomonads represent a rather distinct group with unique features not found in class I and class III enzymes.

Similar to the catalytic triad found in α/β -hydrolases, the highly conserved amino acid residues (three of the eight) of PHA synthases, such as Cys-149, Asp-302 and His-331 of the class III *A. vinosum* PHA synthase, were identified as being adjacent to the core structure of the threading model of the respective synthase, with the putative active-site nucleophile cysteine located at the elbow of the strand–elbow–helix motif (Figure 6). The catalytic triad was found to reside in the core structure of all hitherto generated threading models of class I–III PHA synthases [4,5,52]. An exception is the class II PHA synthase, where the conserved histidine residue, which functions as general base catalyst in α/β -hydrolases, was functionally replaced by an adjacent histidine residue.

Since PHA synthases utilize a cysteine as an active-site nucleophile, the general base catalyst histidine would be sufficient for nucleophilic activation, as has been shown for cysteine proteases [66]. However, in PHA synthases a second general base catalyst is required to activate the 3-hydroxyl of the 3-hydroxybutyryl-CoA or the bound 3-hydroxybutyryl to enable nucleophilic attack on the acylated enzyme (Figure 7). This function of the conserved aspartate, which has been proposed to constitute the catalytic triad (Figures 2 and 6), has been investigated by generation of a site-specific mutant where aspartate was replaced by asparagine. This mutation still allowed the covalent binding of the trimeric 3HB-CoA thioester in the A. vinosum synthase, but turnover of the substrate 3-hydroxybutyryl-CoA was strictly impaired, i.e. chain elongation was truncated [52]. The essential role of this residue in enzyme activity also was confirmed for class I and II synthases. These data strongly suggested an important role of this conserved aspartate in chain elongation (Figure 7). The α/β hydrolase-based catalytic mechanism, particularly considering

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lipases and cysteine proteases, provides a good model for classes I–III of PHA synthases as indicated by mutational analysis of the *R. eutropha* class I PHA synthase [4,62,64], the *A. vinosum* class III PHA synthase [52] and the *P. aeruginosa* class II PHA synthase [5].

Chain elongation

Griebel et al. [67,68] proposed a chain elongation mechanism that involved two thiol groups of the PHA synthases during the catalytic cycle, as was found in fatty acid synthases [69]. However, in the multiple alignment of PHA synthases, only one cysteine residue (e.g. Cys-319 from R. eutropha PHA synthase) is present in all PHA synthases (Figure 3). Efforts were made to identify the second thiol group [26,63]. The essential role of the conserved cysteine of PHA synthases for the reaction mechanism was obtained from site-specific mutagenesis and inhibitor analysis [5,52,62]. Firstly, the weakly conserved Cys-459 of the R. eutropha synthase was supposed to be involved in the catalytic cycle, providing the second thiol group. However, site-specific mutagenesis clearly suggested that this amino acid residue is not essential for catalytic activity, which was also consistent with the PHA synthase alignment [62]. The conserved Ser-260 (Figure 3) of the R. eutropha PHA synthase was identified to be a potential target for covalent post-translational modification by 4-phosphopantetheine. This modification would provide the second thiol group as found in fatty acid synthases. Radiolabelling experiments were conducted, expressing PHA synthase genes from R. eutropha, A. vinosum and P. aeruginosa, in E. coli SJ16 (panD) in order to analyse whether the PHA synthases are posttranslationally modified by 4-phosphopantetheine. E. coli SJ16 is a β -alanine auxotroph, and specific radiolabelling of 4phosphopantetheinylated proteins occurred when cells were fed with $[2^{-14}C]\beta$ -alanine. These experiments indicated that the PHA synthases from R. eutropha and A. vinosum belonging to class I and class III enzymes, respectively, were labelled by 4phosphopantetheine, but not the class II PHA synthase from P. aeruginosa [5,26,62]. However, detailed analysis revealed that only a small portion of total PHA synthase was labelled [35]. Functional low level expression of PHA synthase genes from R. eutropha in E. coli SJ16 and also in β -alanine auxotrophic mutants of R. eutropha, with subsequent analysis of 4phosphopantetheinvlated proteins, gave no evidence for covalent post-translational modification by 4-phosphopantetheine [63]. Exchange of amino acid residue Ser-260 with alanine and threonine, respectively, by site-specific mutagenesis, abolished in vivo and in vitro activity of PHA synthase from R. eutropha [63]. In addition, no peptide derived from PHA synthase could be isolated that was covalently modified by 4-phosphopantetheine [26]. Since PHA synthase genes from bacteria have been functionally expressed in various organisms from different kingdoms, specific post-translational modification of PHA synthases seems to be rather unlikely [70-73]. The current model of active PHA synthase involves two subunits forming a homodimer in class I and II PHA synthases, and forming a multimeric heterodimer (PhaC and PhaE) in the case of class III PHA synthases. Accordingly, class I, II and III PHA synthases possess two thiol groups provided by the conserved cysteine residue of the PhaC subunit with at least two subunits of PhaC in the active PHA synthase [3,35,63].

The development of structural models for classes I–III PHA synthases based on identity with enzymes belonging to the α/β -hydrolase superfamily, and mutational analysis of various highly conserved residues in these PHA synthases, led to the proposal

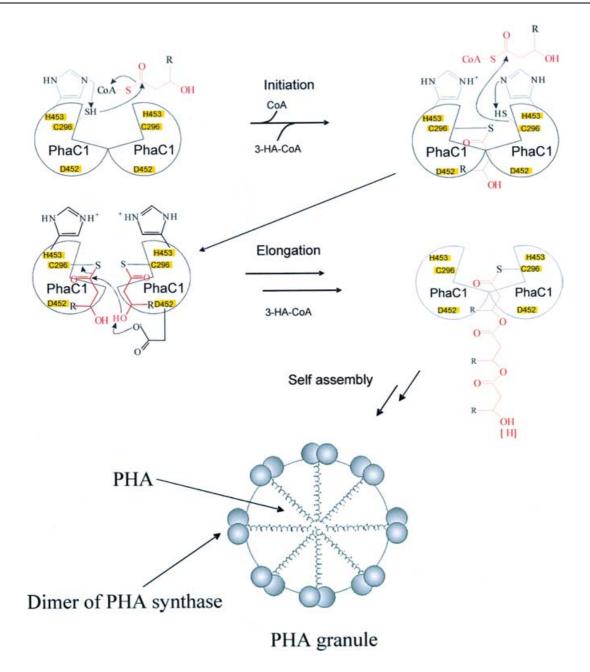


Figure 7 Proposed α/β -hydrolase-based catalytic mechanism of the *P. aeruginosa* class II PHA synthese [5]

of a new catalytic mechanism for PHA synthases [4,5,52]. The previously postulated catalytic mechanism, which was based on the reaction mechanism of fatty acid synthases (β -ketoacyl acylcarrier protein synthases) [67], has now been replaced by a reaction mechanism employed by α/β -hydrolases. In this new model, two thiol groups are proposed to play key roles in covalent catalysis. One thiol group serves as the loading site for 3-hydroxybutyryl-CoA and the second thiol group serves as the priming and elongation site. The highly conserved cysteine residues have been demonstrated to be involved in covalent catalysis [5,35,49]. However, from the above-indicated experiments, it cannot be excluded that the conserved serine (Figure 3) acts as loading site. Some evidence for this alternative reaction mechanism has been provided: (i) replacement of conserved Ser-260 of the *R*. *eutropha* PHA synthase abolished enzyme activity [63], (ii) use of the serine-specific inhibitor, PMSF, inhibited synthases [5,52], and (iii) the conserved serine residues reside close to the core structure of the respective synthase models.

Since no experimental evidence for covalent modification by 4-phosphopantetheinylation of PHA synthases and no sequence similarities of β -ketoacyl acyl-carrier protein synthases, or chalcone synthases, with PHA synthases were found, a new catalytic mechanism related to the catalytic mechanism of lipases was postulated. The lipases belong to the α/β -hydrolase superfamily of proteins [74,75] and this superfamily comprises enzymes with marked differences in substrate specificity, including: thioesterases [76], dienelactone hydrolases [77], and cholesterol esterases [78]. The hydrolases are all proposed to possess catalytic

triads composed of the active site nucleophile (serine, cysteine or aspartate), an acidic amino acid (aspartate or glutamate) and histidine, always being in this order with respect to the primary structure. The nucleophile has always been found located at the elbow of a strand–elbow–helix motif (see Figure 6). Moreover, lipases are characterized by interfacial activation acting at the lipid-water interface. This is comparable with PHA synthases which catalyse polymerization of a water-insoluble polyester and which are located at the polyester–water interface, i.e. attached to the surface of PHA granules. Additionally, the attached synthase showed a significantly increased enzymic activity. The respective water-soluble substrate is presumably bound to waterexposed regions of the PHA synthase, enabling the oriented synthesis of the growing polymer chain.

Interestingly the B. glumae lipase structure, which was used as template structure the R. eutropha class I PHA synthase, was obtained in the 'closed' conformation exhibiting the active site buried underneath a helical segment (α 5), called a 'lid' or a 'flap' [60,79]. In the PHA synthase model the active site was also buried underneath this structurally conserved helical segment (Figure 6), which corresponds to helix $\alpha 5$ of the *B. glumae* lipase. However, during transition to the open conformation of the lipase, due to interfacial activation, the active site becomes accessible to the solvent and a hydrophobic surface is exposed by the movement of the lid. The conformational changes can range from a simple rigidbody hinge-type motion to complex reorganizations involving changes in the secondary structures. Generally speaking, various structural studies suggested that the hydrophobic lipid-binding site is opened up by the rolling back of the lid from the active site at an oil-water interface. However, even in the absence of an oilwater interface, there may be a subtle equilibrium between the two conformations of the enzyme. It is believed that the opening of the lid is essential, but not sufficient, to explain the interfacial activation. In addition to providing access to the active site, the structural rearrangements also change the surface properties of the enzymes and in some cases form the oxyanion hole. In each case described, the movement of the lid exposes a large hydrophobic surface area surrounding the active site. This movement results in an amphipathic molecule which could be properly oriented for interaction of the active site with a lipid interface [80,81].

Accordingly, the soluble PHA synthase turns into an amphipathic molecule upon availability of substrate and covalent synthesis of the hydrophobic polyester chain [1]. This leads to the formation of so-called PHA granules with the hydrophobic PHA in the core and the active PHA synthases at the surface, which represents the water-PHA interface [82]. Consistently the granuleassociated PHA synthase from R. eutropha exerted an approx. $40 \times$ higher activity compared with the soluble enzyme [83]. This indicated that interfacial activation occurred and that a lid-like structure, as found in lipases and exhibited in the R. eutropha PHA synthase model, might also function in PHA synthases [4]. The permissive double mutant GS3 (R386C, K139R) of the *R*. eutropha synthase contained one mutation site, R386C, located in the lid region [4]. Two site-specific mutants (A391T, T393A) were generated by Taguchi et al. [65], which resided in the lid region and still exhibited PHA synthase activity. These data suggested that the proposed surface-exposed lid-like structure is not essential for enzyme function.

Modified polyester synthases obtained by random mutagenesis

For the production of tailor-made biopolyester and for enhancement of PHA production, the PHA synthases were considered as major targets for directed evolution experiments [4,65,84–86].

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The Aeromonas punctata FA440 synthase was chosen as a target for PCR-based *in vitro* evolution, since it can catalyse formation of a PHA random copolyester of 3-HB and 3-hydroxyhexanoate that is a tough and flexible material compared with PHB homopolyester [85]. Two single mutations, N149S and D171G, which occurred at positions that are not highly conserved among the PHA synthase family, resulted in significantly increased *in vivo* and *in vitro* enzyme activity. Interestingly, increases in the 3hydroxyhexanoate fraction (up to 16–18 mol%) were observed for both mutants compared with the wild-type (10 mol%).

In vitro evolution was also applied to obtain highly active mutants of *R. eutropha* polyester synthase. To search for mutations which enhance activity of the enzyme, multi-step mutations were conducted, including activity loss and intragenic-suppression-type activity reversion. This approach led to the identification of a modified PHA synthase with the F420S mutation, which was found to exhibit a 2.4-fold increase in specific activity towards 3HB-CoA, compared with the wild-type [86].

Rehm et al. [4] first employed single gene shuffling of a PHA synthase. However, only modified synthases with reduced activity were obtained. One of the most promising approaches was the in vivo random mutagenesis of the PHA synthase gene from Aeromonas punctata, which was performed employing the mutator strain E. coli XL1-Red [84]. Approx. 200000 mutants were screened on Nile Red-containing medium and five mutants with enhanced fluorescence were selected. Four of these mutants exhibited enhanced in vivo and in vitro PHA synthase activity. Mutant M1, which carried the single mutation F518I, showed a 5-fold increase in specific PHA synthase activity, whereas the corresponding mediated PHA accumulation increased by 20%, as compared with the wild-type PHA synthase. Mutant M2, which carried the single mutation V214G, showed a 2-fold increase in specific PHA synthase activity and PHA accumulation only increased by 7%. Overall, the in vitro activities of the overproducing mutants ranged from 1.1- to 5-fold more than the wildtype activity, whereas the amounts of accumulated PHA ranged over 107–126% of that of the wild-type. Moreover, all mutants mediated synthesis of PHAs with an increased weight-average molar mass, but the molar fractions of 3-hydroxybutyrate and 3-hydroxyhexanoate remained almost constant. In vivo random mutagenesis proved to be a versatile tool to isolate mutants exerting improved properties with respect to PHA biosynthesis [84].

Although it was possible to isolate modified PHA synthases with enhanced activity and changed substrate specificity, the functional role of the affected amino acid residues contributing to modified enzyme properties remains unclear.

Overall biochemical and enzymological studies of wild-type PHA synthase, as well as modified PHA synthases, will further illuminate structure–function relationships and the catalytic mechanism of the PHA synthases. Moreover, resolution of the threedimensional structure of the PHA synthase by X-ray analysis will be a breakthrough for the mechanistic understanding of this interesting class of enzymes.

SUBSTRATE SPECIFICITY OF POLYESTER SYNTHASES

Only the *in vitro* substrate specificities of *R. eutropha* and *A. vinosum* PHA synthases have been partially analysed [26,87]. The substrate specificities of these enzymes have been determined with analogues of varied chain length and branching, OH group position within the chain, and thioesters. The results suggested that, *in vitro*, both PHA synthases are very specific and provide further support for their active-site structural similarities. However, it is not clear why the *in vitro* results differed from studies *in vivo*.

Since only a few PHA synthases have been purified to homogeneity, the substrate specificity of almost any PHA synthase can only be estimated in vivo from cultivation experiments with precursor substrates provided as carbon source. The subsequent analysis of the chemical composition of the accumulated PHAs was used as a measure of the in vivo substrate specificity [1]. The value of these studies is limited for three reasons: (i) several bacteria, such as pseudomonads, harbour more than one PHA synthase gene, (ii) the physiological background in which PHA synthases are produced, and particularly the capability to provide hydroxy fatty acid CoA thioesters derived from the carbon source as substrate for the enzyme, may vary considerably, and (iii) synthetic CoA thioesters of hydroxy fatty acids cannot be analysed by this approach. Recently the substrate range of PHA synthases was studied in recombinant E. coli and various 3-hydroxy fatty acid CoA thioesters were provided in vivo by metabolic engineering [31,32,88-90]. In these studies a rather broad substrate specificity was observed, which was indicated by, for example, the ability of the class I PHA synthase from R. eutropha to accept also medium-chain-length 3-hydroxy fatty acid CoA thioesters as substrate [31,32]. For the first time, these studies allowed the independent analysis of the substrate range of the two class II PHA synthases PhaC1 and PhaC2 from P. aeruginosa, showing that these PHA synthases exert a similar substrate specificity and that 3-hydroxydecanoyl-CoA is the main substrate. Considering the growing number of natural and synthetic constituents found in PHAs accumulated by bacteria, it is evident that these synthases show an extremely broad substrate specificity [91,92]. Accordingly, the use of 3-mercaptopropionic acid as carbon source for R. eutropha resulted in the formation of a novel polyester, which is composed of 3-hydroxybutyric acid and 3-mecaptopropionic acid linked via thioester bonds [10]. Although it has not been confirmed by *in vitro* experiments with purified synthase and 3-mercaptopropionyl-CoA, this provides evidence that PHA synthases catalyse formation of polythioester.

BIOGENESIS AND STRUCTURE OF POLYESTER INCLUSIONS

In vivo PHA biosynthesis starts as soon as substrate, (R)-3hydroxyacyl-CoA thioesters, are provided intracellularly. PHA synthase is constitutively produced, although at a rather low level, and upon availability of substrate these enzymes start to catalyse the formation of a high molecular mass polyester (n > 1000). The growing polyester chain, which remains covalently attached to the enzyme [6], converts the initially soluble enzyme into an amphipathic molecule. The amphipathic molecules undergo a self-assembly process, which is supposed to be similar to micelle formation. Small water-insoluble inclusions are formed with an amorphous polyester core and PHA synthase covalently attached to the surface [93,94] (Figure 8). These PHA granules increase in size while the attached PHA synthases continuously incorporate precursor from the cytosol into the growing polyester chain. It remains to be determined whether larger granules occur due to fusion events or whether simple increase in size due to ongoing polymerization takes place. Usually from 5 to 8 PHA granules are deposited intracellularly, constituing the entire cell volume, when maximum PHA accumulation is achieved [82]. PHA granules are surrounded by a phospholipid membrane [67] with embedded or attached proteins [95] consisting of the PHA synthase [50,51,94,96], the intracellular PHA depolymerase [2,97–99], amphiphilic phasin proteins [100–104], PHA-specific regulator proteins [105-108] and additional proteins with as yet unknown functions [109]. The intracellular depolymerase is

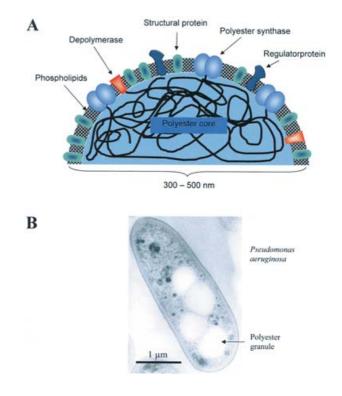


Figure 8 (A) Schematic presentation of a polyester granule, and (B) electron microscopy image of *Pseudomonas aeruginosa* harbouring polyester granules

required for mobilization of the reserve polyester. The phasin proteins function as structural proteins that promote PHA biosynthesis and their copy number has an impact on PHA granule size [103,110]. Kinetic simulation of the self-assembly process revealed that phasins have an impact on the kinetics of granule formation by reducing the lag phase [100]. There are PHAspecific regulators such as PhbR from R. eutropha [105,106], PhaF from pseudomonads [108,111] and PhaR from Paracoccus denitrificans [40,107]. Additional granule-associated proteins were found in pseudomonads, the functions of which have not yet been clarified [108,109,112]. However, these proteins (PhaI, PhaD, PhaS) are considered as structural proteins also involved in biosynthesis and mobilization. According to one model these proteins are embedded in, or associated with, a phospholipid monolaver, whereas other models propose a much more complex membrane structure with two phospholipid membranes [51,82,93,113]. Evidence was provided by NMR analysis that water molecules are present in the core structure of the granules and that these compounds function as a plasticizer [114]. These observations strongly suggest that the enzyme(s) responsible for PHA biosynthesis and consumption operate only on mobile hydrated material and that the solid granules characteristic of dried cells are partially artifactual.

IN VITRO SYNTHESIS OF BIOPOLYESTER

Analysis of *in vitro* PHA synthesis and the formation of macroscopic PHA granules has been made easier as more purified PHA synthases, from various micro-organisms, have been made available; sources include: *R. eutropha*, *A. vinosum*,

P. aeruginosa (PhaC1 and PhaC2) and *P. oleovorans* (PhaC1) [48,50,62,115,116]. *In vitro* PHB synthesis was first obtained by applying recombinantly produced and purified *R. eutropha* PHA synthase [83]. The granules formed in a matter of minutes when the purified synthase was exposed to synthetically prepared (*R*)-3-hydroxybutyryl-CoA, thereby establishing the minimal requirements for PHB granule formation. The artificial granules are spherical with diameters of up to 3 μ m and significantly larger than their native counterparts (0.5 μ m). The isolated PHB was characterized by ¹H- and ¹³C-NMR, gel-permeation chromatography, and chemical analysis. The *in vitro* polymerization system yields PHB with a molecular mass > 10 × 10⁶ Da, exceeding by an order of magnitude the mass of PHAs typically extracted from microorganisms.

Preliminary kinetic analysis of de novo granule formation confirms earlier findings of a lag time for the enzyme but suggests the involvement of an additional granule assembly step. Since substrate analogues lacking the adenosine 3',5'-bisphosphate moiety of (R)-3-hydroxybutyryl-CoA were not accepted by the PHA synthase, evidence was provided that this structural element of the substrate is essential for catalysis [83]. That study also demonstrated that the molar mass of the polymer can be controlled by the initial PHA synthase concentration. Increasing PHA synthase concentration resulted in decrease of the weightaverage molar mass of the in vitro synthesized PHB [83]. These observations were recently transferred to in vivo studies, which confirmed the dependency of the weight-average molar mass on the amount of PHA synthase and which demonstrated that the in vitro synthesis studies are useful tools to mimic the in vivo situation [117]. In vitro PHB synthesis was also obtained by applying only the purified class III PHA synthase from A. vinosum with 3-hydroxybutyryl-CoA as substrate [118]. Macroscopic PHB granules were obtained when MgCl₂ was also added. Interestingly the rate of PHA synthesis in vitro appeared to be 200-fold higher than in vivo. Doi [119] calculated that only approx. two 3-hydroxybutyryl-CoA units were added to a propagating PHB chain(s) in vivo in R. eutropha. Various components were investigated for their effect on in vitro PHB synthesis, and the most striking observation was that CoA acts as a competitive inhibitor of the PHA synthase [35,120]. Therefore, in vitro coupled enzyme systems were developed which recycle CoA due to synthesis of the respective CoA thioester, in order to reduce the free CoA level and to reduce costs [120]. Since synthesis of CoA thioester required hydrolysis of ATP, regeneration of the expensive ATP was investigated. Moreover, ATP regeneration using the cheaper poly(P) was successfully achieved by employing adenylate kinase and polyphosphate kinase [121]. Another coupled enzyme system for in vitro PHB synthesis was established starting from 3hydroxybutyrate and employing the butyrate kinase, the phosphotransbutyrylase as well as the class III PHA synthase from A. vinosum [122]. Again this in vitro system could be successfully transferred as a new PHB biosynthesis pathway in recombinant E. coli producing the respective enzymes [123]. These results suggested that pathway modelling can be, to some extent, simulated by in vitro synthesis experiments.

It was only recently possible to purify the class II PHA synthase from *P. aeruginosa* and to apply this PHA synthase for *in vitro* PHA synthesis [48,115]. The purified soluble class II PHA synthase, PhaC1, and the enzymatically synthesized 3-hydroxydecanoyl-CoA [124] as substrate were sufficient for the *in vitro* synthesis of poly(3-hydroxydecanoate) [115]. The purified enzyme showed a specific activity of only approx. 37 mU mg⁻¹, which might be one of the reasons why soluble class II PHA synthases have not been characterized previously with respect to enzymic and catalytic properties. This specific activity

was approx. 3000-fold lower than the specific activity from the previously characterized class I and class III PHA synthases [35,83]. However, the specific activity of the purified class II PHA synthase was approx. 20-fold lower than the estimated specific activitiy of granule-bound PHA synthase [125]. Therefore, various components were tested with respect to their effect on PHA synthase activity. The phasin GA24 from R. eutropha showed an enhancing effect on the PHA synthase, whereas CoA was also a competitive inhibitor of the class II PHA synthase. A coupled enzyme system was developed employing the acyl-CoA synthetase and the class II PHA synthase from P. aeruginosa, in order to recycle CoA and to achieve a quantitative amount of poly(3hydroxydecanoate). Quantification of the produced polymer and determination of the weight-average molar mass, which was in a typical range of approx. 100000 g · mol⁻¹, as well as knowledge about the amount of enzyme in the reaction mixture allowed calculation of the number of polymer chains synthesized by one PHA synthase molecule. Calculations revealed that one PHA synthase molecule synthesized 0.6 polymer chains, which indicated that no chain transfer reaction occurred [115]. Interestingly similar results were obtained from experiments with class IPHA synthase from R. eutropha [83], whereas class III PHA synthases showed chain transfer (25 chains per PhaC/E complex) during in vitro synthesis based on the calculation mentioned above [118].

FACTORS DETERMINING THE MOLECULAR MASS AND COMPOSITION OF BIOPOLYESTER

Obviously, PHAs synthesized in biological systems by class I PHA synthases exhibit a higher molecular mass than PHAs synthesized by class II PHA synthases with molecular masses ranging from approximately 500000 to several millions or from only approx. 50000 to 500000, respectively. Class III PHA synthases seem to synthesize PHAs with molecular masses that are in between. The molecular mass of PHAs depends on several factors.

(i) The metabolic background is important with respect to the provision of 3-hydroxyacyl-CoA thioesters, i.e. the concentration of substrate for PHA synthases, and also with respect to the availability of enzymes that hydrolyse PHAs, such as intracellular PHA depolymerases [8] or unspecific esterases and lipases [126,127]. If the physiological background does not provide such enzymes then PHAs of higher molecular mass might be produced. This may be one of the reasons why recombinant *E. coli* expressing the *R. eutropha* PHA synthase produce PHB with much higher molecular mass than PHB accumulated by *R. eutropha* [128].

(ii) The level of expression of active PHA synthase protein in the cells is also very important. The higher the concentration of active PHA synthase protein in the cells, the lower is the molecular mass of the accumulated polyester [117,125]. Since the molecular mass of technical polymers is important with respect to their technical properties and processibility, it will be very important to engineer biological production systems which provide PHAs with the appropriate molecular masses. The composition of the PHA depends strictly on the substrate specificity of the PHA synthase and the metabolic potential of the respective organism to provide (R)-3-hydroxyacyl-CoA thioester from the provided carbon source [1]. Metabolic engineering is a powerful tool to vary the pool of certain thioesters and thereby change the composition of PHA. Constraints for the design of PHAs are the substrate specificity of the PHA synthase and the metabolism of the respective organism to provide precursor for PHA biosynthesis.

The number of different PHAs with new constituents, varying composition and molecular mass, which exert a broad range of material properties, has tremendously increased over the last decade. An increasing number of patents for various applications, particularly in the medical field, have been approved, which clearly supports the relevance of these biopolymers [129]. However, for an economically feasible and biotechnological production process it is important to obtain these polyesters from simple and cheap carbon sources [130]. Preferentially the carbon source should be renewable, such as carbohydrates and lipids that are produced by agriculture. In the ideal case the carbon source should be CO_2 . Alternatively, and as a second preference, the carbon source may be derived from waste or residual materials such as lactose in whey. In order to produce PHAs other than PHB from CO₂ or renewable resources it will be necessary to link central metabolic pathways with PHA synthases, i.e. to utilize central anabolic or catabolic pathways for the synthesis of 3-hydroxyacyl-CoA thioesters and to channel metabolic flux towards a synthesis of the respective 3-hydroxyacyl-CoA thioesters. In recent reviews it has been outlined that amino acid metabolism, citric acid cycle, fatty acid de novo synthesis pathway and fatty acid β -oxidation pathways are the most promising candidates for this purpose [1,131,132]. A 3-hydroxyacyl-acyl carrier protein-CoA transacylase and an R-specific enoyl-CoA hydratase linking the fatty acid *de novo* synthesis or fatty acid β -oxidation to PHA synthesis have been identified in various bacteria and characterized at the biochemical and molecular level [124,133-139]. These enzymes were successfully applied to establish the respective metabolic route in various bacteria [140-144] Knowledge about these two enzymes and availability of the genes will have significant impacts on metabolic engineering of PHA biosynthesis pathways from CO₂, or simple carbon sources, to PHAs in other organisms. Recently it has been shown that engineering of the precursor-providing transacylase enabled production of a new polyester [133]. Metabolic engineering of the β -oxidation pathway in *E. coli* employing *fad* mutants harbouring class II PHA synthase genes and/or the use of inhibitors for β oxidation in various micro-organisms led to efficient recombinant medium-chain-length PHA accumulation [89,90,143,145]. The provision of defined substrates by metabolic routing in E. coli represents a valuable tool to determine the *in vivo* substrate specificity of PHA synthases [32]. Beside the use of the respective biopolyesters, more and more interest has been attracted by the enantioselectivity of PHA biosynthesis enzymes. Since only the *R*-enantiomer of 3-hydroxy fatty acids, which appears to be an interesting compound for medical drug biosynthesis, was found as a constituent, efforts were undertaken to either overproduce these chiral compounds by metabolic engineering [146] or to obtain these compounds by hydrolysis of the respective polyester [147,148]. Details about metabolic pathways of PHA biosynthesis were recently summarized [149–151].

CONCLUSIONS AND OUTLOOK

Although the biochemical and molecular analysis of PHA synthases has revealed a tremendous amount of knowledge about the catalytic mechanism and quaternary structure, several open questions remain to be addressed. Much research still has to be undertaken to understand the PHA synthase reaction mechanism more completely and to utilize this knowledge for the production of tailor-made biopolyesters. The more data are available about structure-function relationships the more effort will be undertaken for rational design of synthases. The defined engineering of polyester synthases with certain substrate specificity would enable the production of new and designed polyesters with interesting material properties. Random mutagenesis approaches, employing a recently developed viable-colony staining method for simple screening of modified PHA synthases [152], have been proven to be successful. Several different biopolyesters, particularly as biomaterials, are most probably in the pipe-line and will be commercialized in the future. An emerging field is the recently achieved in vitro synthesis of biopolyesters consisting of 3-hydroxybutyrate and/or 3-hydroxyvalerate as well as novel medium-chain-length biopolyesters recently achieved by employing the purified enzymes from R. eutropha [83], A. vinosum [118,120] and P. aeruginosa [115]. One promising approach is the molecular breeding of transgenic plants expressing functionally active biopolyester biosynthesis pathways and to produce biopolyester directly by agriculture [132,153-155]. However, besides the engineering of biological systems, many other studies and research activities must be performed by technical engineers and polymer chemists to achieve a feasible production process resulting in commercialization of biopolyesters.

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REFERENCES

- Rehm, B. H. A. and Steinbüchel, A. (1999) Biochemical and genetic analysis of PHA synthases and other proteins required for PHA synthesis. Int. J. Biol. Macromol. 25, 3–19
- 2 Gao, D., Maehara, A., Yamane, T. and Ueda, S. (2001) Identification of the intracellular polyhydroxyalkanoate depolymerase gene of *Paracoccus denitrificans* and some properties of the gene product. FEMS Microbiol. Lett. **196**, 159–164
- 3 Rehm, B. H. A. and Steinbüchel, A. (2001) PHA synthases: key enzymes of PHA biosynthesis, in 'Biopolymers' (Steinbüchel, A. and Doi, Y. eds), Polyesters I, 3a, pp. 173–215, Wiley-VCH, Weinheim
- 4 Rehm, B. H. A., Antonio, R. V., Spiekermann, P., Amara, A. A. and Steinbüchel, A. (2002) Molecular characterization of the poly(3-hydroxybutyrate) (PHB) synthase from *Ralstonia eutropha*: in vitro evolution, site-specific mutagenesis and development of a PHB synthase protein model. Biochim. Biophys. Acta **1594**, 178–190
- 5 Amara, A. A. and Rehm, B. H. A. (2003) Replacement of the catalytic nucleophile Cys-296 by serine in class II polyhydroxyalkanoate synthase from *Pseudomonas aeruginosa*-mediated synthesis of a new polyester: Identification of catalytic residues. Biochem. J. **374**, 413–421
- 6 Hezayen, F. F., Tindall, B. J., Steinbüchel, A. and Rehm, B. H. (2002) Characterization of a novel halophilic archaeon, *Halobiforma haloterrestris* gen. nov., sp. nov. and transfer of *Natronobacterium nitratireducens* to *Halobiforma nitratireducens* comb. nov. Int. J. Syst. Evol. Microbiol. **52**, 2271–2280
- 7 Quaiser, A., Ochsenreiter, T., Klenk, H. P., Kletzin, A., Treusch, A. H., Meurer, G., Eck, J., Sensen, C. W. and Schleper, C. (2002) First insight into the genome of an uncultivated crenarchaeote from soil. Environ. Microbiol. 4, 603–611
- Jendrossek, D. and Handrick, R. (2002) Microbial degradation of polyhydroxyalkanoates. Annu. Rev. Microbiol. 56, 403–432
- 9 Anderson, A. J. and Dawes, E. A. (1990) Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol. Rev. 54, 450–472
- 10 Lütke-Eversloh, T., Bergander, K., Luftmann, H. and Steinbüchel, A. (2001) Identification of a new class of biopolymer: bacterial synthesis of a sulphur-containing polymer with thioester linkages. Microbiology (Reading, U.K.) 147, 11–19
- 11 Reusch, R. N. and Sadoff, H. L. (1988) Putative structure and functions of a poly-betahydroxybutyrate/calcium polyphosphate channel in bacterial plasma membranes. Proc. Natl. Acad. Sci. U.S.A. 85, 4176–4180

- 12 Das, S., Seebach, D. and Reusch, R. N. (2002) Differential effects of temperature on *E. coli* and synthetic polyhydroxybutyrate/polyphosphate channels. Biochemistry **41**, 5307–5312
- 13 Das, S. and Reusch, R. N. (2001) pH regulates cation selectivity of poly-(R)-3hydroxybutyrate/polyphosphate channels from *E. coli* in planar lipid bilayers. Biochemistry **40**, 2075–2079
- 14 Das, S. and Reusch, R. N. (1999) Gating kinetics of *E. coli* poly-3-hydroxybutyrate/ polyphosphate channels in planar bilayer membranes. J. Membr. Biol. **170**, 135–145
- 15 Reusch, R. N. (2000) Transmembrane ion transport by polyphosphate/poly-(*R*)-3hydroxybutyrate complexes. Biokhimiya (Moscow) **65**, 280–295
- 16 Reusch, R. N., Huang, R. and Bramble, L. L. (1995) Poly-3-hydroxybutyrate/ polyphosphate complexes form voltage-activated Ca²⁺ channels in the plasma membranes of *Escherichia coli*. Biophys. J. **69**, 754–766
- 17 Reusch, R. N., Huang, R. and Kosk-Kosicka, D. (1997) Novel components and enzymatic activities of the human erythrocyte plasma membrane calcium pump. FEBS Lett. 412, 592–596
- 18 Castuma, C. E., Huang, R., Kornberg, A. and Reusch, R. N. (1995) Inorganic polyphosphates in the acquisition of competence in *Escherichia coli*. J. Biol. Chem. **270**, 12980–12983
- 19 Erdmann, S. and Holler, E. (1988) Recent progress on the structure of polymalate. Biol. Chem. **369**, 1090
- 20 Qi, Q. and Rehm, B. H. (2001) Polyhydroxybutyrate biosynthesis in *Caulobacter crescentus*: molecular characterization of the polyhydroxybutyrate synthase. Microbiology 147, 3353–3358
- 21 Slater, S., Gallaher, T. and Dennis, D. (1992) Production of poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) in a recombinant *Escherichia coli* strain. Appl. Environ. Microbiol. 58, 1089–1094
- 22 Slater, S. C., Voige, W. H. and Dennis, D. E. (1988) Cloning and expression in *Escherichia coli* of the *Alcaligenes eutrophus* H16 poly-beta-hydroxybutyrate biosynthetic pathway. J. Bacteriol. **170**, 4431–4436
- 23 Schubert, P., Steinbüchel, A. and Schlegel, H. G. (1988) Cloning of the Alcaligenes eutrophus genes for synthesis of poly-beta-hydroxybutyric acid (PHB) and synthesis of PHB in Escherichia coli. J. Bacteriol. **170**, 5837–5847
- 24 Peoples, O. P. and Sinskey, A. J. (1989) Poly-beta-hydroxybutyrate (PHB) biosynthesis in *Alcaligenes eutrophus* H16. Identification and characterization of the PHB polymerase gene (phbC). J. Biol. Chem. **264**, 15298–15303
- 25 Liebergesell, M., Schmidt, B. and Steinbüchel, A. (1992) Isolation and identification of granule-associated proteins relevant for poly(3-hydroxyalkanoic acid) biosynthesis in *Chromatium vinosum* D. FEMS Microbiol. Lett. **78**, 227–232
- 26 Yuan, W., Jia, Y., Tian, J., Snell, K. D., Muh, U., Sinskey, A. J., Lambalot, R. H., Walsh, C. T. and Stubbe, J. (2001) Class I and III polyhydroxyalkanoate synthases from *Ralstonia eutropha* and *Allochromatium vinosum*: characterization and substrate specificity studies. Arch. Biochem. Biophys. **394**, 87–98
- 27 McCool, G. J. and Cannon, M. C. (2001) PhaC and PhaR are required for polyhydroxyalkanoic acid synthase activity in *Bacillus megaterium*. J. Bacteriol. **183**, 4235–4243
- 28 Fukui, T. and Doi, Y. (1997) Cloning and analysis of the Poly(3hydroxybutyrate-co-3-hydroxyhexanoate) biosynthesis genes of *Aeromonas caviae*. J. Bacteriol. **179**, 4821–4830
- 29 Liebergesell, M., Rehalkar, S. and Steinbüchel, A. (2000) Analysis of the *Thiocapsa pfennigii* polyhydroxyalkanoate synthase: subcloning, molecular characterization and generation of hybrid synthases with the corresponding *Chromatium vinosum* enzyme. Appl. Microbiol. Biotechnol. **54**, 186–194
- 30 Matsusaki, H., Manji, S., Taguchi, K., Kato, M., Fukui, T. and Doi, Y. (1998) Cloning and molecular analysis of the Poly(3-hydroxybutyrate) and Poly(3-hydroxybutyrate-co-3-hydroxyalkanoate) biosynthesis genes in Pseudomonas sp. strain 61–63. J. Bacteriol. 180, 6459–6467
- 31 Dennis, D., McCoy, M., Stangl, A., Valentin, H. E. and Wu, Z. (1998) Formation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) by PHA synthase from *Ralstonia eutropha*. J. Biotechnol. **64**, 177–186
- 32 Antonio, R. V., Steinbüchel, A. and Rehm, B. H. (2000) Analysis of in vivo substrate specificity of the PHA synthase from *Ralstonia eutropha*: formation of novel copolyesters in recombinant *Escherichia coli*. FEMS Microbiol. Lett. **182**, 111–117
- 33 Schubert, P., Krüger, N. and Steinbüchel, A. (1991) Molecular analysis of the Alcaligenes eutrophus poly(3-hydroxybutyrate) biosynthetic operon: identification of the N-terminus of poly(3-hydroxybutyrate) synthase and identification of the promoter. J. Bacteriol. **173**, 168–175
- 34 Kyte, J. and Doolittle, R. F. (1982) A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157, 105–132
- 35 Müh, U., Sinskey, A. J., Kirby, D. P., Lane, W. S. and Stubbe, J. (1999) PHA synthase from *Chromatium vinosum*: Cys 149 is involved in covalent catalysis. Biochemistry **38**, 826–837

- 36 Satoh, Y., Minamoto, N., Tajima, K. and Munekata, M. (2002) Polyhydroxyalkanoate synthase from *Bacillus* sp INT005 is composed of PhaC and PhaR. J. Biosci. Bioeng. 94, 343–350
- 37 Hezayen, F. F., Steinbüchel, A. and Rehm, B. H. A. (2002) Biochemical and enzymological properties of the polyhydroxybutyrate synthase from the extremely halophilic archaeon strain 56. Arch. Biochem. Biophys. 403, 284–291
- 38 Steinbüchel, A. and Schlegel, H. G. (1991) Physiology and molecular genetics of poly(beta-hydroxy-alkanoic acid) synthesis in *Alcaligenes eutrophus*. Mol. Microbiol. 5, 535–542
- 39 Cevallos, M. A., Encarnacion, S., Leija, A., Mora, Y. and Mora, J. (1996) Genetic and physiological characterization of a *Rhizobium etli* mutant strain unable to synthesize poly-beta-hydroxybutyrate. J. Bacteriol. **178**, 1646–1654
- 40 Maehara, A., Ueda, S., Nakano, H. and Yamane, T. (1999) Analyses of a polyhydroxyalkanoic acid granule-associated 16-kilodalton protein and its putative regulator in the pha locus of *Paracoccus denitrificans*. J. Bacteriol. **181**, 2914–2921
- Mandon, K., Michel-Reydellet, N., Encarnacion, S., Kaminski, P. A., Leija, A., Cevallos, M. A., Elmerich, C. and Mora, J. (1998) Poly-beta-hydroxybutyrate turnover in *Azorhizobium caulinodans* is required for growth and affects nifA expression. J. Bacteriol. 180, 5070–5076
- 42 Tombolini, R., Povolo, S., Buson, A., Squartini, A. and Nuti, M. P. (1995) Poly-betahydroxybutyrate (PHB) biosynthetic genes in *Rhizobium meliloti* 41. Microbiology **141**, 2553–2559
- 43 Valentin, H. E. and Steinbüchel, A. (1993) Cloning and characterization of the Methylobacterium extorquens polyhydroxyalkanoic-acid-synthase structural gene. Appl. Microbiol. Biotechnol. **39**, 309–317
- 44 Choi, J. I., Lee, S. Y. and Han, K. (1998) Cloning of the Alcaligenes latus polyhydroxyalkanoate biosynthesis genes and use of these genes for enhanced production of Poly(3-hydroxybutyrate) in *Escherichia coli*. Appl. Environ. Microbiol. 64, 4897–4903
- 45 Rodrigues, M. F., Valentin, H. E., Berger, P. A., Tran, M., Asrar, J., Gruys, K. J. and Steinbüchel, A. (2000) Polyhydroxyalkanoate accumulation in *Burkholderia* sp.: a molecular approach to elucidate the genes involved in the formation of two homopolymers consisting of short-chain-length 3-hydroxyalkanoic acids. Appl. Microbiol. Biotechnol. **53**, 453–460
- 46 Sudesh, K., Fukui, T. and Doi, Y. (1998) Genetic analysis of *Comamonas acidovorans* polyhydroxyalkanoate synthase and factors affecting the incorporation of 4-hydroxybutyrate monomer. Appl. Environ. Microbiol. **64**, 3437–3443
- 47 Cuff, J. A., Clamp, M. E., Siddiqui, A. S., Finlay, M. and Barton, G. J. (1998) JPred: a consensus secondary structure prediction server. Bioinformatics 14, 892–893
- 48 Rehm, B. H. A., Qi, Q. S., Beermann, B. B., Hinz, H. J. and Steinbüchel, A. (2001) Matrix-assisted in vitro refolding of *Pseudomonas aeruginosa* class II polyhydroxyalkanoate synthase from inclusion bodies produced in recombinant *Escherichia coli*. Biochem. J. **358**, 263–268
- Wodzinska, J., Snell, K. D., Rhomberg, A., Sinskey, A. J., Biemann, K. and Stubbe, J. (1996) Polyhydroxybutyrate synthase: Evidence for covalent catalysis. J. Am. Chem. Soc. 118, 6319–6320
- 50 Liebergesell, M., Sonomoto, K., Madkour, M., Mayer, F. and Steinbüchel, A. (1994) Purification and characterization of the poly(hydroxyalkanoic acid) synthase from *Chromatium vinosum* and localization of the enzyme at the surface of poly(hydroxyalkanoic acid) granules. Eur. J. Biochem. **226**, 71–80
- 51 Mayer, F., Madkour, M. H., PieperFurst, U., Wieczorek, R., Gesell, M. L. and Steinbüchel, A. (1996) Electron microscopic observations on the macromolecular organization of the boundary layer of bacterial PHA inclusion bodies. J. Gen. Appl. Microbiol. 42, 445–455
- 52 Jia, Y., Kappock, T. J., Frick, T., Sinskey, A. J. and Stubbe, J. (2000) Lipases provide a new mechanistic model for polyhydroxybutyrate (PHB) synthases: characterization of the functional residues in *Chromatium vinosum* PHB synthase. Biochemistry **39**, 3927–3936
- 53 Guex, N. and Peitsch, M. C. (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis 18, 2714–2723
- 54 Karplus, K., Barrett, C. and Hughey, R. (1998) Hidden Markov models for detecting remote protein homologies. Bioinformatics 14, 846–856
- 55 MacCallum, R. M., Kelley, L. A. and Sternberg, M. J. E. (2000) SAWTED: Structure Assignment With Text Description – Enhanced detection, of remote homologues with automated SWISS-PROT annotation comparisons. Bioinformatics 16, 125–129
- 56 Fischer, D. and Eisenberg, D. (1996) Protein fold recognition using sequence-derived predictions. Protein Sci. 5, 947–955
- 57 Laskowski, R. A., Moss, D. S. and Thornton, J. M. (1993) Main-chain bond lengths and bond angles in protein structures. J. Mol. Biol. 231, 1049–1067
- 58 Lüthy, R., Bowie, J. U. and Eisenberg, D. (1992) Assessment of Protein Models with 3-Dimensional Profiles. Nature (London) 356, 83–85
- 59 Kelley, L. A., MacCallum, R. M. and Sternberg, M. J. E. (2000) Enhanced genome annotation using structural profiles in the program 3D-PSSM. J. Mol. Biol. 299, 499–520

- 60 Noble, M. E. M., Cleasby, A., Johnson, L. N., Egmond, M. R. and Frenken, L. G. J. (1994) Analysis of the Structure of *Pseudomonas glumae* Lipase. Protein Eng. 7, 559–562
- 61 Kalousek, S., Dennis, D. and Lubitz, W. (1992) Genetic engineering of PHB synthase from Alcaligenes eutrophus H16. FEMS Microbiol. Rev. 103, 426–427
- 62 Gerngross, T. U., Snell, K. D., Peoples, O. P., Sinskey, A. J., Csuhai, E., Masamune, S. and Stubbe, J. (1994) Overexpression and purification of the soluble polyhydroxyalkanoate synthase from *Alcaligenes eutrophus*: evidence for a required posttranslational modification for catalytic activity. Biochemistry **33**, 9311–9320
- 63 Hoppensack, A., Rehm, B. H. A. and Steinbüchel, A. (1999) Analysis of 4-phosphopantetheinylation of polyhydroxybutyrate synthase from *Ralstonia eutropha*: Generation of beta-alanine auxotrophic Tn5 mutants and cloning of the panD gene region. J. Bacteriol. **181**, 1429–1435
- 64 Jia, Y., Yuan, W., Wodzinska, J., Park, C., Sinskey, A. J. and Stubbe, J. (2001) Mechanistic studies on class I polyhydroxybutyrate (PHB) synthase from *Ralstonia eutropha*: class I and III synthases share a similar catalytic mechanism. Biochemistry **40**, 1011–1019
- 65 Taguchi, S., Maehara, A., Takase, K., Nakahara, M., Nakamura, H. and Doi, Y. (2001) Analysis of mutational effects of a polyhydroxybutyrate (PHB) polymerase on bacterial PHB accumulation using an in vivo assay system. FEMS Microbiol. Lett. **198**, 65–71
- 66 Chen, J. M., Rawlings, N. D., Stevens, R. A. and Barrett, A. J. (1998) Identification of the active site of legumain links it to caspases, clostripain and gingipains in a new clan of cysteine endopeptidases. FEBS Lett. 441, 361–365
- 67 Griebel, R., Smith, Z. and Merrick, J. M. (1968) Metabolism of poly-beta-hydroxybutyrate. I. Purification composition and properties of native poly-beta-hydroxybutyrate granules from *Bacillus megaterium*. Biochemistry **7**, 3676–3681
- 68 Griebel, R., Werth, J. and Merrick, J. M. (1968) Poly-beta-hydroxybutyrate synthetase a possible inhibitor of poly-beta-hydroxybutyrate depolymerization. Fed. Proc. Fed. Am. Soc. Exp. Biol. 27, 785–791
- 69 Witkowski, A., Joshi, A. K. and Smith, S. (1997) Characterization of the interthiol acyltransferase reaction catalyzed by the beta-ketoacyl synthase domain of the animal fatty acid synthase. Biochemistry 36, 16338–16344
- 70 Leaf, T. A., Peterson, M. S., Stoup, S. K., Somers, D. and Srienc, F. (1996) Saccharomyces cerevisiae expressing bacterial polyhydroxybutyrate synthase produces poly-3-hydroxybutyrate. Microbiology 142, 1169–1180
- 71 Bohmert, K., Balbo, I., Kopka, J., Mittendorf, V., Nawrath, C., Poirier, Y., Tischendorf, G., Trethewey, R. N. and Willmitzer, L. (2000) Transgenic *Arabidopsis* plants can accumulate polyhydroxybutyrate to up to 4 % of their fresh weight. Planta **211**, 841–845
- 72 Poirier, Y., Somerville, C., Schechtman, L. A., Satkowski, M. M. and Noda, I. (1995) Synthesis of high-molecular-weight poly([*R*]-(-)-3-hydroxybutyrate) in transgenic Arabidopsis thaliana plant cells. Int. J. Biol. Macromol. **17**, 7–12
- 73 Williams, M. D., Fieno, A. M., Grant, R. A. and Sherman, D. H. (1996) Expression and analysis of a bacterial poly(hydroxyalkanoate) synthase in insect cells using a baculovirus system. Protein Expression Purif. 7, 203–211
- 74 Ollis, D. L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S. M., Harel, M., Remington, S. J., Silman, I., Schrag, J., et al. (1992) The alpha/beta-hydrolase fold. Protein Eng. 5, 197–211
- 75 Schrag, J. D. and Cygler, M. (1997) Lipases and alpha/beta hydrolase fold. Methods Enzymol. 284, 85–107
- 76 Lawson, D. M., Derewenda, U., Serre, L., Ferri, S., Szittner, R., Wei, Y., Meighen, E. A. and Derewenda, Z. S. (1994) Structure of a myristoyl-Acp-specific thioesterase from *vibrio-harveyi*. Biochemistry **33**, 9382–9388
- 77 Pathak, D. and Ollis, D. (1990) Refined structure of dienelactone hydrolase at 1.8 Å. J. Mol. Biol. 214, 497–525
- 78 Pletnev, V., Addlagatta, A., Wawrzak, Z. and Duax, W. (2003) Three-dimensional structure of homodimeric cholesterol esterase-ligand complex at 1.4 Å resolution. Acta Crystallogr., Sect. D: Biol. Crystallogr. 59, 50–56
- 79 Noble, M. E. M., Cleasby, A., Johnson, L. N., Egmond, M. R. and Frenken, L. G. J. (1993) The crystal-structure of triacylglycerol lipase from *Pseudomonas glumae* reveals a partially redundant catalytic aspartate. FEBS Lett. **331**, 123–128
- 80 Cygler, M. and Schrag, J. D. (1997) Structure as basis for understanding interfacial properties of lipases. Methods Enzymol. 284, 3–27
- 81 Schrag, J. D., Li, Y. G., Cygler, M., Lang, D. M., Burgdorf, T., Hecht, H. J., Schmid, R., Schomburg, D., Rydel, T. J., Oliver, J. D., et al. (1997) The open conformation of a *Pseudomonas* lipase. Structure **5**, 187–202
- 82 Steinbüchel, A., Aerts, K., Babel, W., Follner, C., Liebergesell, M., Madkour, M. H., Mayer, F., PieperFurst, U., Pries, A., Valentin, H. E. and Wieczorek, R. (1995) Considerations on the structure and biochemistry of bacterial polyhydroxyalkanoic acid inclusions. Can. J. Microbiol. 41, 94–105
- 83 Gerngross, T. U. and Martin, D. P. (1995) Enzyme-catalyzed synthesis of poly[(R)-(-)-3-hydroxybutyrate]: formation of macroscopic granules in vitro. Proc. Natl. Acad. Sci. U.S.A. 92, 6279–6283

- 84 Amara, A. A., Steinbüchel, A. and Rehm, B. H. (2002) In vivo evolution of the Aeromonas punctata polyhydroxyalkanoate (PHA) synthase: isolation and characterization of modified PHA synthases with enhanced activity. Appl. Microbiol. Biotechnol. 59, 477–482
- 85 Kichise, T., Taguchi, S. and Doi, Y. (2002) Enhanced accumulation and changed monomer composition in polyhydroxyalkanoate (PHA) copolyester by in vitro evolution of *Aeromonas caviae* PHA synthase. Appl. Environ. Microbiol. **68**, 2411–2419
- 86 Taguchi, S., Nakamura, H., Hiraishi, T., Yamato, I. and Doi, Y. (2002) In vitro evolution of a polyhydroxybutyrate synthase by intragenic suppression-type mutagenesis. J. Biochem. (Tokyo) **131**, 801–806
- 87 Haywood, G. W., Anderson, A. J. and Dawes, E. A. (1989) The importance of PHB-synthase substrate specificity in polyhydroxyalkanoate synthesis by *Alcaligenes eutrophus*. FEMS Microbiol. Lett. **57**, 1–6
- 88 Qi, Q., Rehm, B. H. and Steinbüchel, A. (1997) Synthesis of poly(3-hydroxyalkanoates) in Escherichia coli expressing the PHA synthase gene phaC2 from *Pseudomonas* aeruginosa: comparison of PhaC1 and PhaC2. FEMS Microbiol. Lett. **157**, 155–162
- 89 Qi, Q., Steinbüchel, A. and Rehm, B. H. (1998) Metabolic routing towards polyhydroxyalkanoic acid synthesis in recombinant *Escherichia coli* (fadR): inhibition of fatty acid beta-oxidation by acrylic acid. FEMS Microbiol. Lett. **167**, 89–94
- 90 Langenbach, S., Rehm, B. H. A. and Steinbüchel, A. (1997) Functional expression of the PHA synthase gene PhaC1 from Pseudomonas aeruginosa in *Escherichia coli* results in poly(3-hydroxyalkanoate) synthesis. FEMS Microbiol. Lett. **150**, 303–309
- 91 Steinbüchel, A. and Valentin, H. E. (1995) Diversity of Bacterial Polyhydroxyalkanoic Acids. FEMS Microbiol. Lett. **128**, 219–228
- 92 Steinbüchel, A., Fuchtenbusch, B., Gorenflo, V., Hein, S., Jossek, R., Langenbach, S. and Rehm, B. H. A. (1998) Biosynthesis of polyesters in bacteria and recombinant organisms. Polymer Degrad. Stab. 59, 177–182
- 93 Mayer, F. and Hoppert, M. (1997) Determination of the thickness of the boundary layer surrounding bacterial PHA inclusion bodies, and implications for models describing the molecular architecture of this layer. J. Basic Microbiol. **37**, 45–52
- 94 Gerngross, T. U., Reilly, P., Stubbe, J., Sinskey, A. J. and Peoples, O. P. (1993) Immunocytochemical analysis of poly-beta-hydroxybutyrate (PHB) synthase in *Alcaligenes eutrophus* H16: localization of the synthase enzyme at the surface of PHB granules. J. Bacteriol. **175**, 5289–5293
- 95 Štuart, E. S., Tehrani, A., Valentin, H. E., Dennis, D., Lenz, R. W. and Fuller, R. C. (1998) Protein organization on the PHA inclusion cytoplasmic boundary. J. Biotechnol. 64, 137–144
- 96 Valentin, H. E., Stuart, E. S., Fuller, R. C., Lenz, R. W. and Dennis, D. (1998) Investigation of the function of proteins associated to polyhydroxyalkanoate inclusions in *Pseudomonas putida* BMO1. J. Biotechnol. **64**, 145–157
- 97 York, G. M., Lupberger, J., Tian, J. M., Lawrence, A. G., Stubbe, J. and Sinskey, A. J. (2003) *Ralstonia eutropha* H16 encodes two and possibly three intracellular poly[p-(-)-3-hydroxybutyrate] depolymerase genes. J. Bacteriol. **185**, 3788–3794
- 98 Saegusa, H., Shiraki, M., Kanai, C. and Saito, T. (2001) Cloning of an intracellular poly[b(–)-3-hydroxybutyrate] depolymerase gene from *Ralstonia eutropha* H16 and characterization of the gene product. J. Bacteriol. **183**, 94–100
- 99 Handrick, R., Reinhardt, S. and Jendrossek, D. (2000) Mobilization of poly(3-hydroxybutyrate) in *Ralstonia eutropha*. J. Bacteriol. **182**, 5916–5918
- 100 Jurasek, L. and Marchessault, R. H. (2002) The role of phasins in the morphogenesis of poly(3-hydroxybutyrate) granules. Biomacromolecules. 3, 256–261
- 101 Pieper-Furst, U., Madkour, M. H., Mayer, F. and Steinbüchel, A. (1995) Identification of the region of a 14-kilodalton protein of *Rhodococcus ruber* that is responsible for the binding of this phasin to polyhydroxyalkanoic acid granules. J. Bacteriol. **177**, 2513–2523
- 102 Wieczorek, R., Steinbüchel, A. and Schmidt, B. (1996) Occurrence of polyhydroxyalkanoic acid granule-associated proteins related to the *Alcaligenes eutrophus* H16 GA24 protein in other bacteria. FEMS Microbiol. Lett. **135**, 23–30
- 103 York, G. M., Junker, B. H., Stubbe, J. A. and Sinskey, A. J. (2001) Accumulation of the PhaP phasin of *Ralstonia eutropha* is dependent on production of polyhydroxybutyrate in cells. J. Bacteriol. **183**, 4217–4226
- 104 York, G. M., Stubbe, J. and Sinskey, A. J. (2001) New insight into the role of the PhaP phasin of *Ralstonia eutropha* in promoting synthesis of polyhydroxybutyrate. J. Bacteriol. **183**, 2394–2397
- 105 Potter, M., Madkour, M. H., Mayer, F. and Steinbüchel, A. (2002) Regulation of phasin expression and polyhydroxyalkanoate (PHA) granule formation in *Ralstonia eutropha* H16. Microbiology (Reading, U.K.) **148**, 2413–2426
- 106 York, G. M., Stubbe, J. and Sinskey, A. J. (2002) The *Ralstonia eutropha* PhaR protein couples synthesis of the PhaP phasin to the presence of polyhydroxybutyrate in cells and promotes polyhydroxybutyrate production. J. Bacteriol. **184**, 59–66
- 107 Maehara, A., Taguchi, S., Nishiyama, T., Yamane, T. and Doi, Y. (2002) A repressor protein, PhaR, regulates polyhydroxyalkanoate (PHA) synthesis via its direct interaction with PHA. J. Bacteriol. **184**, 3992–4002

- 108 Prieto, M. A., Buhler, B., Jung, K., Witholt, B. and Kessler, B. (1999) PhaF, a polyhydroxyalkanoate-granule-associated protein of *Pseudomonas oleovorans* GPo1 involved in the regulatory expression system for pha genes. J. Bacteriol. **181**, 858–868
- 109 Klinke, S., de Roo, G., Witholt, B. and Kessler, B. (2000) Role of phaD in accumulation of medium-chain-length poly(3-hydroxyalkanoates) in *Pseudomonas oleovorans*. Appl. Environ. Microbiol. **66**, 3705–3710
- 110 Wieczorek, R., Pries, A., Steinbüchel, A. and Mayer, F. (1995) Analysis of a 24-kilodalton protein associated with the polyhydroxyalkanoic acid granules in *Alcaligenes-Eutrophus*. J. Bacteriol. **177**, 2425–2435
- 111 Hoffmann, N. and Rehm, B. H. A. (2003) Transcriptional analysis of polyhydroxyalkanoate biosynthesis genes in *Pseudomonas putida* and *Pseudomonas aeruginosa*: Evidence for a regulatory model. Microbiology (in the press)
- 112 Kessler, B. and Witholt, B. (2001) Factors involved in the regulatory network of polyhydroxyalkanoate metabolism. J. Biotechnol. 86, 97–104
- 113 Hoppert, M. and Mayer, F. (1999) Principles of macromolecular organization and cell function in bacteria and archaea. Cell Biochem. Biophys. 31, 247–284
- 114 Barnard, G. N. and Sanders, J. K. (1989) The poly-beta-hydroxybutyrate granule in vivo. A new insight based on NMR spectroscopy of whole cells. J. Biol. Chem. 264, 3286–3291
- 115 Qi, Q., Steinbüchel, A. and Rehm, B. H. A. (2000) In vitro synthesis of poly(3-hydroxydecanoate): purification and enzymatic characterization of type II polyhydroxyalkanoate synthases PhaC1 and PhaC2 from Pseudomonas aeruginosa. Appl. Microbiol. Biotechnol. **54**, 37–43
- 116 Ren, Q., de Roo, G., Kessler, B. and Witholt, B. (2000) Recovery of active medium-chainlength-poly-3-hydroxyalkanoate polymerase from inactive inclusion bodies using ion-exchange resin. Biochem. J. **349**, 599–604
- 117 Sim, S. J., Snell, K. D., Hogan, S. A., Stubbe, J., Rha, C. and Sinskey, A. J. (1997) PHA synthase activity controls the molecular mass and polydispersity of polyhydroxybutyrate in vivo. Nat. Biotechnol. **15**, 63–67
- 118 Jossek, R., Reichelt, R. and Steinbüchel, A. (1998) In vitro biosynthesis of poly(3-hydroxybutyric acid) by using purified poly(hydroxyalkanoic acid) synthase of *Chromatium vinosum*. Appl. Microbiol. Biotechnol. **49**, 258–266
- 119 Doi, Y. (1992) Microbial synthesis and properties of polyhydroxy-alkanoates. MRS Bull. 17, 39–42
- 120 Jossek, R. and Steinbüchel, A. (1998) In vitro synthesis of poly(3-hydroxybutyric acid) by using an enzymatic coenzyme A recycling system. FEMS Microbiol. Lett. 168, 319–324
- 121 Sato, Y., Tajima, K., Munekata, M. and Erata, T. (2000) In vitro PHA synthesis with CoA recycling and ATP regeneration. The 8th International Symposium of Biological Polyester, Abstract, Cambridge, MA, U.S.A.
- 122 Liu, S. J. and Steinbüchel, A. (2000) Exploitation of butyrate kinase and phosphotransbutyrylase from Clostridium acetobutylicum for the in vitro biosynthesis of poly(hydroxyalkanoic acid). Appl. Microbiol. Biotechnol. 53, 545–552
- 123 Liu, S. J. and Steinbüchel, A. (2000) A novel genetically engineered pathway for synthesis of poly(hydroxyalkanoic acids) in *Escherichia coli*. Appl. Environ. Microbiol. 66, 739–743
- 124 Rehm, B. H. A., Krüger, N. and Steinbüchel, A. (1998) A new metabolic link between fatty acid de novo synthesis and polyhydroxyalkanoic acid synthesis – The phaG gene from *Pseudomonas putida* KT2440 encodes a 3-hydroxyacyl-acyl carrier protein coenzyme A transferase. J. Biol. Chem. **273**, 24044–24051
- 125 Kraak, M. N., Smits, T. H. M., Kessler, B. and Witholt, B. (1997) Polymerase C1 levels and poly(*R*-3-hydroxyalkanoate) synthesis in wild-type and recombinant *Pseudomonas* strains. J. Bacteriol. **179**, 4985–4991
- 126 Mukai, K., Doi, Y., Sema, Y. and Tomita, K. (1993) Substrate specificities in hydrolysis of polyhydroxyalkanoates by microbial esterases. Biotechnol. Lett. 15, 601–604
- 127 Jaeger, K. E., Steinbüchel, A. and Jendrossek, D. (1995) Substrate specificities of bacterial polyhydroxyalkanoate depolymerases and lipases – bacterial lipases hydrolyze poly(omega-hydroxyalkanoates). Appl. Environ. Microbiol. 61, 3113–3118
- 128 Kusaka, S., Abe, H., Lee, S. Y. and Doi, Y. (1997) Molecular mass of poly[(*R*)-3-hydroxybutyric acid] produced in a recombinant *Escherichia coli*. Appl. Microbiol. Biotechnol. **47**, 140–143
- 129 Williams, S. F., Martin, D. P., Horowitz, D. M. and Peoples, O. P. (1999) PHA applications: addressing the price performance issue I. Tissue engineering. Int. J. Biol. Macromol. 25, 111–121
- 130 Lee, S. Y., Choi, J. and Wong, H. H. (1999) Recent advances in polyhydroxyalkanoate production by bacterial fermentation: mini-review. Int. J. Biol. Macromol. 25, 31–36
- 131 Chen, G. Q., Zhang, G., Park, S. J. and Lee, S. Y. (2001) Industrial scale production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Appl. Microbiol. Biotechnol. 57, 50–55

- 132 Steinbüchel, A. and Füchtenbusch, B. (1998) Bacterial and other biological systems for polyester production. Trends Biotechnol. 16, 419–427
- 133 Hoffmann, N., Amara, A. A., Beermann, B. B., Qi, Q., Hinz, H. J. and Rehm, B. H. A. (2002) Biochemical characterization of the Pseudomonas putida 3-hydroxyacyl ACP:CoA transacylase, which diverts intermediates of fatty acid de novo biosynthesis. J. Biol. Chem. 277, 42926–42936
- 134 Hoffmann, N., Steinbüchel, A. and Rehm, B. H. A. (2000) Homologous functional expression of cryptic phaG from *Pseudomonas oleovorans* establishes the transacylase-mediated polyhydroxyalkanoate biosynthetic pathway. Appl. Microbiol. Biotechnol. **54**, 665–670
- 135 Hoffmann, N., Steinbüchel, A. and Rehm, B. H. A. (2000) The *Pseudomonas aeruginosa* phaG gene product is involved in the synthesis of polyhydroxyalkanoic acid consisting of medium-chain-length constituents from non-related carbon sources. FEMS Microbiol. Lett. **184**, 253–259
- 136 Fukui, T., Shiomi, N. and Doi, Y. (1998) Expression and characterization of (*R*)-specific enoyl coenzyme A hydratase involved in polyhydroxyalkanoate biosynthesis by *Aeromonas caviae*. J. Bacteriol. **180**, 667–673
- 137 Hisano, T., Tsuge, T., Fukui, T., Iwata, T., Miki, K. and Doi, Y. (2003) Crystal structure of the (*R*)-specific enoyl-CoA hydratase from *Aeromonas caviae* involved in polyhydroxyalkanoate biosynthesis. J. Biol. Chem. **278**, 617–624
- 138 Tsuge, T., Taguchi, K., Seiichi, Taguchi, and Doi, Y. (2003) Molecular characterization and properties of (*R*)-specific enoyl-CoA hydratases from *Pseudomonas aeruginosa*: metabolic tools for synthesis of polyhydroxyalkanoates via fatty acid ss-oxidation. Int. J. Biol. Macromol. **31**, 195–205
- 139 Matsumoto, K., Matsusaki, H., Taguchi, S., Seki, M. and Doi, Y. (2001) Cloning and characterization of the *Pseudomonas sp.* 61–63 phaG gene involved in polyhydroxyalkanoate biosynthesis. Biomacromolecules 2, 142–147
- 140 Fiedler, S., Steinbüchel, A. and Rehm, B. H. A. (2000) PhaG-mediated synthesis of poly(3-hydroxyalkanoates) consisting of medium-chain-length constituents from nonrelated carbon sources in recombinant *Pseudomonas fragi*. Appl. Environ. Microbiol. 66, 2117–2124
- 141 Fukui, T., Yokomizo, S., Kobayashi, G. and Doi, Y. (1999) Co-expression of polyhydroxyalkanoate synthase and (*R*)-enoyl-CoA hydratase genes of *Aeromonas caviae* establishes copolyester biosynthesis pathway in *Escherichia coli*. FEMS Microbiol. Lett. **170**, 69–75
- 142 Rehm, B. H. A., Mitsky, T. A. and Steinbüchel, A. (2001) Role of fatty acid de novo biosynthesis in polyhydroxyalkanoic acid (PHA) and rhamnolipid synthesis by pseudomonads: Establishment of the transacylase (PhaG)-mediated pathway for PHA biosynthesis in *Escherichia coli*. Appl. Environ. Microbiol. **67**, 3102–3109
- 143 Fiedler, S., Steinbüchel, A. and Rehm, B. H. A. (2002) The role of the fatty acid beta-oxidation multienzyme complex from *Pseudomonas oleovorans* in polyhydroxyalkanoate biosynthesis: molecular characterization of the fadBA operon from *P. oleovorans* and of the enoyl-CoA hydratase genes phaJ from *P. oleovorans* and *Pseudomonas putida*. Arch. Microbiol. **178**, 149–160
- 144 Matsumoto, K., Nakae, S., Taguchi, K., Matsusaki, H., Seki, M. and Doi, Y. (2001) Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyalkanoates) copolymer from sugars by recombinant *Ralstonia eutropha* harboring the phaC1Ps and the phaGPs genes of *Pseudomonas* sp. 61–63. Biomacromolecules. **2**, 934–939
- 145 Green, P. R., Kemper, J., Schechtman, L., Guo, L., Satkowski, M., Fiedler, S., Steinbüchel, A. and Rehm, B. H. A. (2002) Formation of short chain length/medium chain length polyhydroxyalkanoate copolymers by fatty acid beta-oxidation inhibited *Ralstonia eutropha*. Biomacromolecules **3**, 208–213
- 146 Gao, H. J., Wu, Q. N. and Chen, G. Q. (2002) Enhanced production of p-(-)-3-hydroxybutyric acid by recombinant *Escherichia coli*. FEMS Microbiol. Lett. **213**, 59–65
- 147 Lee, S. Y., Lee, Y. and Wang, F. (1999) Chiral compounds from bacterial polyesters: sugars to plastics to fine chemicals. Biotechnol. Bioeng. 65, 363–368
- 148 de Roo, G., Kellerhals, M. B., Ren, Q., Witholt, B. and Kessler, B. (2002) Production of chiral *R*-3-hydroxyalkanoic acids and *R*-3-hydroxyalkanoic acid methylesters via hydrolytic degradation of polyhydroxyalkanoate synthesized by pseudomonads. Biotechnol. Bioeng. **77**, 717–722
- 149 van Wegen, R. J., Lee, S. Y. and Middelberg, A. P. (2001) Metabolic and kinetic analysis of poly(3-hydroxybutyrate) production by recombinant *Escherichia coli*. Biotechnol. Bioeng. **74**, 70–80
- 150 Park, S. J., Park, J. P. and Lee, S. Y. (2002) Metabolic engineering of *Escherichia coli* for the production of medium-chain-length polyhydroxyalkanoates rich in specific monomers. FEMS Microbiol. Lett. **214**, 217–222
- 151 Madison, L. L. and Huisman, G. W. (1999) Metabolic engineering of poly(3-hydroxyalkanoates): From DNA to plastic. Microbiol. Mol. Biol. Rev. 63, 21–53

- 152 Spiekermann, P., Rehm, B. H., Kalscheuer, R., Baumeister, D. and Steinbüchel, A. (1999) A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. Arch. Microbiol. **171**, 73–80
- 153 Poirier, Y. (2001) Production of polyesters in transgenic plants. Adv. Biochem. Eng. Biotechnol. 71, 209–240

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- 154 Williams, S. F. and Peoples, O. P. (1996) Biodegradable plastics from plants. Chemtech. 26, 38–44
- 155 Vanderleij, F. R. and Witholt, B. (1995) Strategies for the Sustainable Production of new biodegradable polyesters in plants a review. Can. J. Microbiol. **41**, 222–238
- 156 Feng, D. F. and Doolittle, R. F. (1987) Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J. Mol. Evol. 25, 351–360