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Research article

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Bacterial variations on the methionine salvage pathway Agnieszka Sekowska^{1,2,6}, Valérie Dénervaud³, Hiroki Ashida⁴,

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Abstract

Background: The thiomethyl group of S-adenosylmethionine is often recycled as methionine from methylthioadenosine. The corresponding pathway has been unravelled in *Bacillus subtilis*. However methylthioadenosine is subjected to alternative degradative pathways depending on the organism.

Results: This work uses genome in silico analysis to propose methionine salvage pathways for *Klebsiella* pneumoniae, Leptospira interrogans, Thermoanaerobacter tengcongensis and Xylella fastidiosa. Experiments performed with mutants of *B. subtilis* and *Pseudomonas aeruginosa* substantiate the hypotheses proposed. The enzymes that catalyze the reactions are recruited from a variety of origins. The first, ubiquitous, enzyme of the pathway, MtnA (methylthioribose-I-phosphate isomerase), belongs to a family of proteins related to eukaryotic initiation factor 2B alpha. *mtnB* codes for a methylthioribulose-I-phosphate dehydratase. Two reactions follow, that of an enolase and that of a phosphatase. While in *B. subtilis* this is performed by two distinct polypeptides, in the other organisms analyzed here an enolase-phosphatase yields 1,2-dihydroxy-3-keto-5-methylthiopentene. In the presence of dioxygen an aci-reductone dioxygenase yields the immediate precursor of methionine, ketomethylthiobutyrate. Under some conditions this enzyme produces carbon monoxide in *B. subtilis*, suggesting a route for a new gaseous mediator in bacteria. Ketomethylthiobutyrate is finally transaminated by an aminotransferase that exists usually as a broad specificity enzyme (often able to transaminate aromatic aminoacid keto-acid precursors or histidinol-phosphate).

Conclusion: A functional methionine salvage pathway was experimentally demonstrated, for the first time, in *P. aeruginosa*. Apparently, methionine salvage pathways are frequent in Bacteria (and in Eukarya), with recruitment of different polypeptides to perform the needed reactions (an ancestor of a translation initiation factor and RuBisCO, as an enolase, in some Firmicutes). Many are highly dependent on the presence of oxygen, suggesting that the ecological niche may play an important role for the existence and/or metabolic steps of the pathway, even in phylogenetically related bacteria. Further work is needed to uncover the corresponding steps when dioxygen is scarce or absent (this is important to explore the presence of the pathway in Archaea). The thermophile *T. tengcongensis*, that thrives in the absence of oxygen, appears to possess the pathway. It will be an interesting link to uncover the missing reactions in anaerobic environments.

Background

Availability of a huge number of genome sequences (1004 ongoing or completed genome sequencing programmes are listed at http://wit.integratedgenomics.com/GOLD/ on February 17th, 2004) now allows scientists to explore new avenues in the deciphering of metabolic pathways. While sulfur is ubiquitously used in life, it is a highly reactive atom, hence very sensitive to the oxido-reduction potential of its local environment. It is also very sensitive to dioxygen and reactive oxygen species. As a consequence, sparing processes that enable the cell to keep a sulfur atom in its final chemical and electronic environment (inside well characterized groups of atoms) often exists in cells [1]. A case in point is the fate of the ubiquitous S-adenosylmethionine molecule (AdoMet), which can donate each one of its sulfur connected groups: methyl-, aminobutyryl- (usually, after decarboxylation as aminopropyl-), and adenosyl- (ribosyl-). A vast number of AdoMet mediated methylation events continuously happen in most, if not all organisms. Methylases are present at the level of DNA metabolism, RNA metabolism and protein post-translational modifications [2-4]. They are also involved in formation of a variety of small metabolites (essential coenzymes or prosthetic groups in particular [5,6]). This group transfer results in the synthesis of S-adenosyl homocysteine (AdoHcy). The latter molecule has to be recycled or further metabolized, either after excision of the adenine moiety by hydrolysis or phosphorolysis, or by direct hydrolysis into adenosine and homocysteine. When aminopropyl- is transferred, methylthioadenosine (MTA) is a by-product. It is produced during synthesis of spermidine and spermine, and also of N-acylhomoserine lactone synthases in Gram-negative bacteria [7] and 1-aminocyclopropane-1-carboxylate (ACC) synthase in plants (and possibly in bacteria producing ethylene). Furthermore, other still unknown reactions might exist, transferring the aminopropyl-group to yet unrecognized acceptors (most likely to be nitrogen atoms). Recycling the sulfur-containing compounds Sadenosylhomocysteine and MTA is a challenge for all cells. MTA may regenerate methionine in the process. The biochemical details of the MTA to methionine pathway remains unknown in most organisms. This prompted us to make a thorough analysis of available genome sequences to try and identify some of the metabolic pathways involved in MTA recycling through genomic and functional comparisons with our recent findings [8]. The present work aims at combining comparative genomics and biochemical or genetic data to propose a list of identified and putative genes associated to the corresponding functions. A self-consistent nomenclature will be proposed in parallel, so that homologous functions in various organisms could tentatively be identified from their genome sequences.

Results

MTA can be exported from the cell, or recycled, beginning with two main alternative pathways (Figure 1). The most energy sparing one starts with MTA phosphorolysis and yields methylthioribose-1-phosphate (MTR-1-P) [9], while the second one splits this first step into two steps: the first one being hydrolysis of MTA to methylthioribose (MTR) and adenine, followed by ATP-dependent phosphorylation of MTR by MTR kinase [10,11]. In this latter case, MTR can also be excreted from the cell, especially in organisms that are not challenged by a high level of dioxygen (this happens in facultative anaerobes living in rich media, such as *Escherichia coli* [12]).

MTA phosphorylase

The gene coding for MTA phosphorylase (named *mtnP*, see below) has been identified in many organisms, in all three kingdoms: Archaea, Bacteria and Eukarya. The activity of the enzyme has long been studied as a target to fight some kinds of tumor cells [13]. A PROSITE signature, PDOC00946, [GST]-x-G-[LIVM]-G-x-[PA]-S-x-[GSTA]-Ix(3)-E-L, is common to three classes of nucleoside phosphorylases, purine nucleosidases, uridine nucleosidases and MTA phosphorylase. A second signature, PDOC00954 [LIV]-x(3)-G-x(2)-H-x-[LIVMFY]-x(4)-[LIVMF]-x(3)-[ATV]-x(1,2)-[LIVM]-x-[ATV]-x(4)-[GN]x(3,4)-[LIVMF](2)-x(2)-[STN]-[SA]-x-G-[GS]-[LIVM] labels some other phosphorylases (including MTA phosphorylase), from Eukarya in particular.

This family can be narrowed into three conserved domains families: COG0005, purine nucleoside phosphorylase, pfam00896, phosphorylase family 2, KOG3985, MTA phosphorylase. Interestingly, these purine nucleoside phosphorylase families share most of their sequence in amino acid residues, except for the very first residues, that appear to be made of a particular sequence in the case of MTA phosphorylase. In contrast to other purine nucleoside phosphorylases, it appears likely that the amino-terminal end of MTA phosphorylase starts very early upstream of the G [ST]G sequence that is conserved in purine nucleoside phosphorylases: the exception we find to the rule is the sequence present in Synechocystis PCC6803, but a methionine codon is placed just at the expected beginning of the protein, while translation of the upstream region is not confirmed by biochemical analysis (and it is notorious that start codons are difficult to identify in general and in cyanobacteria in particular). Immediately upstream of this first consensus sequence, a G amino acid residue is found in all known and putative MTA phosphorylases, as well as other features of the region displayed in Figure 2. It was therefore important to explore whether in Pseudomonas aeruginosa gene PA-3004 (mtnP), which has this signature, codes for MTA phosphorylase. The presence of this gene is not ubiq-



Figure I

The methionine salvage pathway. As a reference, the pathway is shown in *B. subtilis* (solid lines). Variants of the pathway are shown in broken lines.

uitous in gamma-proteobacteria, since this gene seems to be absent from *Klebsiella pneumoniae* (the sequence of the complete genome is not publicly available, sequences of the ongoing genome project are available at <u>http://</u> <u>genome.wustl.edu/blast/client.pl</u>).

The *P. aeruginosa* methionine-auxotroph PAO503, which carries a mutation in the *metZ* gene encoding *O*-succinyl-homoserine sulfhydrylase [14], grew well on minimal medium E when supplemented with either methionine, homocysteine or MTA. By contrast, a mutant derived from PAO503 by gene replacement, PAO6422 (*metZ mtnP*), did not grow with MTA, but still responded to methionine and homocysteine (Table 1). These results demonstrate that *P. aeruginosa* has an MTA recycling pathway and that

MTA phosphorylase has an essential function in this pathway.

An alignment of MTA phosphorylase and purine phosphorylase is provided in Figure 2, with sequences from *Thermoanaerobacter tengcongensis* (Firmicutes [15]) and *P. aeruginosa* (gamma-proteobacteria), as well as from an organism that is usually difficult to classify in terms of proteins, *Leptospira interrogans*. In addition to the position of the beginning of the protein (shorter in MTA phosphorylase) residues that are discriminating between general scavenging purine phosphorylase and MTA phosphorylase are indicated in Figure 2, around the common motif [FIMLV]-[IV]-X-G-[ST]-G-X₁₆₋₂₂-P. Noteworthy features are the presence of conserved residues in MTA phosphorylase.

MTA phosphorylase

ORBERTA DESCEN		1933.000		11.30000	C129239 Ad			1 10 10 10 10 10 10 10 10 10 10 10 10 10	030000.032	51045
PSEAE	1	MSVY	AIIGGTGLTQLEGL	TLS	ESL-	PIETPYG	APSA-PLQRGR	YAGRE	VLFL	49
THETE	1	MEK	AIIGGTGVYDVADK		VVQK	KVETKYG	TVEVDVVTVEG	ED	IVFL	45
HOMSA	9	AVKI	GIIGGTGLDDPEIL	EGR	TEK-	YVDTPFG	KPSDALILG-K	IKNVD	CVLL	58
CAEEL	1	MVKV	GIIGGSGLEDPNIL	LDP	VTV-	AVDTPYG	KPSDDVVEG-T	INGVE	CVLL	50
SCHPO	7	PILL	GVIGGSGFYDLPGF	DIV	ESV-	NPITPWG	YPASPISIART	TSG FL	IAFL	57
SYNEC	(35)	MVNAPI	GIIGGSGLYQMEAL	KNV	EEV-	TLDTPFG	APSDSFIVG-E	LAGVR	VAFL	84
MYCTU	5	GRML	GVIGGSGFYTFFGS	DTR	TV	NSDTPYG	QPSAPITIG	TIGVHD	VAFL	53
METJA	1	MI	GIIGGTGIAEILKG	DKE	E	IINTKYG	KA-RVIIDK	ENE	VVLL	42
METTH	1	MI	GIIGGTGIYEMAEY	GRL	ERRG	SLITPYG	KTPEISVFK	LHGRR	VAFI	48
LEPIN	1	MSYNVRA	AIIGGTGLYSLEGM	ELI	EEI-	FPDTPWG	KPSDKIKIG-K	YKGKL	IAFL	53
XYLFA	1	MQTIAL	AVIGGTGVYTLSQL	DDV	QVY-	EVETLYG	RPSGPIRVG-M	LFGQR	VAFF	52

Purine phosphorylase

MYCTU	27	EHDV	AVVLGSGWLPAVAA	LGS	PTT VLP	QAELPGF	VPPTAAGHAGE	LLSVPIGA	HRVL	83
ESCCO	25	TPRV	AFILGSGLGALADQ	IEN	AVAIS	YEKLPGF	PVSTVHGHAGE	LVLGHLQG	VPVV	80
SACCE	37	PPRT	LIICGSGLGGISTK	LSR	DNPPPVTVP	YQDIPGF	KKSTVPGHSGT	LMFGSMNG	SPVV	96
BACSU	19	SPKI	GLILGSGLGILADE	IEN	PVKLK	YEDIPEF	PVSTVEGHAGQ	LVLGTLEG	VSVI	74
GEOST	20	SPQI	GLILGSGLGVLADE	IEQ	AIKIP	YSDIPNF	PVSTVEGHAGQ	LVYGQLEG	ATVV	75
BOSTA	24	RPQV	AVICGSGLGGLVNK	LTQ	AQTFD	YSEIPNF	PESTVPGHAGR	LVFGILNG	RACV	79

Figure 2

Comparison of purine and MTA phosphorylases. The beginning of MTA phosphorylases is aligned with that of purine phosphorylases extracted from phylogenetically widely diverse organisms: PSEAE *Pseudomonas aeruginosa*; THETE *Thermoanaerobacter tengcongensis*; HOMSA *Homo sapiens*; CAEEL *Caenorhabditis elegans*; SCHPO *Schizosaccharomyces pombe*; SYNEC *Synechocystis* PCC 6803; MYCTU *Mycobacterium tuberculosis*; METJA *Methanococcus jannaschii*; METTH *Methanobacterium thermoautotrophi-cum*; LEPIN *Leptospira interrogans*; XYLFA *Xylella fastidiosa*; ESCHO *Escherichia coli*; SACCE *Saccharomyces cerevisiae*; BACSU *Bacillus subtilis*; GEOST *Geobacillus stearothermophilus*; BOSTA *Bos taurus*. Conserved residues in both classes of enzymes are emphasized [54]. In red are shown conserved residues that are specific to one class, while conservative replacements are indicated in blue. The three residues in green indicate a region that suffered an insertion/deletion event in the course of evolution that separated the two classes. The position of the first residue of the alignment shown is displayed in blue. Note that for *Synechocystis* the published sequence starts earlier, but that the residue indicated is likely to be the real start of the protein.

Table 1: Growth of Pseudomonas aeruginosa mutants on various sulfur sources

Strain	МТА	Methionine	Homocysteine
PAO503 metZ9011	+	+	+
PAO6412 metZ9011 mtnB::Ω-Sp/Sm	-	+	+
PAO6422 metZ9011 mtnP::Ω-Sp/Sm	-	+	+a

^a weaker growth as compared to the other constructs.

ylase, that differ from those at the same position in the other phosphorylases: G upstream of the highly conserved G-[ST]-G motif and a T is present upstream of the partially conserved P residue, with a downstream aromatic residue followed by a G in MTA phosphorylase (respectively L or I and F in other phosphorylases). The product of the reaction is MTR-1-P, which undergoes further processing as detailed below.

MTA hydrolysis and methylthioribose phosphorylation

AdoMet-dependent methylases produce AdoHcy, while aminopropyl-group transfer produces MTA. It was once thought that the metabolism of AdoHcy was split in various organisms into two alternative pathways [16]: the molecule was recycled either by AdoHcy hydrolase that generates homocysteine and adenosine, or by the sequential action of AdoHcy nucleosidase that generates adenine and S-ribosylhomocysteine, followed by a S-ribosylhomocysteine-cleavage enzyme. The hydrolase has been now found in many organisms, such as *Pseudomonas* sp., *Homo sapiens, L. interrogans, Aquifex* sp. and *Thermotoga maritima* but it is not present in *E. coli* nor in *Bacillus subtilis,* at least with a related sequence [17].

In *E. coli* the nucleosidase (coded by gene *pfs*) has in fact a lower specificity since it hydrolyses both AdoHcy and MTA [18]. In B. subtilis it has been experimentally demonstrated that the gene initially termed *yrrU* has this activity [19]. The counterpart of this enzyme exists in K. pneumoniae (Figure 3). In many bacteria (including E. coli) the fate of the MTR thus produced is not known, but the molecule is found to accumulate in the external medium, suggesting the presence of an export system [12]. In B. subtilis experiments using radioactive labelling have shown that a kinase exists that transforms MTR into MTR-1-P (Figure 1). It is encoded by a gene belonging to a S-box riboswitch-controlled operon [20,21], ykrT (renamed here *mtnK*, see below) [22]. In contrast with the situation in *E*. coli, a counterpart of this gene exists in K. pneumoniae (Figure 3), consistent with the existence of a methionine salvage pathway in the latter. Interestingly, this enzyme that has been crystallized recently [23] does not belong to the ribo- or deoxyribokinase family, but, rather, to the choline/ethanolamine kinase family [24].

The elusive dowstream section of the methionine salvage pathway

It has long been known that MTA, once converted to adenine and MTR-1-P was able to regenerate methionine. This was found in organisms as distant as *H. sapiens* or *K. pneumoniae*. The overall reaction scheme has been unraveled by Abeles and coworkers in *K. pneumoniae* [25]. However, the actual enzymes or genes involved for each step have not been uncovered in totality. A gene inactivation study in *B. subtilis* revealed the involvement of several genes [26], but the exact nature of the enzymes needed for each step in this organism was only discovered when appropriate biochemical experiments were set up [8]. Briefly, the pathway in B. subtilis begins as follows: MTR-1-P is converted into MTRu-1-P by the isomerase encoded by gene *ykrS* (now *mtnA*, see new proposed nomenclature below) [8,22]. Remarkably, it has been observed that this gene, expressed at a level similar to that of ykrT (mtnK) [22], codes for a protein with a sequence annotated as similar to that of eukaryotic initiation factor eIF-2B (alpha subunit). Figure 4 shows a high degree of conserved residues in the corresponding alignment with genes from Firmicutes, gamma-proteobacteria, plants and humans. Interestingly this sequence is similar, especially in its carboxy-terminal end (see the Mus musculus sequence), with eIF-2B-alpha, but, as can be seen in the Figure, this similarity, although unambiguous, should perhaps not have been emphasized in genome annotations.

The next step is dehydration of MTRu-1-P by MTRu-1-P dehydratase encoded by gene ykrY (now mtnB), a member of operon ykrWXYZ, under the control of an S-box riboswitch [20,21]. The product of the reaction is the diketone 2,3-diketo-5-methylthiopentyl-1-phosphate. A counterpart of this gene can be found in many organisms (Figure 5), where it is usually associated to other genes involved in the pathway. This is the case, for example in K. pneumoniae. In P. aeruginosa, the operon aroC, PA-1682, PA-1683, PA-1684, mas http://bioinfo.hku.hk/AeruList encodes proteins similar to chorismate synthase, a metabolite transporter, MTRu-1-P dehydratase, 1,2-dihydroxy-3keto-5-methylthiopentene dioxygenase and enolasephosphatase, respectively. Indeed, the methionine-auxotroph (metZ [14]) P. aeruginosa strain PAO503, which grew well on minimal medium E when supplemented with either methionine, homocysteine or MTA, lost the ability to use MTA when the *mtnB* gene (PA-1683) was inactivated by gene replacement in strain PAO6412 (metZ *mtnB*) (Table 1). Thus, in addition to the MTA phosphorylase, P. aeruginosa has an MTRu-1-P dehydratase which has an essential function in the methionine salvage pathway (Table 1). The MtnB sequence is a member of a large cluster of proteins that form class II aldolases (COG235, Pfam0096). Among these enzymes one finds L-ribulose-5-phosphate-4-epimerase, suggesting that recognition of the ribulose backbone is common to a variety of enzymes of the class. The N-terminal domain of these proteins form class KOG2631, which appears to overlap exactly with the dehydratase.

The dehydratase product is further metabolized by 2,3diketo-5-methylthiopentyl-1-phosphate enolase, encoded in *B. subtilis* by gene *ykrW* (now *mtnW*). Interestingly, this enzyme is highly similar to RuBisCO [8,26,27]. It produces 2-hydroxy-3-keto-5-methylthiopentenyl-1-

nenn .	Meth	yithioadenosine nucleosidase		
BACSU	1	MRLAVIGAME EEVTILRNKLEN AKTETIA HCEFTTGE YEGTEVILLKSGIG KVNA	AAISTT	60
ESCCO	1	MKIGI IGAME EEVTLLR DKIEN ROT ISLGGCEIY TGOLNGTEVALLKS GIGKVAA	AALGAT	60
KLEPN	1	MKIGIIGAME EEVTLLRDKIENRQTITIGGSEIYTGQLHGVDVALLKSGIGKVAA	AAMGAT	60
BACSU	61	LLL DR YKPD YVINTGS AGG FHH TLN VGDV VI STDVRH HDVDVT AF DYEYGQ VPGI	LPAAYA	120
ESCCO	61	LLLEHCKPDVIINTGS AGG LAPTLKVGDIVVSDEARY HDADVTAF GYEYGQ LPG C	CPAGFK	120
KLEPN	61	LLL ERCOPDVIINTGS AGGLAS TLK VGDI VVSDEARY HDADVTAF GYEYGQLPGC	CPAGFK	120
BACSU	121	ADEKLISITEEAVSELDGIQVAKGTIATGDSFMNDPKRVEEVRARFSDLYAVEME	EAAAVA	180
ESCCO	121	ADDKLIAAAEACIAELN-LNAVRGLIVSGDAFINGSVGLAKIRHNFPQAIAVEME	ATAIA	179
KLEPN	121	ADEKLVAAAESCIKALD-LNAVRGLIVSGDAFINGSVGLAKIRHNFPQAIAVEME	EATAIA	179
BACSU	181	QVCHQFKTPFVVIRALSDIAGKESHVSFDQFLEQAAVHSTELVLKVIKRI 2	230	
ESCCO	180	HVCHNFNVPFVVVRAISDVADQQSHLSFDEFLAVAAKQSSLMVESLVQKLAHG 2	232	
KLEPN	180	HVCHNFKVPFVVVRAISDVADQQSHLSFEEFLAVAARQSTLMVENLVQNL 2	229	
MtnK :	Meth	ylthioribose kinase		5.0
MtnK : BACSU	Meth	ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSOVHTFTAHDAVAYAOOFAGIDNESELVSAGEVGDGNLNYVFHIYDQE	HDRAL I	59
MtnK : BACSU KLEPN XYLFA	Meth; MHP	ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL*	EHDRAL I QGVSRA I	59 55 55
MtnK : BACSU KLEPN XYLFA BACSU	Meth MHP 60	ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL*	EHDRALI OGVSRAI	59 55 55 119
MtnK : BACSU KLEPN XYLFA BACSU KLEPN	Meth MHP 60 56	ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV	EHDRALI QGVSRAI VMEDLSH VMEDLSD	59 55 55 119 115
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU	Meth MHP 60 56 120	ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV UKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI	EHDRAL I OGVSRA I VMEDLS H VMEDLS D LCD ITER	59 55 55 119 115 179
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN	Meth MHP 60 56 120 116	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRQ PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM</pre>	EHDRAL I QGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED	59 55 55 119 115 179 175
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU	Meth MHP 60 56 120 116 180	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQFHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM</pre>	EHDRAL I QGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED DLHTGSI	59 55 55 119 115 179 175 239
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU	Meth MHP 60 56 120 116 180 176	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQFHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM LVFTDPFFDHDTNDFEEERPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD LVFTDPFFDHDTNDFEEERPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD</pre>	EHDRAL I OGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED DLHTGS I DIHS GS I	59 55 55 119 115 179 175 239 235
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU	Meth MHP 60 56 120 116 180 176 240	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTIDRARIEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM LVFTDPFFDHDTNDFEEELRPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD LFFNDPYQIHERNNYPAELEADVAALRDDAQLKLAVAALKHRFFAHAEALLHGD FASEHETKVIDPEFAFYGPIGFDVGQFIANLFLNALSRDGAD-REPI</pre>	EHDRAL I QGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED DLHTGSI DIHSGSI LYE HVNQ	59 55 55 119 115 179 175 239 235 291
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU KLEPN BACSU	Meth 60 56 120 116 180 176 240 236	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQFHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM LVFTDPFFDHDTNDFEEELRPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD LFFNDPYQIHERNNYPAELEADVAALRDDAQLKLAVAALKHRFFAHAEALLHGD FASEHETKVIDPEFAFYGPIGFDVGQFIANLFLNALSRDGAD-REPI FVAEGSLKAIDAEFGYFGPIGFDIGTAIGNLLLNYCGLPGQLGIRDAAAAREQF</pre>	EHDRAL I OGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED DLHTGSI DIHSGSI LYE HVNQ RLNDIHQ	59 55 55 119 115 179 175 239 235
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU KLEPN BACSU	Meth MHP 60 56 120 116 180 176 240 236 292	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM LVFTDPFFDHDTNDFEEELRPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD LVFTDPFFDHDTNDFEEELRPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD FASEHETKVIDPEFAFYGPIGFDVGQFIANLFLNALSRDGAD-REPI FVAEGSLKAIDAEFGYFGPIGFDIGTAIGNLLLNYCGLPGQLGIRDAAAAREQF VWETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI VMETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEFT</pre>	EHDRAL I QGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED D LHT GS I D LHT GS I D LHT GS I LYE HVNQ LLAHVAD L	59 55 55 119 115 179 175 239 235 291 295 351
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU KLEPN BACSU KLEPN	Meth 60 56 120 116 180 176 240 236 292 296	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQFHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTLDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM LVFTDPFFDHDTNDFEEERPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD LFFNDPYQIHERNNYPAELEADVAALRDDAQLKLAVAALKHRFFAHAEALLHGD FASEHETKVIDPEFAFYGPIGFDVGQFIANLFLNALSRDGAD-REPI FVAEGSLKAIDAEFGYFGPIGFDIGTAIGNLLLNYCGLPGQLGIRDAAAAREQF VWETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI LWTTFAERFQALAAEKTRDAALAYPGYASAFLKKVWADAVGFCGSELIRRSVGI KUNAAAAAAANGCGCGSELIRRSVGI</pre>	EHDRAL I OGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED DLHTGSI DIHSGSI LYE HVNQ RLNDIHQ LAHVAD I	59 55 55 119 115 179 175 239 235 291 295 351 355
MtnK : BACSU KLEPN XYLFA BACSU KLEPN BACSU KLEPN BACSU KLEPN BACSU KLEPN BACSU	Meth MHP 60 56 120 116 180 176 240 236 292 296 352	<pre>ylthioribose kinase MGVTKTPLYETLNESSAVALAVKL-GLFPSKSTLTCQEIGDGNLNYVFHIYDQE MSQYHTFTAHDAVAYAQQFAGIDNPSELVSAQEVGDGNLNLVFKVFDRC PDNALKKQPHLEALHQSSATALLVLINLSRRNSATATLRAAGNTPSALTMPCL* IKQAVPYAKVVGESWPLTIDRARIESSALIRQGEHVPHLVPRVFYSDTEMAVTV VKQALPYVRCVGESWPLTIDRARLEAQTLVAHYQHSPQHTVKIHHFDPELAVMV LKIARKGLIEGENYPHLSQHIGEFLGKTLFYSSDYALEPKVKKQLVKQFTNPEI HRIWRGELIANVYYPQAARQLGDYLAQVLFHTSDFYLHPHEKKAQVAQFINPAM LVFTDPFFDHDTNDFEEELRPFVEKLWNNDSVKIEAAKLKKSFLTSAETLIHGD LFFNDPYQIHERNNYPAELEADVAALRDDAQLKLAVAALKHRFFAHAEALLHGD FASEHETKVIDPEFAFYGPIGFDVGQFIANLFLNALSRDGAD-REPI FVAEGSLKAIDAEFGYFGPIGFDIGTAIGNLLLNYCGLPGQLGIRDAAAAREQF VWETFEETFSEAWQKDSLDVYANIDGYLTDTLSHIFEEAIGFAGCELIRRTIGI LWTTFAERFQALAAEKTRDAALAYPGYASAFLKKVWADAVGFCGSELIRRSVGI DTIVPFDKRIGRKRLALETGTAFIEKRSEFKTITDVIELFKLLVKE 398</pre>	EHDRAL I QGVSRA I VMEDLS H VMEDLS D LCD ITER MCE ITED D LHT GSI D LHT GSI D LHT GSI LYE HVNQ RLND IHQ LAHVAD I	59 55 55 119 115 179 175 239 235 291 295 351 355

The MTA nucleosidase / MTR kinase pathway in *B. subtilis* and *K. aeruginosa*. When the methionine salvage pathway exists, while the organism does not code for a MTA phosphorylase, the combination of MTA nucleosidase and MTR kinase produce MTR-I-P from MTA (Figure I). *B. subtilis* (BACSU) and *K. pneumoniae* (KLEPN) possess this pathway, while *E. coli* (ESCCO) has only the nucleosidase. Conservative replacements are shown in blue following Dayhoff's rule [54]. The short protein that may be a truncated MtnK in *X. fastidiosa* is shown for comparison (identities with the *B. subtilis* sequence in orange, and similarities in violet).

BACSU	WTHSFAVPRSVEWKETAITILN QQKLPDETEYLELTTKEDVFDAIVTL
SYNEC	MTQSTNFDDVYPVQWRDDQVILIDQTRLPLEYKEVAIADYEAMGHAIKSM
ATLFA	
FSEAL	CONTRACT CON
KLEPN	33333333333333333333333333333333333333
LEFIN	
HOMSA	MTLEA IRVS RGS LOLLOOL LEPKOSRY EAVG SVHOAWEA TRAM
ARATH	
CAEEL	MTSK INKI VNGP I PD SFLFDESK RLDS LKFDGTN LEVLOOLL LPHEFKY I PVEGVSDAFAV I KSM
SACCE	MSLEAIVFDR SEPENVSVKVLDOLLLPYTTKYVPIHTIDDGYSVIKSM
Consensus	*
MUSMU_IF2	MEDGELIEYFKSQMKGDPKMASAVAAIQTLLEF
BACSU	KVRGAPAIGITAAFGLALAAKDIETDNVTEFRRRLEDIKQYLNSSRPTAINLS
SYNEC	VVRGAPAIGVAAAYGMYLGARRIQTTELREFVVQLEFVADQLRQTRPTAVNLF
XYLFA	IVRGAPAIGIVAGWGAVLAAREIEAVDGIEALRKLEPALQRLHAARPTAVNLA
PSEAE	VVRGAPAIGISAAYGIVLGARARLAQGGDWRAALEEDFRLLADSRPTAVNLF
KLEPN	RVRGAPLIGLSASLLLALLAQRGLNRDALQQALETLRAARPTAVNLM
LEPIN	VVRGAPAIAITGAFGITLYWNSLVSKPSFSELKLKLSELLESRPTAVNLR
AQUAE	AVRGAPAIGCTAAIGPVLGVKVQKEDPEKVIETLKNIKPIAIKLP
ARATH	VVRGAPATALA VOLDA VELOAGAGOGG
CAFEL	OVECAPLIAVYCSIGULETOKA-
SACCE	OVRGAPAIAIVGSLSVLTEVOLIKHNPTS DVATLYSLVNWE STKTVLNKRLDFLLSSRPTAVNLS
Consensus	-****-*::*:*:*:*:*:*:*:
MUSMU_IF2	LKRDKGETLQGLRANLTYAIKTLCGVDSSVAVSSGGELFLR
DECCU	
BACSU	WALE KLSH SVENAI SVNE AKTN LVHE AIQ IQVE DEETCRL I GQNALQLFKK GDR
SINEC	WAID WELL VALUES CENVEQ I ANVED SUCCESSION OF CONCENTER FOR CONCENT OF CONCENT
DCEAF	WALNESS TERAARADWRYARARD AES LAKE DUTANKURGATGAALLPIGSG
KLEPN	NNLDRMKOALARFDVPOALEAFALRIVEEDKOLCARIAEIKKUUGSF
LEPIN	LATERESS REPESING SESSION OF THE AND A DECAMPACING AND A DECAMPACI
AQUAE	WALDRMMKALKEGKD IEEE AKE IEKE DYEANKRMGEIG GETUPI.N
HOMSA	RAARDLAD VAAR EAE REGATEEA VRER VICCTEDMLEK DLEDNEST GDLCARHLIP DVADSCC
ARATH	DAALKLKHVIAKALAT-ATEAKSIFKAYIEASEDMLEDDVVSNKAIGNFGLSLLROOAKNPD
CAEEL	NSLNGLKPILESODYSDVVKLEKCRSYLLNVYTDEKLONRILVWNAYOELLSAFPDKE
SACCE	NSLVEIKNILKSSSDLKAFDGSLYNYVCELIDEDLANNMKMGDNGAKYLIDVLOKDGFKD
Consensus	
MUSMU_IF2	FISLTSLEYSDYSKCKKIMIERGELFLRRISLSRN
B1000	
SACSO	
XVI.FA	
PSEAE	-ON LLTHONT GALAT GGF GTAL GVI BAAHLE
KLEPN	
LEPIN	-LNIITHCNTGALATAGHGTALGVIRSLRDAGHSLTVFADETRPYLQG
AQUAE	-AKVLTHCNTGALATAGWGTALGVIRSAHYGGKNIFVWVDETRPYLQG
HOMSA	KVTVLTHCNTGALATAGY GTALGVIRS LHSL
ARATH	KLSVLTHCNTGSLATAGYGTALGVIRALHTQGILERAYCTETRPFNQG
CAEEL	KLTVMTICNTGSLATISWGTALGVIRALHSENRLKLVYVLETRPYNQG
SACCE	EFAVLTICNTGSLATSGYGTALGVIRSLWKDSLAKTDKADSGLDNEKCPRMGHVFPLETRPYNQG
MUSMU TF2	
BACSU	SRLTAWELMQGGIDVTLITDSMAAHTMKEKQISAVIVGADRIAKNGDTANKIGTYGLAILANA
SYNEC	AKLTVWECVQAGIPVTLITDNMAAHCMQQQRIDAVVVGADRIARNGDIANKIGTYGLAVLAKM
XYLFA	ARLTVWELQQDGIDVTLIADSAAAHLMKSGQVQWVIVGADRICANGDAANKIGTYQLAITARH
FSEAL	SRETAWELAREGIPVSINVDSAAAHEARTERITWVIVGADRITANGDVARRIGTVGLAVRAMH
LEPN	GRETAWELGELG VPTQE TADSMAASEMAQGQ V DAVWY GAD RTAANGDV ANKT GTTS LAVLAHT
AOUAE	AR LTAWELVERS TOHE I TTOSTA CEL MEKCHVDLVTVCADE TAK COVANEL CTYTLAVICEE
HOMSA	ARLTAFELVYEOIPATLITDSMVAAAMAHRGVSAVVVGADRVVANGDTANKVGTYOLAIVAKH
ARATH	SRLTAFELVHEK IPATLIADSAAAALMKDGRVDGVIVGADRVASNGDTANKIGTYSLALCAKH
CAEEL	IRLTSIELLHGEVPFKLITDSMAAWAMKNHQVDCVLTGADNVARNGDTANKIGTYMLAVLCKH
SACCE	SRLTAYELVYDKIPSTLITDSSIAYRIRTSPIPIKAAFVGADRIVRNGDTANKIGTLQLAVICKQ
Consensus	-:***::::***-:***
MUSMU_IF2	GKKMAKALSHLNVPVTVVLDAAVGYIMEKADLVIVGAEGVVENGGIINKIGTNQMAVCAKA
BACSU	FD IPFFVAAPLSTFDTKVKCGAD IPIEERDPEEVRQISGVRTAPSN
SYNEC	HAIPFFVAAPLSTVDFALDDGSQIPIEERDPVEIYQPEGNRITPEG
XYLFA	HGVKFMVVASAATVDMDTTAGEAIEIEQRDPEELLGVSGVRTVAEG
PSEAE	HG VRFMVVAP SSTIDMNLESGED IFIEERDGRELLE IGGRRVAAE
KLEPN	HQIPFYVAAPQTTLDRYCPNGAAIPIEQRAAAEVTGVAGSFGAVQWAPTG
LEPIN	HGVPFYVAATAKSMDFRIPNGSYIPIEMRKEEEITSF-GFLKDSDGKPLLKEGVIAPKG
HOMSA	
CE 2025 (A	IN VERTICAAL IS IT DON'T AS CALL IN PROPERTY AVEC GOLATAR AS
ARATH	NV VF I VAAP 101 I DOMING DE I II DENDE DE VAV GOGGANAP KE HG IPF VAAP SSCOLRLETCKE I I EERPGQELTD VNGVRIAAPG- HC IPF VVAAP ISSCOLRISSCKET VI EERSKET NUMBERGE DE TANDO
ARATH	IN UT I VAR I DI LONI NO VER LI DENDE O IN VOLCALITA FE- HO I PTVA PI SSCULLETCE I I DENDE OLTO VACUALITA FG- HO I PTVA PI LSVULSLESCRE I VI EERSPK ELMI HI GGLERI AAPG- IN INFVVV PTVI INKI ACTER I KI EERSPK ELMI TI GGLERI AAPG-
ARATH CAEEL SACCE	IN VFT I VAR I DI I JONIASUEDI I ERROPETAVI VOUGANA MA- HG IPFYVAA SISCILALETEKETI I ERROPETAVI VOUGANA MA- HG IPFYVAA FLTSVOLSLSSGKE IVI ERROPETAVI VOUGANA MA- HN INTYTVI FTT INNIATGEE IKI ERROPETAVI VOTTVI INPINASI LILMESGUD FVGVUTA
ARATH CAEEL SACCE Consensus	IN OFFIVAR / SI I SMILSGE II ERROFENVOGGARAFNE- HOIPFVVASISSICULETEREI II ERROFENVOVIAA HOIPFVVASISSICULETEREI II ERROFENTIONVAVIAA HINTYTVYPTII INSIATASILSSIKII VI ERROFENTIONVAVIAA HINTYTVYPTII INSIATASILSSIKII II ERROFENTIONVAVIAA FOIRFVVASIKTII INSIATASI
ARATH CAEEL SACCE Consensus MUSMU_IF2	IN UT I VAC IS I LONING VERI LEROPE IN CONCALMANT. IN UTIL VALUES SECONDECTION IN TERPORTEUR VOLUCIANA IN UNITY VALUES VOLUS CREVING IN TERPORTEUR VOLUCIAN FG IRFTVAR ITTI UNITECODI I VERMPEERKUVTCTVINPENGS LILMESGEP ITGK VGIA ONNEPTVVAR STRFV VALP FLADE - DVDVFKYKADTLK SVQTQD-
ARATH CAEEL SACCE Consensus MUSMU_IF2	IN VET I VAC I DI L'ONIASUEDI I ENDOPENN CONCALANA. IN UPTVAN I SISCILLETTE I ENTOPENN CONCALANA. IN UNTYVA I SISCILLETTE I ENTOPENNE I ENTOPENNE ANTONIA IN UNTYVA I SISCILLETTE I ENTOPENNE I ENTOPENNE VICINA FOI KETVVA I KTIL UNTITICO I I VERNIPEETKVITCTI UN ENGS LILLE SGEP I TGKVGLA OKKPTYVA KTIL UNTITICO I I VERNIPEETKVITCTI UN ENGS LILLE SGEP I TGKVGLA OKKPTYVA KTIL UNTITICO I I VERNIPEETKVITCTI UN ENGS LILLE SGEP I TGKVGLA
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU	HN UPT I VAR 19 I SON ILSUE I LEADYE IN VOIGANAR MA- HO IPT VAR 19 I SON ILSUE I LEADYE IN VOIGANAR MA- HO IPT VAR 15% VOLLOS CREIVI ERRYREININGCICER IARG- HN INTY VAR 15% VOLLOS CREIVI ERRYREININGCICER IARG- FO IRTY VAR KTI INNYTETOD I VERNIPEERK VITOT VIN PIRGS LIAR SCEP I TORVOLA ONREPT VVAR SFREVRLEPENDE-DV POI FRYRADILS SVOTOGO-
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC	IN UPT VVAR 15 I 1 SMILSUES II ERROPENVOUGALARME. HI IPT VVAR ISSICULATERE (III ERROPENVOUGALARME. HI INTYTVP VPT INSILAUSKI III ERROPENVOUGANARME. FO IRTYVAR VTI INSILAUSKI III ERROPENVOUGANARME. FO IRTYVAR KTII INVITCIOD II VERMEERK VVTOTVIMPEKSILLUE SGEP ITGEVGIA I I I I I I I I I I I I I I I I I I I
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XXLFA	IN OFF VVAL 59 I SON LASSEE I LEADYEAN CORALINA NA HE IFF VVAL SISCICALE TYPE IN THE REPORT FROM VOLUME AND HE IFF VVAL INVOLUSS CRUTTEREN OFFICIAL SOURCE NANC- HE INVERTION OF THE INSTANCE IN THE REPORT FROM VOLUME FOR THE VAL FOR THE SOURCE OF THE SOURCE OF THE SOURCE OF THE SOURCE OF ONREFIT VVAL STREAM OF THE SOURCE OF THE SOUR
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XYLFA PSEAE	HI UPT VVAN 13 I I SUN LASUE II LEARY PE VN OGATAV KALINKA HI UPT VVAN 13 I I SUN LASUE KI I LEARY PE UN VVV I AN KALINKA HI UPT VVAN I UT SV ULASUK KI EURO SALVAN VVV I AN KALINKA FI I KRYT VN VN TI I SUN TATE KI EURO SALVAN VVV I AN KALINE SGEP I TAK VA I ONKP FYVVAN KTI I UNVTET OD I I V EXHPE EIRV VT AT VI HPENGS LI LINE SGEP I TAK VA I ONKP FYVVAS SFKF VRLF PLAQE – D V POLFKYK ADTLK SVQTGQ D VPVT HPA FD I TP HDLI SG-I I TE KGI MTANYE EI EQLERGEKVI AEFY HPA FD I TP HDLI SG-I I TE KGI MTANYE EI EQLERGEKVI
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XYLFA PSEAE KLEPN	HIVPT VVALUS VISUALISSEE II EEROPEENVOUGALIAKA HIIPTVVALUSSIOLALISSEKSI UI EEROPEENVOUGALIKKA HIINTYTVVTISSIOLALISSEKSI VIEEROPEENKUVUUTAVULUU POIKPTVVALUSVOLLUSSEKSI VIEEROPEENKUVUUTAVULUU POIKPTVVALUSVOLLUSSEKSI VIEEROPEENKUVUTAVUUUU ONNPTVVALUSVESTKIVUUTAUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XYLFA PSEAE KLEPN LEPIN	HI UPF VVA. 19 I I SUN LSUE I I ERROPENN CUGALAR ME HI UPF VVA. 19 I I SUN LSUE II ERROPENN CUGALAR ME HI UPF VVA. 17.5V ULSUS CREVI I ERROPENN VICANAR ME HI NITYEV SUS CLAUETER KIE ERROPENNE VOLULAN FEIRYEVA. 17.5V ULSUS CREVIELE ERROPENNE VICAN VICANA FEIRYEVA. 17.5V ULSUS CREVIELE ERROPENNE VICAN VICANA ONKEPTVVAE SFRFVELFPLNQE – DV PRIFYKADTLK SVQTQQD
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XYLFA PSEAE KLEPN LEPIN AQUAE	IN UPP TVAN 13 IS SUCILALIZETER II TERROPETAN CONTRAINED. IN UPPTVAN 13 IS SUCILALIZETER II TERROPETAN VICUUSIAN IN UNPTVV PTTI INSULASCERE IVI EERSPELMININGCIER NAPC- FOLKEVVAN KTUI UNPTTI CONTACTION VICUUSIAN ONKP FYVVAN KTUI UNPTTI CONTACTION VICUUSIAN
ARATH CAEEL SACCE Consensus NUSNU_IF2 BACSU SYNEC XYLFA PSEAE KLEPN LEFIN AQUAE HONSA	HI UPPTVAAL SISICILALETKKIII IERRIVQETTUKUVUKAATAA HI UPPTVAAL SISICILALETKKIII IERRIVQETTUKUVUKAATAA HI UPPTVAALITSVULASISKKIVI EERRIVAATAANVUKAISA FI UPPTVAALITSVULASISKKIVI EERRIVAATAANVUKAISA FI UPPTVAALITSVULASISKKIVI EERRIVAATAANVUKAISA ONKPTYVAAESIKKIVITTUKUTETUD II VEERRIPEERKVUTUTUTUHPIKSILLAESGEPITUKVULA ONKPTYVAAESIKKIVETUD II VEERRIPEERKVUTUTUKUVAA ONKPTYVAAESIKKIVITTUHUU
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XYLFA PSSAE KLEPN LEPIN AQUAE HOMSA ARATH	IN UPT VIAN 13 I I SUN LSUE 11 EEROPETUVUKUKAINA HI UPT VIAN 13 I I SUN LSUE 11 EEROPETUVUKUKAINA HI UPT VIAN 11 SUULSUSKE VI UEROPETUVUKUKAINA HI NITYT VIAN 11 SUULSUSKE VI EEROPETUVUKUKAINA FO LKYTVAN KTI I UNVETTOD I I VERMEEERVVUTOT VIHPEKUS LILLE GGE I TOKVOLA OKKEPTYVAS SFKFVALF PLAQE-D V POI FEYKADTLK SUUTOON
ARATH CAEEL SACCE Consensus MUSNU_IF2 BACSU SYNEC XYLFA PSEAE KLEPN LEPIN AQUAE HONSA ARATH CAEEL	IN UPT VVAL 19 I SUBILISUEL I LEBRO QELTVOVALAN AL. HE IPT VVAL 15 I SUBICIALET VELT I EBRO QELTVOVALAN AL. HE IPT VVAL 15 V LASS CREVI I EBRO QELTVOVALAN AL. HE IPT VVAL 15 V LASS CREVI I EBRO ADELTO VALON AL. FO IRT VVAL 15 V LASS CREVI I EBRO ADELTO VALON AL. FO IRT VVAL 15 V LASS CREVI I EBRO ADELTO VALON AL. FO IRT VVAL 15 V LASS CREVI I EBRO ADELTO VALON AL. ONRE PTVVALS STRT VALE PLANE AD DO FORTAVISTI I EBRO ADELTO VALON AL. ONRE PTVVALS STRT VALE PLANE AD DO FORTAVISTI I EBRO ADELTO VALON AL. ONRE PTVVALS STRT VALE PLANE AD LASS CREVENTATI I EBRO ADELTO VALON AL.
ARATH CAEEL SACCE Consensus MUSMU_IF2 BACSU SYNEC XYLFA PSEAE NLEPN LEPIN LEPIN AQUAE HOMSA ARATH CAATH SACCE	HI UPP IVAN JS IS COLLETE KI I EER OPEN CONTAINEN. HI UPP IVAN JS IS COLLETE KI I EER OPEN CONTAINEN. HI UPP IVAN JS IS COLLETE KI I EER OPEN CONTAINEN. HI UPP IVAN JS IS COLLETE KI I EER OPEN CONTAINEN. FO IRP IVAN SI IS COLLETE KI EER OPEN CONTAINEN. FO IRP IVAN SI IS COLLETE KI EER OPEN CONTAINEN. FO IRP IVAN SI IS COLLETE KI EER OPEN CONTAINEN. FO IRP IVAN SI IS COLLETE KI EER OPEN CONTAINEN. ONKP PYVVAE SPRP VELF PLAQE - D'Y POIR FYKADTLK SVQTGOD.
ARATH CAEEL SACCE Consensus MUSNU_IF2 BACSU SYNEC XYLFA FSEAE KLEFN LEFIN LEFIN LEFIN LEFIN AQUAE HOMSA ARATH CAEEL SACCE Consensus WICHLIFT	IN INF IVAN IS SICOLALETKE I LERRA DELTO VIGUELA VALUELA VALUE

Alignment of methylthioribose-I-phosphate isomerases. Conserved residues are indicated in red, while conservative replacements are indicated in blue. Organisms are labelled as in Figure 2, with a few additional organisms: AQUAE Aquifex aeoliqus; ARATH Arabidopsis thalina and MUSMU Mus musculus. The sequence of mouse initiation factor eIF-2B (alpha subunit) is shown for comparison. The § symbol at the beginning and the end of the K. aeruginosa sequence corresponds to regions that have not been identified from the ongoing genome programme sequences.

HOWEN	MSCCDARECOCC SERCCAODEERERY T DELCEORY ULCHUTCHC CCT ST EUCDE
ADATH NTO TO	
ARATH_NIEIM	
CAEEL	MVEFVNDPNFKDLVVDTTIDNKNDEEPMTIRNFTELMIQFIKLGWARGSGGAAGCISGSE
SCHPO	MDDFLKKDLGCLRSGDLKKCGELICEICRDLYTSGWVTGTGDAIVI
SACCE	MSSQDVL1HSDDPCHPANL1CTLCKQFFHNNWCTGTGGG1S1KDPNT
BACSU	WAAKQERWRELAEVKRELAERDWFPATSGNLSIKVTDE
LEPIN	GTAGNLSIR IPEE
PSEAE	MNDNREQLTQQIIDAGRFLYGRGWSPATSSNYSARLDEQ
KLEPN	\$QERLAQLVTTCHWIGAKGWAPATGGNMSVRQDDT
XYLFA	MNATSTP SLPY DANR LRELAHLL IGTI GEFAQAGWTP ATS SNFSHR LDEH
AQUAE	MNVELFKKFSEKVEEIIEAGRILHSRGWVPATSGNISAKVSEE
Consensus	**
HOMS A	IYIAPSGVQKERIQPEDMFVCDINEKDISGPSPSKKLKKSQCTPLFMN
ARATH_NTerm	SIPK PEQLIVM SPS GVQKERMQPEDMYILSANGSIISTP SPKPYPNKPPKCTDCAPLFMK
CAEEL	LMISPSALQKERIREQDVFVYNMKDKTEVQRPPNKRITVSSCSVLFSL
SCHPO	APS GVQK ERMEL HHLF VMSL ITRE YMRM PAL RLKP SQC TPLFLA
SACCE	NYYYLAPSGVQKEKMIPEDLFVMDAQTLEYLRSPKLYKPSACTPLFLA
BACSU	PHTFLVTASGKDKRKETVEDFLLVDONGEPAESGHSLKPSAETLLHTH
LEPIN	SGEWV SGSGLDKNLLNK BNFL YVDLESGK PVDS KNTK -ATKGLKP SARTSTHRA
PSEAE	RALLTVSGKHKGOLGEDDVLATDLAGNSLEPGK
KLEDN	
NUTEN	
AILFA	VIAL TO SERVICE AND ADDRESS AN
AQUAE	YIAI TAS GKHK GKETPEDILLIDI EGRPVGGGKPSAE TLLHTT
Consensus	
HOMSA	AYT-MRGAGAVIHTHSKAAVMATLLFPGREFKITHQEMIKGIKKCTSGGY
ARATH_Nterm	AYE-MRNAGAVIHSHGMESCLVTMLNPQAKEFRITHMEMIKGIQGHGY
CAEEL	IMK-ETGSECVIHTHSKCANLITQLIKSNVFEISHQEYIKGIYDPFSGKA
SCHPO	VYTSLRDAYACIHTHSQEAILLSTLFADSDHFSATGFEVLSYIPKGSKNNGFHKPT-
SACCE	CYQ-KKNAGAIIHTHSQNAVICSLLFGDEFRIANIEQIKAIPSGKVDPVTKKPMA
BACSU	LYN-KTNAGCCLHVHTVNNNVISELYGDQKKITFKGQEIIKALGL
LEPIN	VYRALDGVGCGLHVHTLESNLICANTSKNEPIVLFELPAIEILKAYGI
PSEAE	LYAWNPAIGAVLHTHSVNATVLSRLVRGDRLVLQDYELQKAFAGV
KLEPN	VYRLFPEANVVLHVHTVNATVLSRIEKSDTLALQGYEMQKTLSGQ
XYLFA	LYRRFPEIGCVLHTHSLTQTVASRVYAGAGHISLKDYELLKAFEGH
AQUAE	VYKLFPEVNAVVHTHSPNATVISIVEKKDFVELEDYELLKAFPDI
Consensus	*****
HOMSA	YRYDDMLVVPIIENTPEEKDLKDRMAHAVNEYPDSCAV-
ARATH NTerm	YDELVYPTTENTAYENELTDSLTKATEAV PKATAV-
CAFEL	LKY SDTLTTPITDNMPSESOLUCATNE SPMOPDCFTNTALFOEPTRGVLENV POATAV-
SCUDO	
SUCCE	
DACCH	UPENARUMUD LIEN DAULD
BACSU	WEENAEVTVPIIEN PAHIPTLAALFAEEISEDSGAV-
LEPIN	WEENPRVIVPIIINPPNVQDISDCLERILREIRPHVPPC
PSEAE	TTHEGQVEVPIFDNDQDIARLASRVQPWLEAHPHCPGY-
KLEPN	HSHLDTVPIAIFDNDQDIDALAARIADYAQTRPLRYGF-
XYLFA	STHETTLDVPVFCNTQNMNILAAQVDTLL-DKQRMWGY-
AQUAE	HTHEVKIKIPIFPNEQNIPLLAKEVENYFKTSEDKYGF-
Consensus	
HOMSA	LVRRHGVYVWGETWEKAKTMCECYDYLFDIAVSMKKVGLDPSQLPVGENGIV
ARATH_Nterm	LVRNHGVYIWGDSWIHAKTQAECYHYLFDAAIKLHQLGLDAATPDHGPIRRTIHSQIKDS
CAEEL	LVRNHGLFVWGPTWESTKIMTECIDYLLELSIEMLKNNIPLVNEEAFEKEDNLSDKMRTL
SCHPO	IVRDHGIYCWGDTWQDTKMNTEAVEFLFQAYLRRRLQKPE
SACCE	IVRRHGIFVWGPTIDKAKIFNEAIDYLMELAIKMYQMGIPPDCGIGEEKKHLKMASP
BACSU	LIRNHGITAWGKTAFEAKRVLEAYEFLFSYHLKLKTLEHQLVK
LEPIN	IIEKHGITVWGKDAVQANRNLEATDFILKYMTSWKSFSYSGEPRKLSVSDVLGQDRREIF
PSEAE	LIRGHGLYTWGARMSDALRQVEAFEFLFECELKVLSLSR
KLEPN	LLRGHGLTCWGKDIQEARRQLEGLEFLFECEL \$
XYLFA	LINGHGMYTWGNTLADARRHLEALEFLLHCELNLLKLRGYL
AQUAE	LIRGHGLYTWGRSMEEALIHTEALEFIFECELKLLSFHS
Consensus	;;**;**;*;;
	. 61 (10.876) 181 (191 (191

Alignment of methylthioribulose-I-phosphate dehydratases. Important residues are indicated as in Figure 2. For A. *thaliana* (sequence from At5g53850) the N-terminal end of the protein is aligned. The § symbol at the beginning and the end of the K. *aeruginosa* sequence corresponds to regions that have not been identified from the ongoing genome programme sequences.

phosphate. At this stage (outcome of the enolase reaction) 2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate phosphatase, encoded in *B. subtilis* by gene *ykrX* (*mtnX*), produces 1,2-dihydroxy-3-keto-5-methylthiopentene (Figure 1).

In other organisms, such as K. pneumoniae, the corresponding enzymes appear usually to consist of a single enolase-phosphatase, allowing the cell to bypass the last step. Interestingly, in Arabidopsis thaliana, the protein carrying the dehydratase (Figure 5) is a two-domain protein, the carboxy-terminal domain coding for the enolasephosphatase (Figure 6). This further substantiates the proposed identification of the corresponding steps in the pathway. The enolase-phosphatase corresponds to the KOG family KOG2630. The product of the enolase-phosphatase reaction is then submitted to the action of a dioxygenase, as demonstrated in K. pneumoniae [28]. It has also been shown that dioxygen is required for the methionine salvage pathway in *B. subtilis* [8,26]. The sequence of the proteins is illustrated in Figure 7. The acireductone dioxygenase that creates 2-keto-4-methylthiobutyrate (KMTB) has counterparts in many Bacteria and Eukarya, including in mammals, belonging to family COG1791. It seems to be absent from Archaea. Interestingly Abeles and co-workers discovered that the enzyme has two activities, depending on its metal co-factor. When iron was involved, the enzyme produced KMTB, the immediate precursor of methionine, together with formate. In contrast, when nickel was involved, carbon monoxide was produced in addition to formate, together with 3-methylthiopropionate [29]. The enzyme has been crystallized [30]. In B. subtilis, using palladium salt reduction, we found that CO was likely to be produced when the cells were grown in the presence of MTR. A significant difference between the wild type and a *mtnD* mutant could be seen: the wild type makes more reducing gasses when MTR is the sulfur source (16.7 ppm/hour, Table 2). However, B. subtilis produced other reducing gasses as shown by the presence of a reddish-black hue in the precipitate, even in the mutant. To test for another possible source of reducing gasses we investigated reducing gas production in a *yflM* mutant. Its gene product, YflM, is highly similar to an NO synthase. As shown in Table 2, an *yflM* mutant produced somewhat less reducing gasses. The amount produced in our conditions, about 6.7 ppm/hour less than the wild type in sulfate, and 10 ppm/hour less in MTR, is however not much higher than the uncertainty of the experiment (ca 5 ppm/hour). In this background, the effect of MTR was still visible and significant as compared to growth with sulfate as sulfur source (13.4 ppm/hour). However a significant level of palladium precipitation remains in these mutants. The background level found in the absence of bacteria was of the order of 12.5 ppm/hour. Other gasses such as methanethiol and hydrogen sulfide are likely to contribute to the reducing power of the organism.

When MTA is recycled, the two last steps of the methionine salvage pathway appear to be common to all organisms that grow in the presence of dioxygen. The counterpart of the dioxygenase is not known in organisms that live under anaerobic growth conditions. KMTB is transaminated to methionine. There are many analogs of the final aminotransferase, however they can more or less complement one another (as is often the case with aminoacid aminotransferases, see e.g. [31]). In B. subtilis, putative aspartate aminotransferases, the yugH, ywfG, ykrV, aspB, and patA gene products, have been cloned, expressed, and characterized for methionine regeneration activity. In this family, only YkrV (encoded by ykrV, adjacent to the *ykrWXYZ* operon) was able to convert KMTB to methionine, and it catalyzed the reaction only when glutamine was used as amino donor [32]. This suggests a link with nitrogen metabolism, substantiating our previous observations [33]. However the bulk of the activity resulted from transaminase YbgE, using either branched chain aminoacids or phenylalanine or tyrosine as donors could also perform a similar reaction [32]. In K. pneumoniae it is the same enzyme that makes methionine from KMTB and tyrosine from its ketoacid precursor [34].

Nomenclature

We propose the following nomenclature, taking into consideration previous names of some of the genes, as well as previous usage of names for some other groups of genes, trying to avoid naming conflicts. The acronym "*mtn*" (for <u>methylthionucleoside</u>) will be used.

Because the early steps are variable, we shall use as a mnemonic the capital letter of the enzyme's name, following Demerec's rule. Thus, *mtnN* will code for MTA <u>n</u>ucleosidase, *mtnK* will code for MTR <u>k</u>inase, *mtnP* will code for MTA <u>p</u>hosphorylase.

Considering that the common part of the pathway starts with MTR-1-P, we shall name the following genes sequentially: *mtnA* will code for MTR-1-P isomerase (*ykrS* in *B. subtilis*) and *mtnB* will code for MTRu-1-P dehydratase (*ykrY*).

In *B. subtilis*, the pathway is probably unusual, with *mtnW* (*ykrW*) coding for the enolase and *mtnX* (*ykrX*) for the phosphatase creating 1,2-dihydroxy-3-keto-5-methylthiopentene. In other organisms, this step is performed by an enolase-phosphatase, encoded by gene *mtnC*, while *mtnD* (*ykrZ*) codes for the aci-reductone dioxygenase.

XYLFA	MSMPQAILTDIEGTTSSLSFVKDVLFPYARRALPDFVREHREHPDVMPW
PSEAE	MTIKAILTDIEGTTSAVSFVFDVLFPYAARHLPDFVREHAGETEVAAQ
KLEPN	§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§§
AQUAE	MVKAILLDIEGTIAPLSFVKEVMFPYSKKKLREFLEKNWEKPEIKKI
SACCE	MVIGQKVLLAR IPKMGDNY STYLLDIEGTVCPISFVKETLFPY FTNKVPQLVQQDTRDSPVSNI
SCHPO	AVKNLLLDIEGTVGS IS FVKDKLFPY AASRY ESYVNENY ESDENLRE
ARATH_CTerm	TIHSQIKDSQYEREWPRRWIVLDIEGTTTPITFVTDVLFPYARENVGKHLNLTYHTAETQED
HOMS A	MVVLSVPAEVTVILLDIEGTTTPIAFVKDILFPYIEENVKEYLQTHWEEEECQQD
LEPIN	MN IKTFEFYLFDIEGTTTPIEFVHKILFPYSVEKFDSFFQSNLLEKEWIEK
CAEEL	MTTTTIQFNALLLDIEGTITSISFVKDELFPYAFENVGNYLEEHYDNPATQII
Consensus	
XYLFA	LDQVANETGTAFSEEALVATLQTWIDTDSKH
PSEAE	LAAVRAESGEADADVERVIAILLQWIAEDRKA
KLEPN	LDNLRRETDAPAASTADLITTLFAFMDEDRKS
AQUAE	VQEVEKIEGRELSLEEAVQLFSRWIDEDRKI
SACCE	LSQFHIDNKEQLQAHILELVAKDVKD
SCHPO	LGKTPEEAL INLRKLHAEGSKE
ARATH CTerm	IKLLRAOVEEDLREGVTGAVPIPHADEGKEKVIAAMVSNVEAMIRADRKI
HOMSA	VSLLRKOAEEDAHLDGAVPIPAASGNGVDDLOOMIOAVVDNVCWOMSLDRKT
LEPIN	LILEG KNDTTYSGKLSDSAFDLSEVCKHLVSLDBKS
CAFEL	VEDLE HIADOOAENDVAVVE DE PERKECTE DVTKNVE HWIK BDK KVVEVFEKECI.KEN
Consensus	
compensas	
XVLFA	TALKALOCMT WASGY ONGDET A HLYP DAVOR LRAWH AANV PLY VYSSGS VP AOO LEFRHS-
PSEAF	TPLKALOGWWAOGYRDGOLKGHVYPDAVOALREWKARGLDLYVYSSGSTOAOKLTEGCS-
KLEDN	
AOUAE	
CLOCE	
SAUCE	
SCHPU	RSFRMVQGRIWKKGIESNELTSHLFPDVVPAIQRSLQLGMRVIIISSGSVPAQKLIFEHS-
ARATH_CTERM	TALKELOGHTWRTGFECDELKATVFEDVADALEKWHSSGIKVYTTSSGSRLAQKLLFGNT-
HOMSA	TALKQLQGHMWRAAFTAGRMKAEFFADVVPAVRKWREAGMKVYIIYSSGSVEAQKLLFGHS-
LEPIN	GILKEIQGRIWKSGYENGELKSSMFSDVPSFLKKIQASKKKSAVYSSGSIQAQKLIFEYS-
CAEEL	IFQLTPMKALQGLIWEEAYQRGDVKGHVYPDVLPVLKIVENRKIPIYIYSSGSVHAQKLLFANS-
Consensus	::*-:**-:*:::-:-:-::*****
XYLFA	HAGDLSGLFSGWFDT-EIGGKRESTSYQRIAEHIGI-AFAGIVFLSDVIEELNAAAQI
PSEAE	EAGDLGSLFSGYFDT-TSGPRRESASYARIAGAIGL-PAAEILFLSDVVQELDAARDA
KLEPN	DEGDITHLFTGYFDT-LVGAKREVQSYRNIAEHLGH-APGTILFLSDIHQELDAAEAA
AQUAE	VYGDIRNLFSGFFDT-KIGSKRERSSYEKIAKEIGL-PPHEILFISDNPEELKAAKEA
SACCE	DPNAPAHDSLDLNSYIDGYFDINTSGKKTETQSYANILRDIGA-KASEVLFLSDNPLELDAAAGV
SCHPO	DAGNLLKYFSGYYDT-TIGLKTECGSYVKIVGNSNPREWLFLSDNINELKAARKV
ARATH_CTerm	DYGDLRKYISGFFDT-TIGNKKESRSYKEIKETLGVDDPAEIMFVTDVYQEAVAAKAA
HOMS A	TEGDILELVDGHFDT-KIGHKVESESYRKIADSIGC-STNNILFLTDVTREASAAEEA
LEPIN	DFGNLTHFFYAYFDT-GVGGKRESSSYSKISEQLGV-APEKILFFTDIKEEADAAKEA
CAEEL	IEGDMTKILYGYFDT-NIGLKGESNSYTKISERIKI-PPSEILFLTDVEAEAAAAKKA
Consensus	::::*-:*-*-*-***:::*-:*
XYLFA	GLNTVLIDRRDDYPTPRHLKDADHHLHLDSFAQLPF
PSEAE	GMRTLGLAREGGSLDGHPTVASSPTSSWSERAGYPEGTLLLGSIAPWVPPSAG
KLEPN	GLRTIQLVRGDRDPASHHPQV\$
AQUAE	GMKVIQSVREGVEPSGDFEKITSFRELEI
SACCE	GIATGLAS RPGNAP VPDGQKYQVYKNFETL
SCHPO	GLHTGLVVRPGNDPVVDTSGFPVYNSFEILFTE
ARATH_Cterm	GLEAIISIRPGNAPLPENHGFKTVTSFSQIFKTVTSFSQI
HOMSA	DVHVAVVVRPGNAGLTDDEKTYYSLITSFSELYLPSST
LEPIN	KLHSAILERPGNYPQPQHSHFKISSFEGLNP
CAEEL	GLQTKLVVRPGNAGLTQEAINAYGTIETFLNIQNSEFEFRMIIRCFQ
Consensus	-:*

Alignment of 2,3-diketo-5-methylthiopentyl-I-phosphate enolase-phosphatases. Important residues are indicated as in Figure 2. For A. thaliana (sequence from At5g53850) the C-terminal end of the protein is aligned. The \S symbol at the beginning and the end of the K. aeruginosa sequence corresponds to regions that have not been identified from the ongoing genome programme sequences.

SCHPO	MRAYIFQDEGDQRKPNDSKIEVSAEDLEAAK-VS-YRHHD-GDL
CAEEL	-MAYFMTDVTKENRQDECRFSPNKEATEEDLLR-IGVECTKVNFDDEHV
ARATH	MQAWFMDDSNEDQRLPHHRNPKELVSLDYLAELGVLYWKLNPENYEN
HOMSA	-MVQAWYMDDAPGDPRQPHRPDPGRPVGLEQLRRLGVLYWKLDADKYEN
SACCE	MVKVYIHDNKVDSDYRAPHNSGTELSLDELAKLGVI-YKYCAN
BACSU	MATIRIHDEANTTIENQEEVASFLDSQEVI-YEQWDITRLPEHLSEKYDLTEEEK
LEPIN	MATI-VRQNGLTPIQETNEVKSFLKERGID-YDHWKVPHNASNLTDKEVLVDTEK
XYLFA	MSRLRIFDDHTPDTPFFVSKEQTQITAELHKIGIT-FERWEATQ-AIEPGATPEQV
PSEAE	MSSLTVYHENQPEQPLKLLTHAEDIASTLAEVGVR-FERWEAAA-PIAAGASQDEV
KLEPN	MSALTLFSVTDPQTPVWHSTDAKAIQAQLNAKGVRFERWQADRD-LGANPSPETV
AQUAE	MSRLRIYTESGELIKDITDPKEIAEELQKINVL-FERWRANA-PLKENASDEEI
Consensus	
SCHPO	HTFADGLMKEYGFKNRDEVVVSRKGLGDRYDNMVKKFFEEHLHEDEEIRLI
CAEEL	ATMPNFDEKLDIFFEEHLHDDAELRVI
ARATH	EKVSNYEEKLKNFFTEHIHKDEEIRYC
HOMSA	DPELEKIRRERNYSWMDIITICKDKLPNYEEKIKMFYEEHLHLDDEIRYI
SACCE	EEEVNEIARQREYKNRDVVNICEGSFKSEAEFNEKLATFYQEHLHEDEEIRYC
BACSU	QQILDTFETEIKDISTRRGYKAQDVISLSDSNPKLDELLENFKREHHHTDDEVRFI
LEPIN	EELLKKLDDRFETLKAKEGYQSRDLIVLHPNVSGLNEMLAKFDKVHYHTDEEVRYI
XYLFA	MAAYRADIDRLIATHGFKTVDVVSIAPDNPKREEMRAKFLEEHFHKEDEVRFF
PSEAE	IAAYAHEIERLKRERGYITVDVVSLNSDHPQKAELRAKFLDEHRHGEDEVRFF
KLEPN	IAAYQHAIDKLVAEKGYQSWDVISLRADNPQKEALREKFLNEHTHGEDEVRFF
AQUAE	INAYKHEIDRLINKYGFQSYDVIAMTPEHPKKDELRQKFLKEHTHSDFEVRYF
Consensus	**-:*-:*-:*-:**-:*************************
SCHPO	LDGNGYFDVRSVDDRWVRIFVEKGDLIILPPGIYHRFTTTTDDYIHAMRLFHENPKWI
CAEEL	KHGVGFFDVRTKDEAWIRIPVRRGDFVFLPAGIYHRFTTDPSEDVVALRLFRNNPKWT
ARATH	LAGSGYFDVRDKDDRWIRIWMQPGDLIVLPAGIYHRFTLDASNYIKLMRLFVGEPVWT
HOMS A	LDGSGYFDVRDKEDQWIRIFMEKGDMVTLPAGIYHRFTVDEKNYTKAMRLFVGEPVWT
SACCE	LEGAGY FDVRDASTPENWIRCLVESGDLLILPPGIYHRFTLTTSNHIKALRLFKDEPKWQ
BACSU	VSGHGIFVIQGQDGTFFDVRLNPGDLISVPENIRHYFTLQEDRKVVAVRIFVTTEGWV
LEPIN	VDGSGVFGFAFKDEKFLVHVYKDDFISVPRNTNHWFYLDDKKRIKAVRYFQDMSGWV
XYLFA	VAGSGLFTVHSGNKVYEIECVKNDLIAIPDGTQHWFDMGAAPYFVAIRFFTEPDGWV
PSEAE	VAGRGLFVLHIEEHVYAVLCERNDLISVPAGTRHWFDMGEHPHFVAVRLFNNPEGWV
KLEPN	VEGAGLFCLHIGDEVFQVLCEKNDLISVPAHTPHWFDMGSEPNFTAIRIFDNPEGWI
AQUAE	VYGDGVFYLHPNDKVYILHCTAGDLISVPPNTKHWFDMGENPNFKCIRLFTTPEGWV
Consensus	*-*-***-*-*-*
SCHPO	ALSRTD STSEELDARKSYLNSIKKSVYV-
CAEEL	AFNR-KADGDEORVROOYLNDISA
ARATH	PYNRPQEEHPVRKKYIHGLTYKFGETVKAH
HOMSA	AYNRPADHFEARGQYVKFLAQTA
SACCE	AINRSNQ-ADSLPVRKDYIALINQY
BACSU	PIYEKDS VNQ
LEPIN	PNYVEETNSLD
XYLFA	GHFTGTDIAQRFPRYIPEGCQSAH
PSEAE	AQFTGDDIASRFPLLED
KLEPN	AQFTGDDIASAYPRL
AQUAE	AEYTGS DIAEKFPKYEEVVNG
Consensus	;

Figure 7 Alignment of aci-reductone dioxygenases. Important residues are indicated as in Figure 2. The N-terminus of the protein is poorly conserved.

Table 2: CO production by Bacillus subtilis on plates using GASTEC dosimeters.

Strain	Sulfur sou	urce used
	MgSO ₄	MTR
WT 168	50.0	66.7
BFS1853 mtnD::lacZ	48.3	51.7
BFS4605 yflM::lacZ	43.3	56.7

CO production is expressed in ppm/hour accumulated during 20 hours of incubation. Background is not subtracted. A difference of 5 in the limit of the palladium metal precipitate is reproducibly visible. The values given correspond to three replicates of each experiment. Note that it is not possible to distinguish between purely black and reddish black precipitates (often due to thiol containing gasses). Background precipitation in the absence of bacteria was of the order of 12.5 ppm/ hour.

Finally, *mtnE* codes for KMTB aminotransferase, when it exists as a specific enzyme. The pathway with this nomenclature is summarized in Figure 1.

Discussion

The beginning of the methionine salvage pathway is variable, even in phylogenetically related organisms. It can either correspond to the tandem reactions, MTA nucleosidase/MTR kinase (as in B. subtilis or K. pneumoniae) or start with MTA phosphorylase as in P. aeruginosa, many Eukarya or presumably in T. tengcongensis, a Firmicute living at high temperature in partially anaerobic conditions. The pathway can further vary at the level of enolase, with either an enolase (RuBisCO-like) and a phosphatase (in B. subtilis, B. cereus and B. anthracis, for example), or, more often, with an enolase-phosphatase as in a variety of organisms, including many Bacteria (e.g. P. aeruginosa), Plants and Animals (Figure 1). An explicit substantiation of this latter pathway can be seen in A. thaliana where gene At5g53850 codes for a protein made of two domains, similar to KOG2631 and KOG2630, with proposed activities of the Class II aldolases and enolase-phosphatases. We do not discuss here the case of Archaea because, although there seems to be a clear counterpart of the methionine salvage pathway, starting from MTA phosphorolysis [27], no clearcut biochemical data are vet available, while the last step (dioxygenase) is probably missing or altered to another type of reaction (many Archaea are strict anaerobes).

In order to further support our description of the pathway we extrapolated from the *B. subtilis* data to other genomes, based on chromosome neighborhood [35] (Figure 8). Here we discuss four cases: *K. pneumoniae*, *P. aeruginosa*, *Xylella fastidiosa*, *L. interrogans* and *T. tengcongensis*.

Most of the methionine salvage pathway has been uncovered in *K. pneumoniae* by the biochemical studies of Abeles and coworkers [25,28,29]. It was therefore interesting to identify the genes corresponding to the pathway. A complete genome sequence is known but not publicly available, while the *K. pneumoniae* genome programme at Washington University is still ongoing. We could however identify the genes for a complete methionine salvage pathway, which begins as in *B. subtilis* (MTA nucleosidase followed by MTR kinase), but differs at the level of the enolase-phosphatase step (Figures 3, 4, 5, 6).

In contrast with the behavior of K. pneumoniae, P. aeruginosa codes for an MTA phosphorylase. Gene PA-3004, located downstream from nagZ (coding for N-acetyl-Dglucosaminidase) and perhaps from transcriptional regulator gene psrA, corresponds to mtnP. Operon aroC(PA-1681)-PA-1682-PA-1683-PA-1684-masA(PA-1685) is likely to code for the core of the pathway in which PA-1682 has an unknown transport function, whereas PA-1683(mtnB), PA-1684(mtnD) and PA-1685(mtnC) correspond to the enzymatic steps described above. Finally, *mtnA* corresponds to gene *PA-3169*, possibly the first gene of a large mixed-function cluster mtnA-gyrA-serC-pheAtyrB-aroE-cmk-rpsA-himD. This cluster is also involved in biosynthesis of aromatic molecules, and the gene tyrB coding for a wide spectrum aminotransferase is probably involved in KMTB transamination, as is its counterpart is in K. pneumoniae [34]. As a support to this analysis, both mtnP and mtnB mutants of P. aeruginosa could not grow on MTA as a sulfur source.

In X. fastidiosa a cluster of genes, presumably constituting an operon, is consistent with the existence of a complete methionine salvage pathway. This operon "serA-xf-2207*xf-2208-xf-2209-xf-2210-xf-2211-xf-2212*" would code for glyceraldehyde-3-phosphate dehydrogenase (serine and one-carbon metabolism), subunits of a transporter, *mtnB*, mtnD, mtnC and a short fragment (N-terminus) of mtnK, respectively (Figure 3). A second gene cluster "xf-2555-xf-2554-mtnA-gyrA-xf-2551" codes for a tRNA synthetase, a protein of unknown function, mtnA, DNA gyrase and a protein of unknown function found in plants. There exists also a clear counterpart of mtnP (xf-2353, see Figure 2). This gene belongs to an operon (xf-2358->xf-2352), coding for an RNA methylase, a putative membrane protein highly conserved in cyanobacteria or bacteria associated to plants, a very short protein with similar sequence found in plants, cell wall hydrolase, purine а phosphoribosyltransferase, MtnP and a cold-shock-like protein. This operon is, at least in part, consistent with MTA and/or S-adenosylhomocysteine production and phosphorolysis. In particular, the first gene product would produce the latter molecule, while the phosphoribosyltransferase could scavenge adenine or a derivative.

Thus, *X. fastidiosa* has all genes needed for a functional methionine salvage pathway. The truncated form of *mtnK* suggests that the core metabolic genes might have been obtained by horizontal gene transfer [36], and that MtnK was useless in the presence of a functional MtnP. Presence of a gene coding for spermidine synthase (*xf*-0143) as well as a counterpart of AdoMet decarboxylase (*xf*-1539) indicates that the substrate, MTA, is indeed synthesized in *X. fastidiosa*. The similarity between the steps of the methionine salvage pathway in *K. pneumoniae*, *P. aeruginosa*, and *X. fastidiosa* suggests that similar pathways will be found in many gamma-proteobacteria.

Leptospira interrogans genome annotation has been particularly difficult because the majority of the genes present in this organism are only poorly related to known genes in other organisms. Indeed, the annotated genome contains a large number of small putative genes that may not really be coding DNA sequences [37]. This prompted us to explore the presence of a methionine salvage pathway in this organism. In order to have better chances to find the genes of the pathway, we started by looking for counterparts of the first gene in the pathway, mtnA. There is a clear counterpart, LA2868, in L. interrogans (Figure 3), as an isolated gene in chromosome I. The gene for the phosphorylase with clear features (molecular footprint) of MTA phosphorylase (Figure 2), mtnP (LA4248), is also present as an isolated gene in the same chromosome. In chromosome II, operon LB294-LB293-LB292-fabG codes for translation factor EFP, MtnD, MtnB and a putative oxoacyl-ACP-reductase. The last enzyme required for the pathway is the enolase-phosphatase (MtnC): it is encoded in an operon present in chromosome I "LA0931-LA0930-LA0929-LA0928". The first gene codes for a peptide that could have a regulatory role, the following gene codes for a permease, then we find *mtnC* (Figure 5). The operon ends with a gene coding for a short peptide that could have features of transcriptional regulators. This indicates that L. interrogans most probably has the complete methionine salvage pathway. Interestingly, this organism has two counterparts of spermidine synthase (one on each chromosome), enzymes that should result in the production of MTA. Gene LB312, on the second chromosome could be the counterpart of *speD* in this genome.

Finally, we tried to explore the pathway in *T. tengcongensis*, because although similar to *B. subtilis*, this organism thrives mostly under anaerobic conditions. In *T. tengcongensis* the phosphorylase would be encoded in a large cluster (either continuous or split into two operons: *tte-1584<-tte-1594*) "*ileS-ssnA-araD-tdh2-gcn3-cbiO2-cbiQ2-pnp2-tte-1586*" upstream of "*metC3-tte-1584*". Both parts of this cluster, typical of a "sulfur island" [38], are preceded by a S-box riboswitch [20,21]. Because of the consistency of the functions encoded in this cluster it is

possible that *ileS* has been mistakenly thought to code for isoleucine tRNA synthetase, but rather codes for methionine tRNA synthetase (both enzymes are usually extremely similar). Because annotations are often propagated by similarity only, it would be most interesting to check experimentally whether this enzyme is an authentic methionine tRNA synthetase. If this were the case, this should prompt reanalysis and annotation of the whole class of cysteine, isoleucine, leucine, methionine and valine tRNA synthetases. The second gene could code for a metal-dependent hydrolase, probably hydrolyzing C-N bonds. The third gene, in the present context, is mtnB, tdh2 could code for a metal-dependent enolase (differing from its counterpart in other organisms, hence tentatively named mtnY, but this has to be substantiated experimentally), gcn3 is mtnA, the next two genes are divalent metal transporters, followed by *mtnP*, while the last gene could code for a component of a transporter. Thus the operon would be "S-box-metS-ssnA-mtnB-tte-1591(mtnY)-mtnAcbiO2-cbiQ2-mtnP-tte-1586". For this organism, it is unlikely that a dioxygenase belongs to the pathway, because it lives mostly in the absence of oxygen. Indeed, we do not find a clear counterpart of mtnD. Starting from the end of the pathway we find that a broad-spectrum aminotransferase (labelled hisC, tte-0380) belongs to a large cluster mostly devoted to divalent-metal transport and synthesis of coenzyme B12, with two unknown genes tte-0378 and tte-0379. Coenzyme B12 is often involved in pathways operating under anaerobic conditions and this should prompt further studies of the methionine salvage pathway under these conditions (T. tengcongensis, that belongs to Bacteria, might provide a missing link to explore the pathway in Archaea).

Sulfur metabolism has probably to be compartmentalized, because of the extreme reactivity of this atom towards dioxygen and radicals. In this respect, it is interesting to observe that, despite their apparent variety of origins, genes of the methionine salvage pathway have a tendency to cluster into operons (translation products of neighboring cistrons are produced in close proximity in the cell), or even fuse and give multidomain proteins (this is frequent in eukaryotes [39]). Another case of gene fusion that might be related to comparmentalization of sulfur containing molecules can be seen in *Aquifex aeoliqus* where the MtnA protein begins with a domain of unknown function:

MDIRTHLGINQELSGKPVKVAEGYAEVLLKTQESMKAD-DKGLVHGGFIFSAADYA SMLAVNHPNVVLAGANVKFLKPVKVGDEIICKARVSED-KGKKKKVLVECFKGEDKVFEGEFFIVFLID

that represents a whole COG domain, COG2050.1, PaaI. This domain has a signature for sulfur metabolite binding.

This further substantiates the idea that sulfur metabolism is compartmentalized.

Finally it was interesting to note that *B. subtilis*, when in the presence of significant amounts of MTR, was likely to produce CO, that may act as a gaseous mediator. This is the more interesting because *B. cereus* and *B. anthracis* possess the same methionine salvage pathway as *B. subtilis*. Furthermore, because this pathway exists in humans, it may be relevant to the ongoing discussion about the role of CO as a neuronal or gastro-intestinal mediator [40,41]. Analysis of similar arrangements in other genomes will lead to recognition of new features of the genetics of microbial sulfur metabolism.

Conclusions

The MTA => methionine salvage pathway is present in many Bacteria. This pathway provides us with a remarkable example of enzyme recruitment in bacteria associated to their biotope. Indeed the enolase-phosphatase step in the pathway is performed by enzymes of entirely different descent, RuBisCO being the most puzzling example. In the same way it seems quite remarkable that a family of GTP binding proteins, previously shown to be involved in translation elongation, universally belong to this pathway (MtnA). It will be an interesting link to compare the reactions in anaerobic environments. This will teach us how we could modify enzyme activities in the future and reprogram biochemical processes.

Methods

Genetic and biochemical experiments Growth conditions

The following B. subtilis strains were used in this work: the wild type strain 168 [42], BFS1853 (mtnD(ykrZ)::lacZ) and BFS4605 (vflM::lacZ). These strains have been constructed during the B. subtilis functional analysis program (http://locus.jouy.inra.fr/cgi-bin/genmic/madbase/progs/ madbase.operl and http://bacillus.genome.ad.jp[26,43]). Escherichia coli strains were grown either on LB or on nutrient yeast broth (NYB) liquid or agar plates at 37°C. B. subtilis was grown in ED minimal medium: K₂HPO₄, 8 mM; KH₂PO₄, 4.4 mM; glucose, 27 mM; Na₃-citrate, 0.3 mM; Lglutamine, 15 mM; L-tryptophan, 0.244 mM; ferric citrate, 33.5 µM; MgSO₄, 2 mM; MgCl₂, 0.61 mM; CaCl₂, 49.5 μM; FeCl₃, 49.9 μM; MnCl₂, 5.05 μM; ZnCl₂, 12.4 μM; CuCl₂, 2.52 µM; CoCl₂, 2.5 µM; Na₂MoO₄, 2.48 µM at 37°C. To assay for CO production, ED minimal medium plates were used with either a MgSO₄ containing medium or a sulfur-free basal medium (where MgSO₄ was replaced by MgCl₂ at 2 mM concentration). In the latter case, 10 µl of the sulfur source under investigation (MTR, 200 mM stock solution) was applicated onto paper discs deposited at the center of the plate, after bacteria had been uniformly spread on the surface of the plate. All experiments were performed in accordance with the European regulation requirements concerning the contained use of Genetically Modified Organisms of Group-I (French agreement N°2735). P. aeruginosa strains were routinely grown in NYB or on nutrient agar plates at 37°C. When required, antibiotics were added to media at the following concentrations: tetracycline (Tc), 25 µg ml⁻¹ (E. coli) or 125 µg ml⁻¹ ¹ (*P. aeruginosa*); kanamycin (Km), 50 µg ml⁻¹ (*E. coli*); ampicillin (Ap), 100 µg ml⁻¹ (E. coli); spectinomycin (Sp), 25 μg ml⁻¹ (E. coli) or 1,000 μg ml⁻¹ (P. aeruginosa); erythromycin plus lincomycin, 1 μ g ml⁻¹ and 25 μ g ml⁻¹ (B. subtilis). To counterselect E. coli S17-1 donor cells in matings with P. aeruginosa for gene replacement, chloramphenicol was used at a concentration of 10 µg ml⁻¹; mutant enrichment experiments were performed with tetracycline at a final concentration of 20 µg ml-1 and carbenicillin at a final concentration of 2,000 µg ml⁻¹. Strain PAO503 and its derivatives were cultivated in NYB, washed and plated out on minimal medium E; 50 µl of methionine, homocysteine or MTA (50 mM each) were then spotted onto sterile filters placed on the bacterial lawn. Growth around the filters was scored after 18 h of incubation. The optical density (OD) of bacterial cultures was measured at 600 nm.

DNA manipulation and cloning procedures

Small- and large-scale preparations of plasmid DNA were carried out by the cetyltrimethylammonium bromide (CTAB) method [44] and with JetStar columns (Genomed, Basel, Switzerland), respectively. Chromosomal DNA was purified from P. aeruginosa with phenol:chloroform (1:1) by a method described previously [45]. Restriction enzyme digestions, PCR, ligation, agarose gel electrophoresis, and electroporation were performed using standard procedures [46,47]. For Southern blotting, chromosomal DNA was digested overnight with the appropriate enzymes, electrophoresed on a 0.8% agarose gel and transferred to a Hybond-N nylon membrane (Amersham Biosciences). Labeling of DNA probes, hybridization and detection were performed using the digoxigenin (DIG) DNA Labeling and Detection Kit (Roche) according to the manufacturer's procedures. Nucleotide sequences of PCR-derived constructs were determined on both strands with a dye terminator kit (Perkin-Elmer product 402080) and an ABI Prism 373 sequencer.

Construction of P. aeruginosa mutant strains

For the construction of strain PAO6412 (*metZ9011 mtnB*::Ω-Sp/Sm), the *mtnB* gene (= PA1683) was PCRamplified using PAO1 genomic DNA as a template and primers PA1683-1 (5'-AAAAGAATTCTGCACCT-GGAATCGCTGGG-3') and PA1683-2 (5'-AAAAGGATC-CCGGTGCTCGTCGAGGAACTTG-3'), tagged with *Eco*RI and *Bam*HI artificial sites (italics), respectively. The *mtnB*



Variety of gene organisation for the methionine salvage pathway in different bacteria. The organisation of the genes coding for the methionine salvage pathway is shown in several bacteria: *B. subtilis*, *P. aeruginosa*, *X. fastidiosa*, *L. interrogans* and *T. tencongensis*. The pathway is as described in Figure I. Genes with the same function are indicated with the same color code. When a gene arrow is broken, this indicates that the function is the same, but that the gene product differs (enolase (MtnW) and phosphatase (MtnX) in *B. subtilis*, vs enolase-phosphatase (MtnC) in other organisms). The stem and loop symbol indicates the presence of a S-box riboswitch. In *B. subtilis* the enolase-phosphatase activity found in other organisms is replaced by two enzymes, a RuBisCO-like enolase and a phosphatase. In *X. fastidiosa*, downstream of the main operon on can see a truncated form of *mtnK* (broken arrow) requiring the presence of *mtnP* instead.

gene was interrupted by a spectinomycin-streptomycin resistance cassette (Ω -Sp/Sm) integrated as a 2-kb *SmaI* fragment into the *SmaI* site of *mtnB*; the resulting 3.3-kb *EcoRI-XbaI* fragment containing *mtnB*:: Ω -Sp/Sm was inserted into the suicide plasmid pME3087 [48] to generate pME3880. This plasmid was mobilized into PAO503 (*metZ9011*) by the donor *E. coli* S17-1 (*thi pro hsdR recA*/ RP4-2 Tc::Mu Km::Tn7) and chromosomally integrated with selection for tetracycline resistance. Excision of the pME3087 carrying *mtnB* by a second crossover was obtained by enrichment for tetracycline-sensitive cells [49]. The tetracycline-sensitive, spectinomycin-resistant

clones (containing $mtnB::\Omega$ -Sp/Sm) were checked by Southern blotting (data not shown).

For the construction of strain PAO6422 (*metZ9011 mtnP*:: Ω -Sp/Sm), the *mtnP* gene (= PA3004) was PCRamplified with primers PA3004-1 (5'-AAAAGGATCCGTC-GAAGGCGGTTTCGAC-3') and PA3004-2 (5'-AAAAAAGCTTCATGTGGCGATTCCCGAAGAC-3') containing artificial sites for *Bam*HI and *Hin*dIII (italics). To generate pME3891, the 2-kb *Sma*I fragment containing the Ω -Sp/Sm cassette was integrated into the *Eco*RV site of *mtnP* and the resulting 3.3-kb *KpnI-Xba*I fragment carrying *mtnP*:: Ω -Sp/Sm was inserted into the suicide plasmid pME3087. The resulting plasmid (pME3891) was delivered to the chromosome of PAO503 as described above, resulting in strain PAO6422, which was checked by Southern hybridization as above.

Preparation of MTR and CO assays

Methylthioribose (MTR) was prepared from MTA (Sigma, D5011) by acid hydrolysis as described by Schlenk et al. [50]. The CO production assay was carried using the GASTEC No.1DL dosimeters (Carbon Monoxide Low Range Passive Dosi-Tubes, Gastec Corporation, Japan). To detect CO production the ED minimal medium plates of 150 mm diameter were inoculated with bacteria and the dosimeter was activated by breaking the tube at its score. Plates were incubated at 37°C for 20 hrs and readings were recorded after the end of incubation period. Comparison of replicates of the same experiment (same volume of the growth medium, same temperature, same incubation time) indicated that the level of significance was better than 5 ppm. Control and MTR experiments were performed on the same day. It is not possible, however, to separate the nature of the different reducing gasses from the production of palladium metal precipitates. Replicates of the experiment on plates without bacteria gave an average level of reducing gasses of ca 12.5 ppm/h.

Computer-mediated analyses

Data were extracted from the microbial genome databases set up at <u>http://bioinfo.hku.hk/genolist.html</u>, using the following databases with their imbedded BLAST browsers and searching using appropriate patterns ("search patterns") when appropriate: AeruList, LeptoList, ThermaList and XylelList. For *B. subtilis*, the reference database was used: <u>http://genolist.pasteur.fr/SubtiList</u>. Analyses of domains were performed using "CD-search" at the NCBI [51]. Annotations were used with the help of SwissProt [52]. Alignments were performed using CLUSTALW and further refined manually [53].

Authors' contributions

All authors participated in the final form of the article. AS set up and performed the experiments meant to detect the presence of reducing gasses in *B. subtilis* and its mutants. VD performed the genetic experiments with *P. aeruginosa*. HA elaborated experiments of the methionine salvage pathway in *B. subtilis*. KM established the nomenclature and harmonized it with the Swissprot requirements. DH conceived the experiments with *P. aeruginosa*. AY established the biochemical protocol needed to identify the steps of the methionine salvage pathway in *B. subtilis*. AD performed the sequence comparisons and wrote the core of the article.

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