Overview on enzymatic mechanisms to break down phosphonates

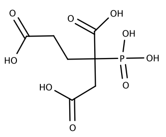
Dr.-Ing. Ramona Riedel

BTU Cottbus/Senftenberg
Chair Biotechnology of Water Treatment

02nd October 2024

Structures of industrial and natural phosphonates

Common industrial phosphonates



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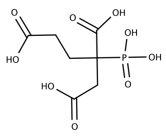

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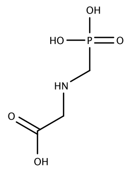
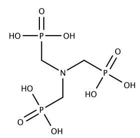


Glyphosat



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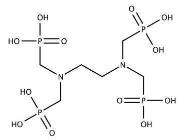

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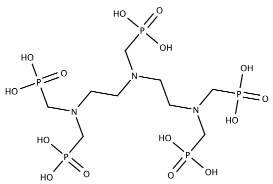

DTPMP

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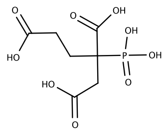

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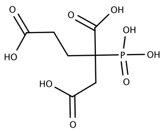

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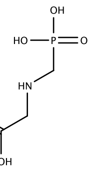

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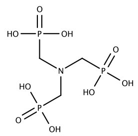

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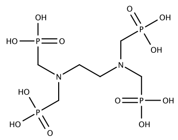

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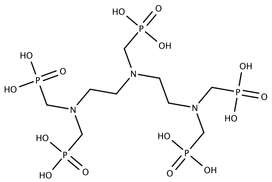
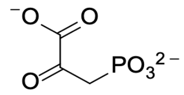
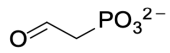


DTPMP

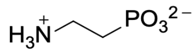
Common natural phosphonates



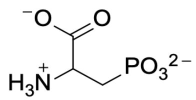
PnPyr



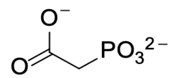
PAA



AEP



PnAla



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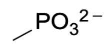
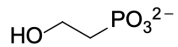
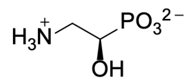


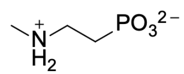
MePn



HEP



(*R*)-HAEP



M_1AEP

Source: Ruffolo et al. (2023); doi: 10.3390/molecules28196863.

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Structures of industrial and natural phosphonates

Common natural phosphonates

Chemical Reviews | Review

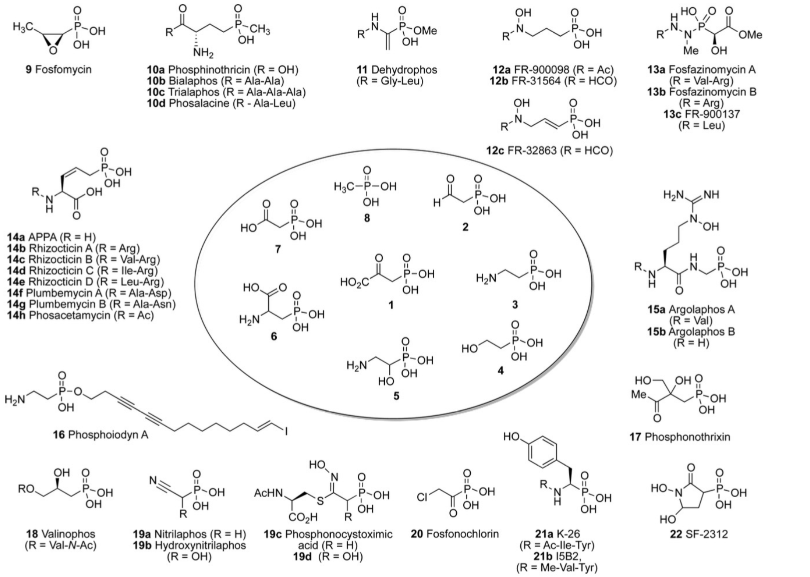


Figure 1. Overview of Pn natural products. Pn within the circle, with the exception of **4**, are distinguished by their dual roles in biosynthesis and sources of Pi. Pn outside the circle are bioactive secondary metabolites.

Source: Horsman and Zechel, 2016; doi: 10.1021/acs.chemrev.6b00536.

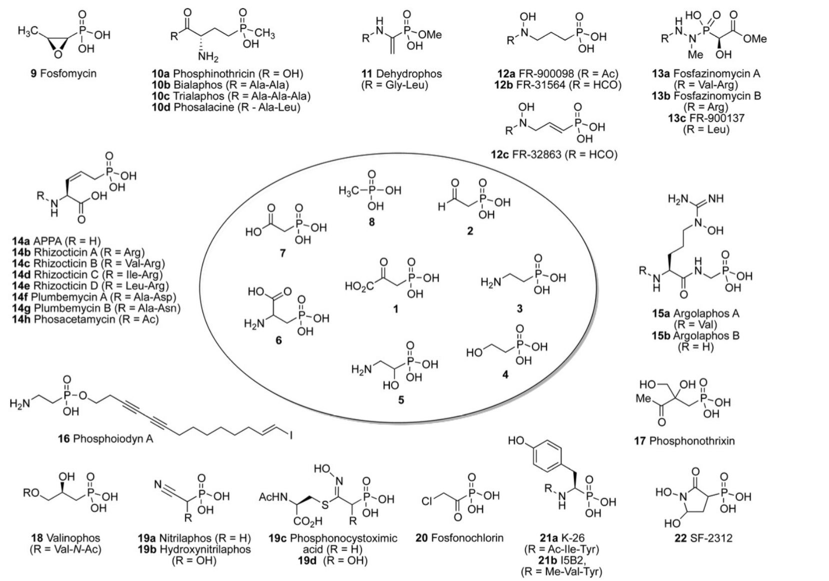
Structures of industrial and natural phosphonates

Function of natural phosphonates

- Antibiotics (Fosfomycin)
- Herbicide (Glyfosinate)
- Part of phosphonolipids
- Part of phosphonoglycans
- Part of exopolysaccharides
- Part of phosphonoglycoproteins

Common natural phosphonates

Chemical Reviews — Review



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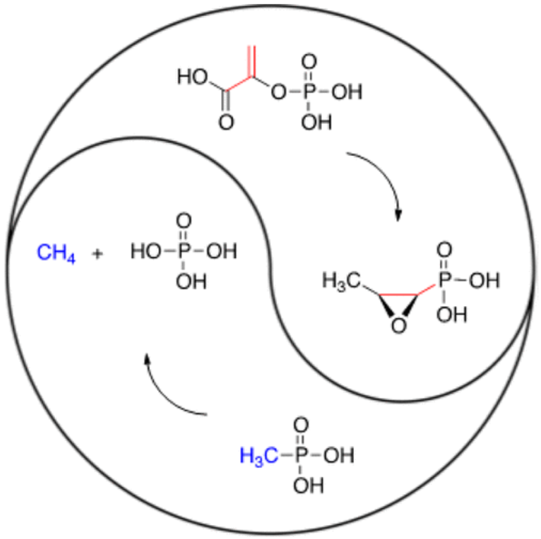
The yin and yang of phosphonates



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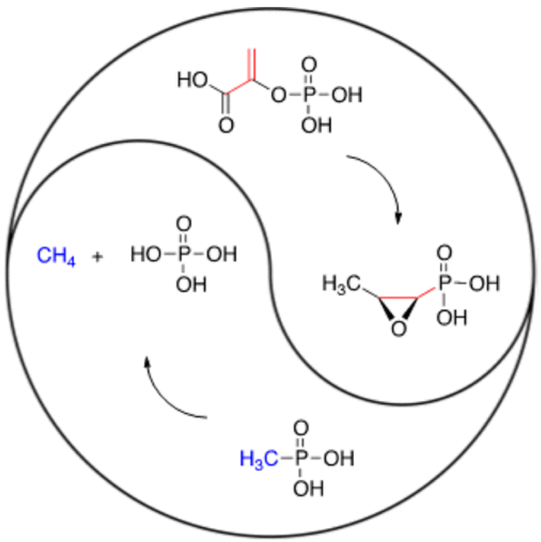

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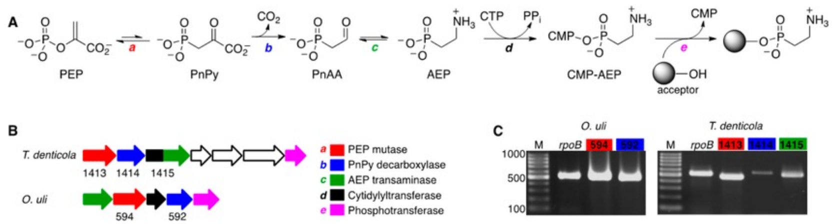
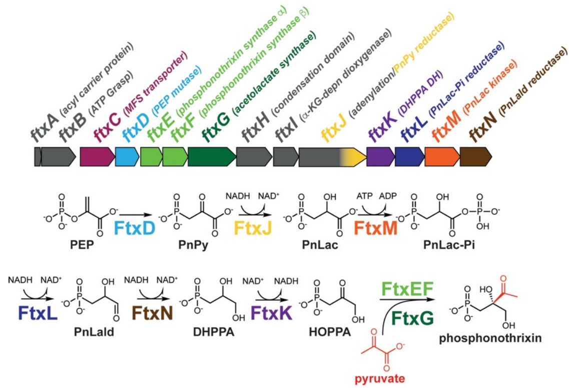
Biosynthesis of natural phosphonates



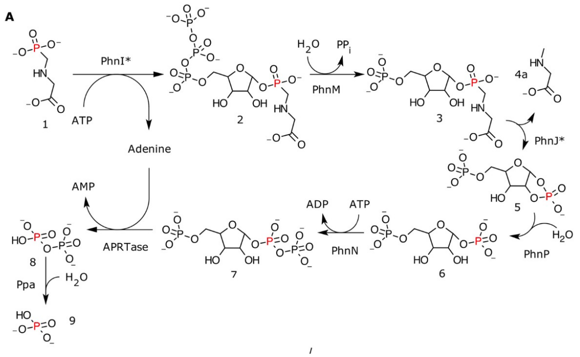
Source: Bartlett at al., 2017; doi: 10.1021/acs.biochem.7b00814.



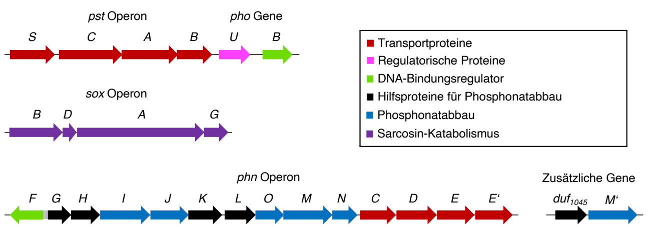
Source: Brown et al. 2023; doi: 10.1128/jb.00485-22.

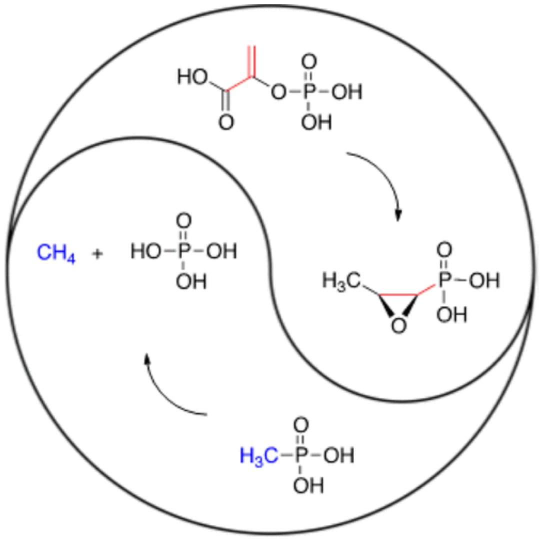
The yin and yang of phosphonates

Catabolism of phosphonates



Source: Hove-Jensen et al. 2014; doi: 10.1128/MMBR.00040-13.





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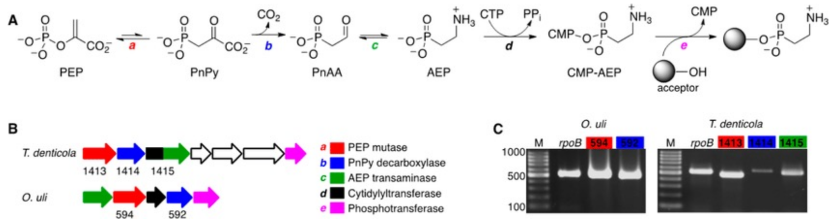

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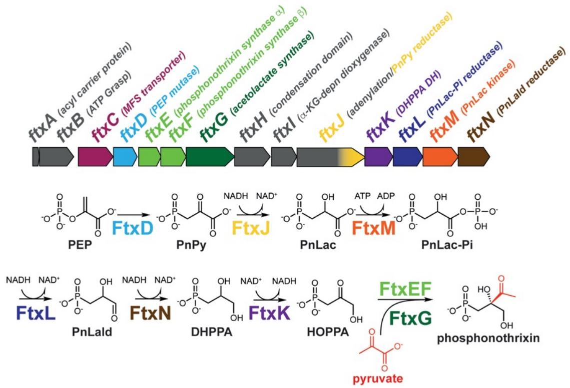

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The enzymatic arsenal to break down phosphonates

1. Hydrolytic C-P bond cleavage
2. Oxidative C-P bond cleavage by PhnY*/PhnZ

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Substrate specific →

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The enzymatic arsenal to break down phosphonates

Substrate specific → 1. Hydrolytic C-P bond cleavage

Substrate specific → 2. Oxidative C-P bond cleavage by PhnY*/PhnZ

Broad spectrum → 3. Radical C-P bond cleavage by carbon-phosphorus lyase

4. …?

The enzymatic arsenal to break down phosphonates

Substrate specific → 1., 2.

Broad spectrum → 3.

1. Hydrolytic C-P bond cleavage
2. Oxidative C-P bond cleavage by PhnY*/PhnZ
3. Radical C-P bond cleavage by carbon-phosphorus lyase
4. …?

Microbial genomes reveal (2012):

- 10 % of all genomes contain genes for Pn biosynthesis
- 40 % of all genomes contain genes for Pn catabolism (some with different pathways)

1. Hydrolytic C-P bond cleavage of phosphonates

Hydrolytic C-P bond cleavage

Agarwal et al. (2011), Cell Chem. Biol. 18, 1230-1240.

Phosphonopyruvate hydrolase	Phosphonoacetaldehyde hydrolase	Phosphonoacetate hydrolase
↓	↓	↓
(PalA)	**(PhnX)**	**(PhnA)**

- Not evolutionarily related
- All three are metal-depending
- Catalyze similar enzymatic reactions → Hydrolytic cleavage due to neighbouring carbonyl or carboxyl group.

Hydrolytic C-P bond cleavage

Agarwal et al. (2011), Cell Chem. Biol. 18, 1230-1240.

Phosphonopyruvate hydrolase

(PalA)

Phosphonoacetaldehyde hydrolase

(PhnX)

Phosphonoacetate hydrolase

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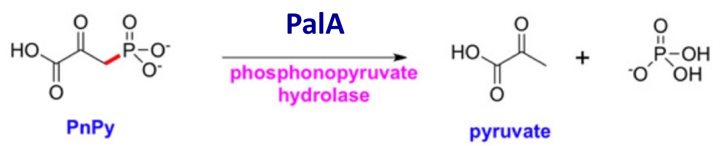

Co^{2+}, Ni^{2+} and Mg^{2+}

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(PhnX)

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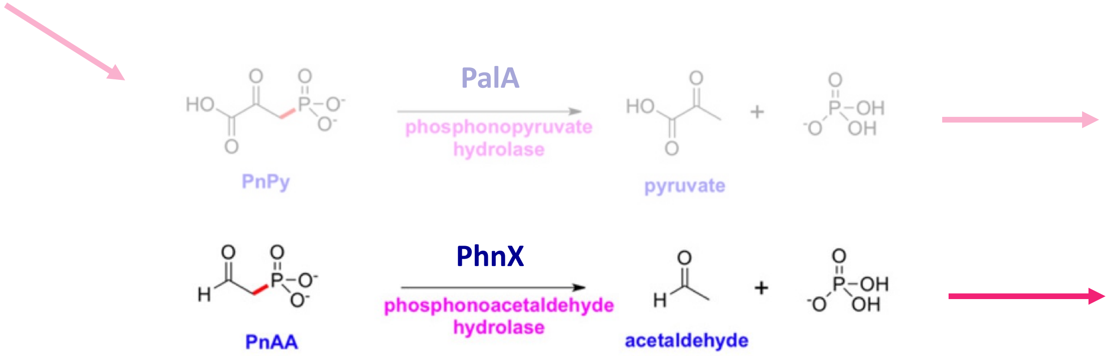

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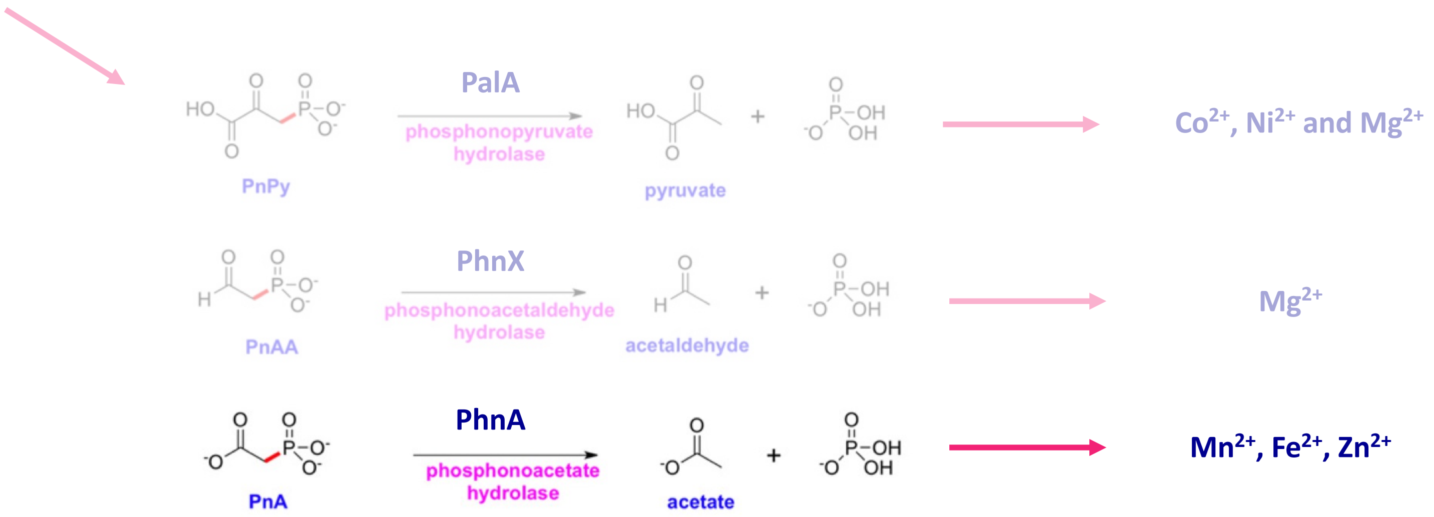
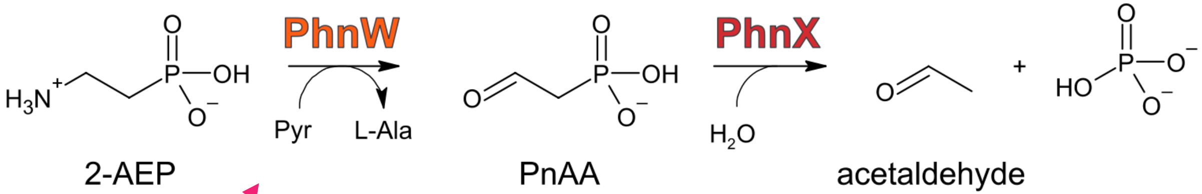


Hydrolytic C-P bond cleavage

Zangelmi (2021), PhD thesis, University of Parma, Italy.

PhnX and PhnA → Commonly degrade 2-AEP, but in 2 different pathways



PhnW - Aminotransferase → Known as the PhnWX pathway

→ Very narrow substrate scope, no conversion of AEP-related structures

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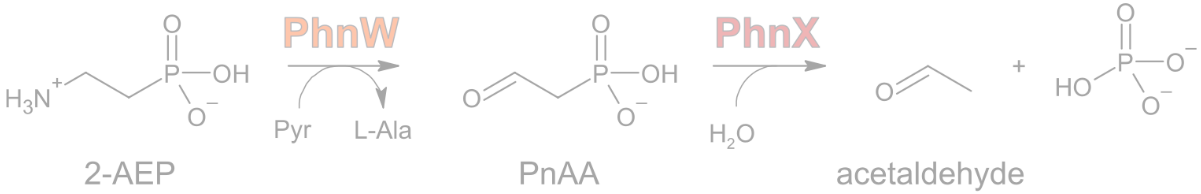
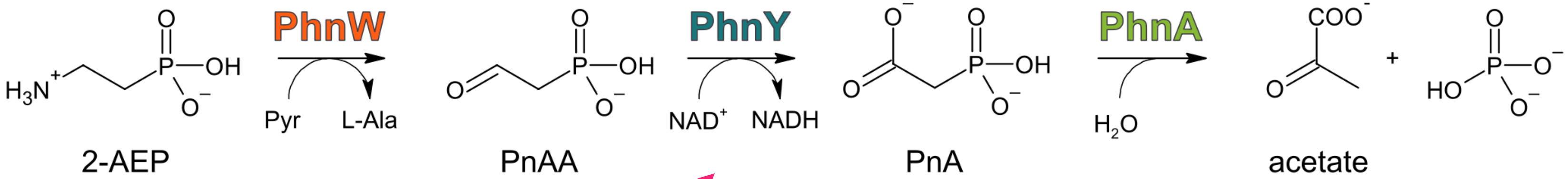




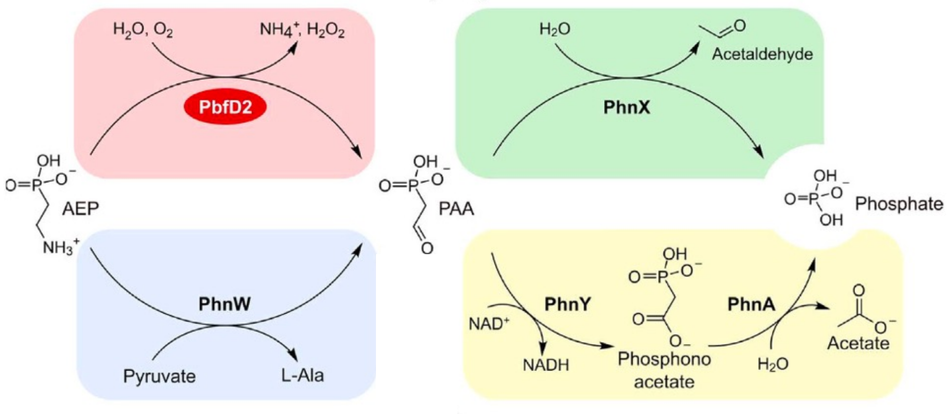
PhnY – Phosphonoacetaldehyde dehydrogenase

Known as the PhnWAY pathway

Hydrolytic C-P bond cleavage

Zangelmi et al. (2023), Iscience 26, 108108.

Other hydrolytic cleavage of AEP and related structures by phosphonate breakdown factors (PbfB, PbfC, PbfD)



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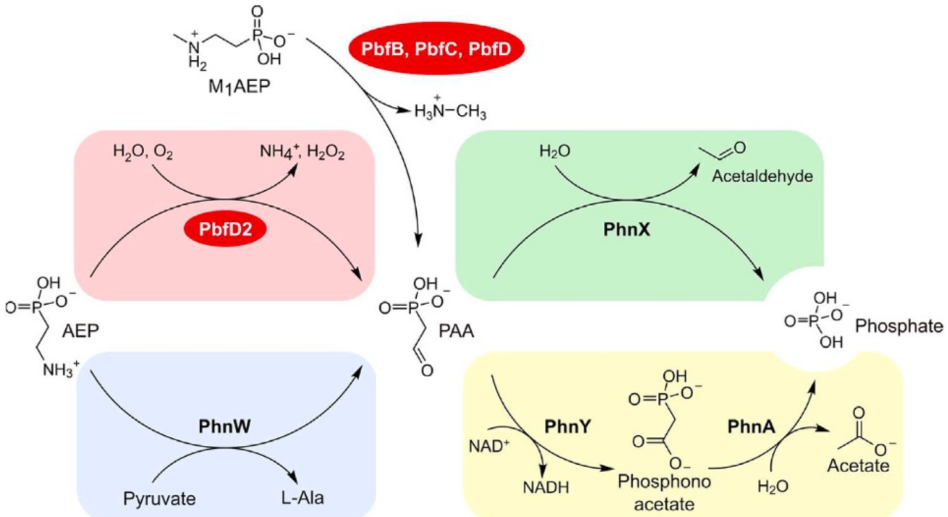

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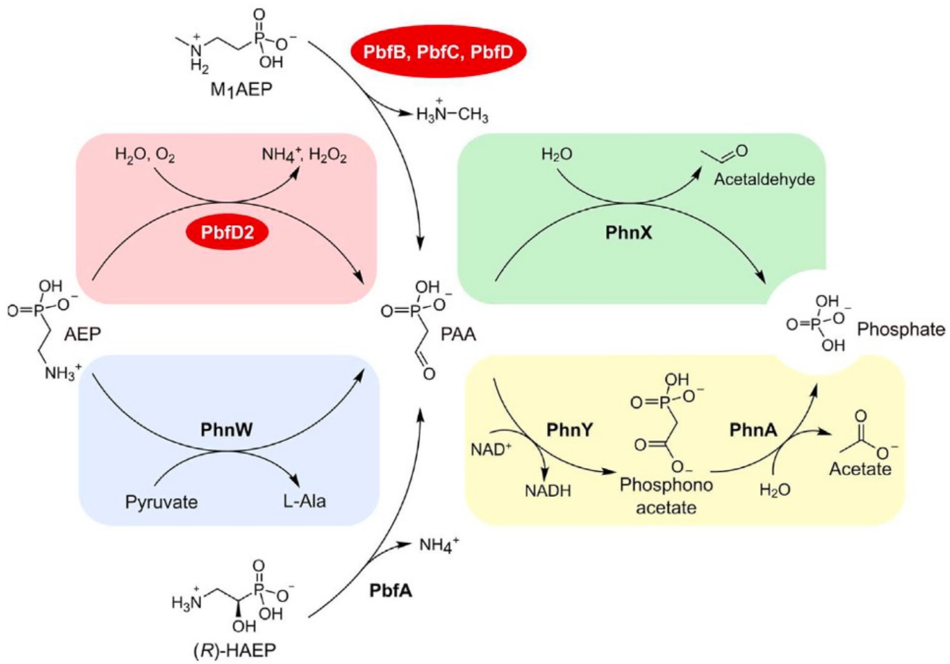

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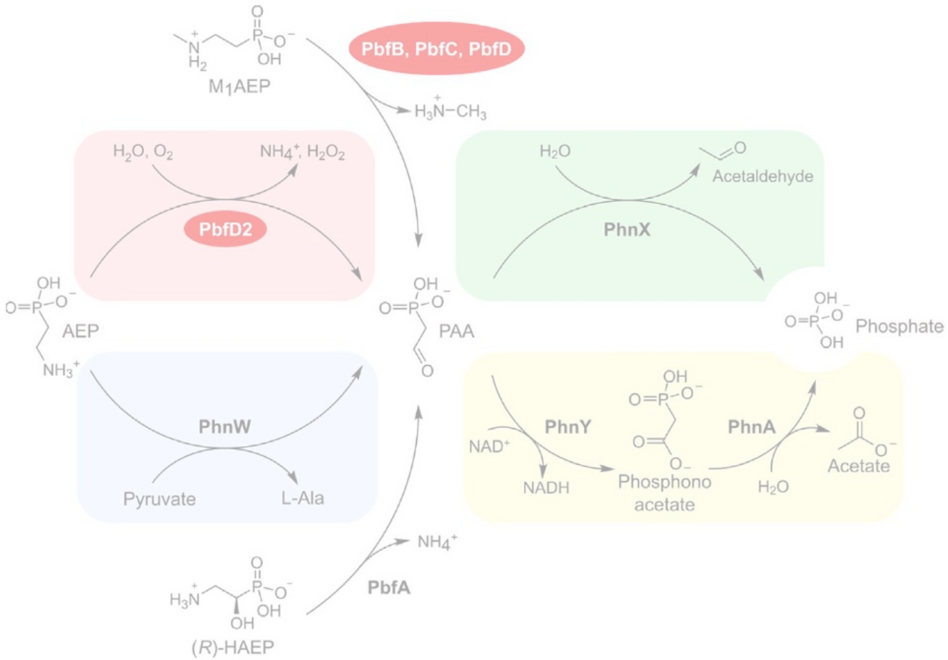

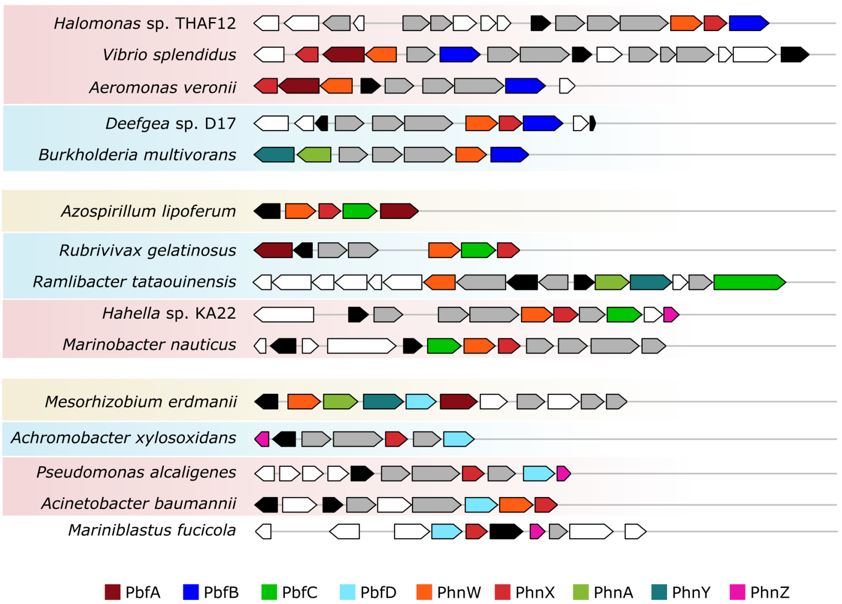

2. Oxidative C-P bond cleavage of phosphonates

Oxidative C-P bond cleavage by PhnY* and PhnZ

- Pathway uses molecular O_2 and ferrous iron for cleavage
- Frist identified in genomes of marine bacteria, later also in terrestrial bacteria

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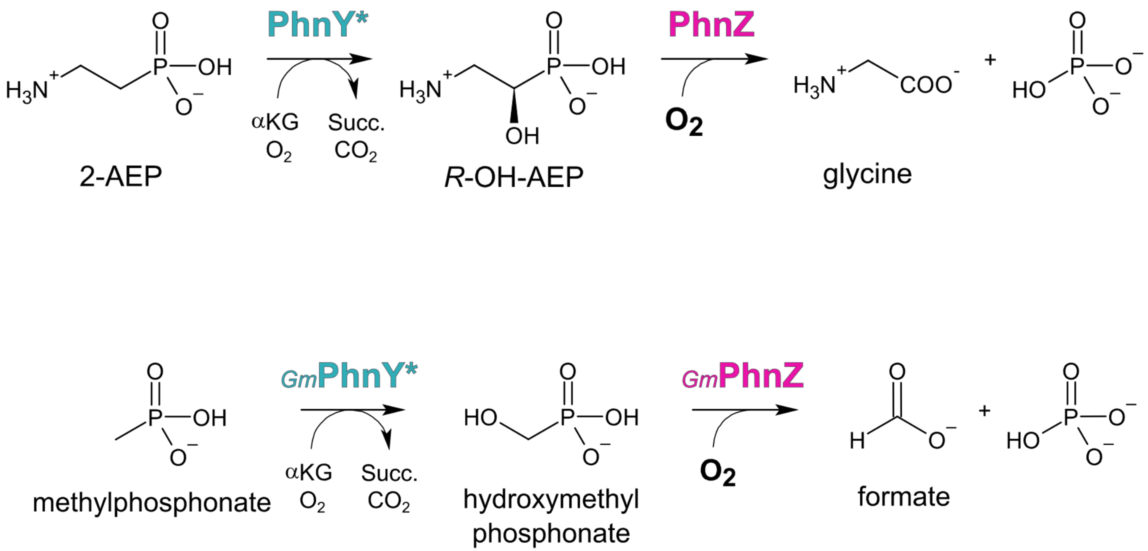
- 2 key enzymes: PhnY* and PhnZ
- PhnY* - 2-AEP dioxygenase
- PhnZ – 1-hydroxy-2-AEP dioxygenase
- PhnY* appear most common in marine bacteria to break down 2-AEP
- PhnZ can also occur in association with *phnWX* gene cluster or associated with the C-P lyase operon

Oxidative C-P bond cleavage by PhnY* and PhnZ

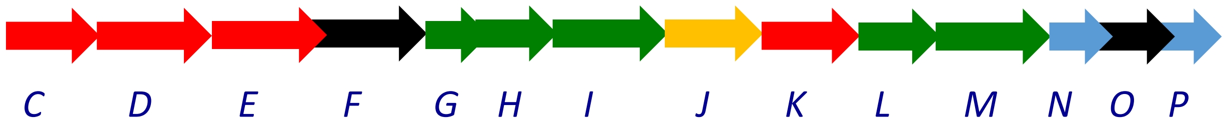
Zangelmi (2021), PhD thesis, University of Parma, Italy.



3. Radical C-P bond cleavage of phosphonates

Radical C-P bond cleavage by carbon-phosphorus lyase

Peck & van der Donk (2013) Current Opinion in Chemical Biology 17, 580-588



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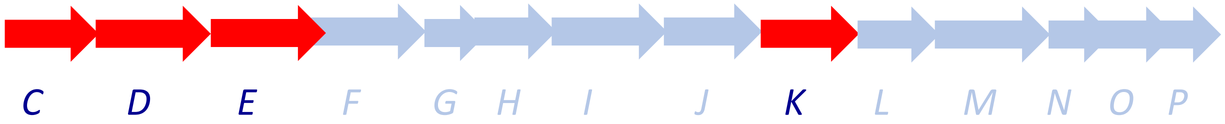


Transporter related

PhnC – Phosphonates-import ATP-binding protein

PhnD – Phosphonates-binding periplasmic protein

PhnE – Phosphonates-import permease protein

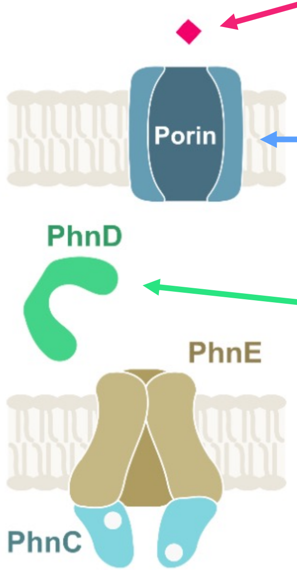
PhnK – Phosphonates C-P lyase system protein

Radical C-P bond cleavage by carbon-phosphorus lyase

Ruffolo et al. (2023) *Molecules* 28, 6863

Phosphonate outside of cell

Outer membrane



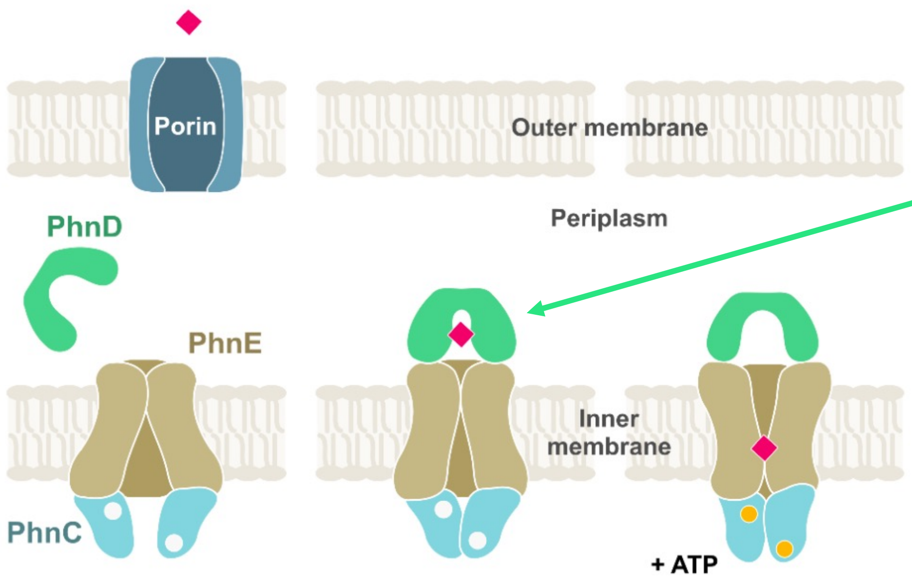
PhoE – porin expressed during P staravtion (controlled by Pho regulon)

PhnD not associated with transporter

Inner membrane

Radical C-P bond cleavage by carbon-phosphorus lyase

Ruffolo et al. (2023) *Molecules* 28, 6863



PhnD interacts with transmembrane complex

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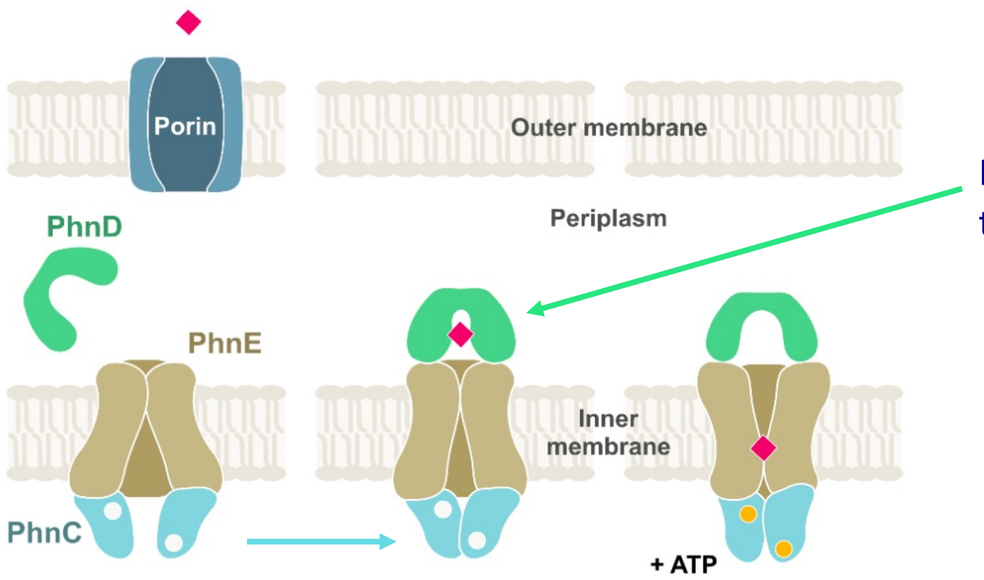

PhnD interacts with transmembrane complex

PhnC binding domain move towards

Radical C-P bond cleavage by carbon-phosphorus lyase

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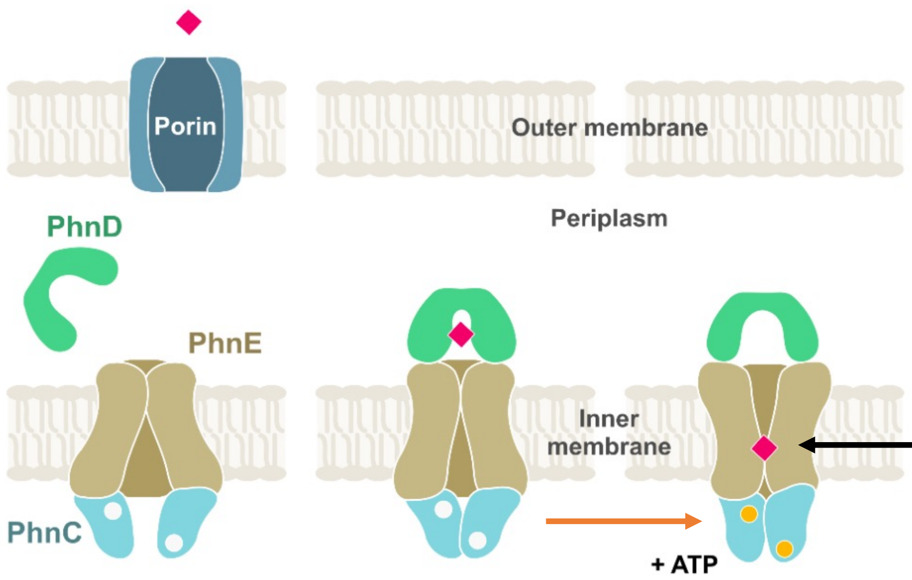

ATP binds to PhnC and allows phosphonate to enter chanel

Radical C-P bond cleavage by carbon-phosphorus lyase

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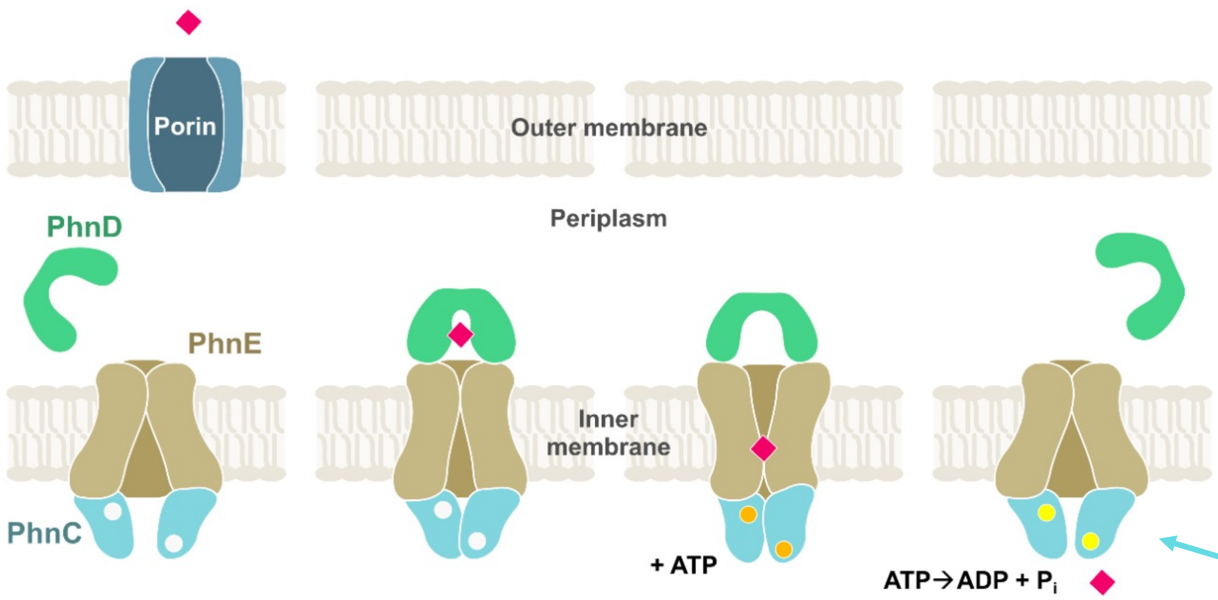
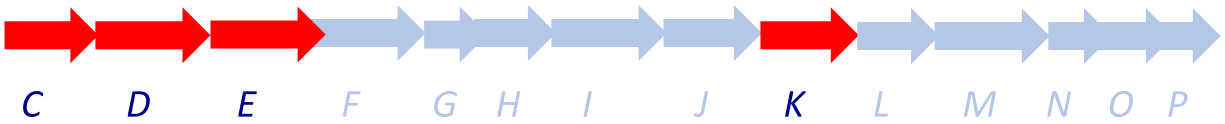


ATP is hydrolyzed & chanel open inwards

Radical C-P bond cleavage by carbon-phosphorus lyase



Transporter related

PhnC – Phosphonates-import ATP-binding protein

PhnD – Phosphonates-binding periplasmic protein

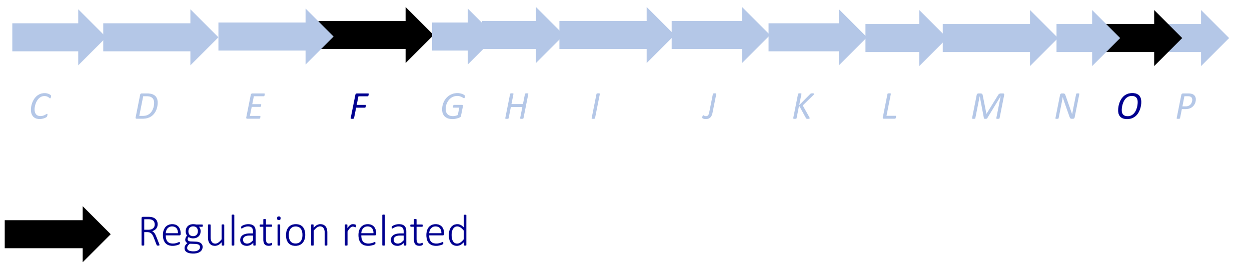
PhnE – Phosphonates-import permease protein

PhnK – Phosphonates C-P lyase system protein

Function still uncertain

Radical C-P bond cleavage by carbon-phosphorus lyase

Peck & van der Donk (2013) Current Opinion in Chemical Biology 17, 580-588

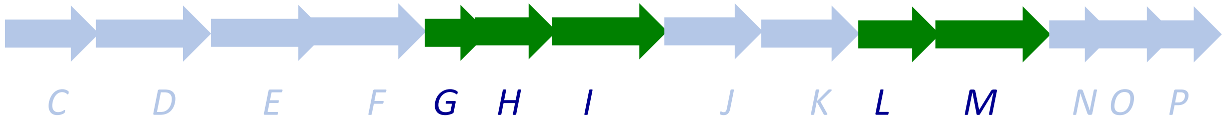


PhnF – Probable transcriptional regulator (repressor)

PhnO – Aminoalkylphosphonate N-acetyltransferase

Radical C-P bond cleavage by carbon-phosphorus lyase

Peck & van der Donk (2013) Current Opinion in Chemical Biology 17, 580-588



Catalytic – **pre** C-P bond cleavage

PhnG – Carbon-phosphorus lyase complex subunit

PhnH, PhnI, PhnL – PRPn* synthase subunits

PhnM – PRPn diphosphonatase

*PRPn – alpha-D-ribose 1-methylphosphonate-5-triphosphate

Radical C-P bond cleavage by carbon-phosphorus lyase

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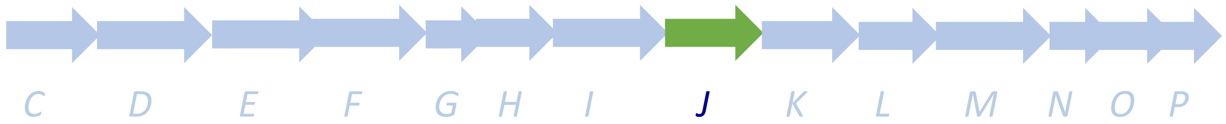
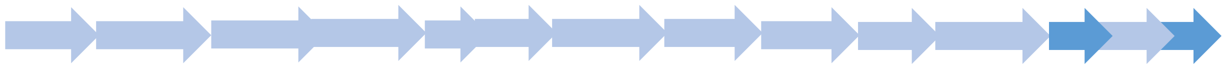


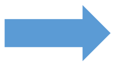
Catalytic – **C-P** bond cleavage

PhnJ – alpha-D-ribose 1-methylphosphonate-5-phosphate **C-P lyase**

Radical C-P bond cleavage by carbon-phosphorus lyase

Peck & van der Donk (2013) Current Opinion in Chemical Biology 17, 580-588



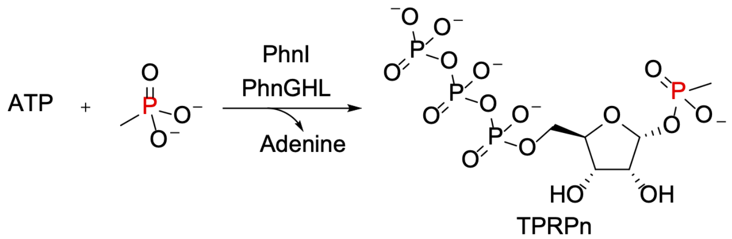
 Catalytic – **post** C-P bond cleavage

PhnN – Ribose-1,5-bisphosphate phosphokinase

PhnP – Phosphoribosyl 1,2-cyclic phosphate phosphodiesterase

Radical C-P bond cleavage by carbon-phosphorus lyase

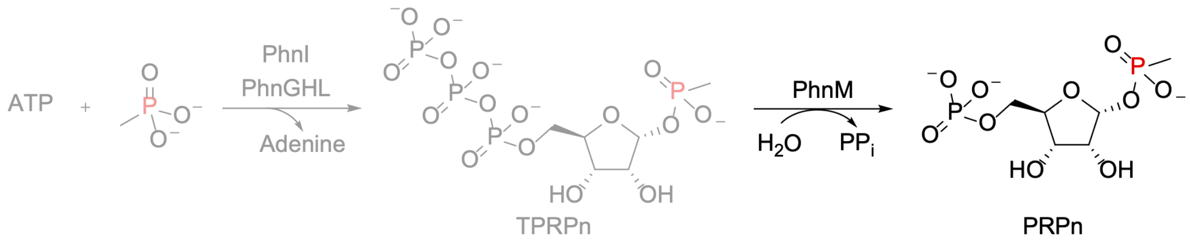
Ruffolo et al. (2023) *Molecules* 28, 6863



5´-phospho-α-d-ribosyl-trisphophate (TRPP)

Radical C-P bond cleavage by carbon-phosphorus lyase

Ruffolo et al. (2023) *Molecules* 28, 6863



Radical C-P bond cleavage by carbon-phosphorus lyase

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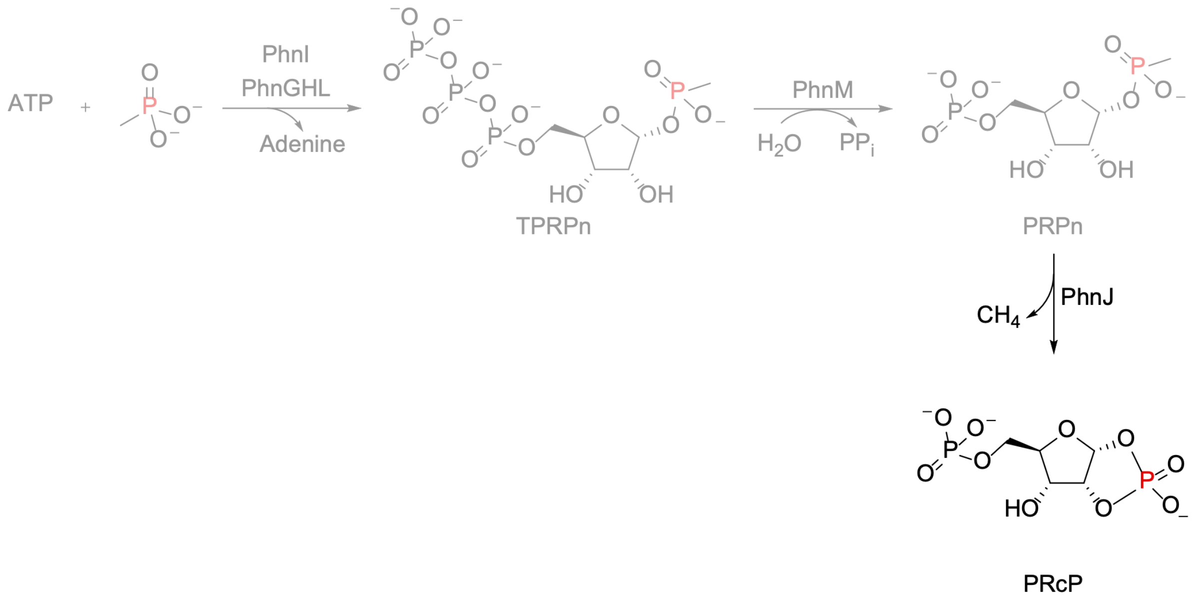

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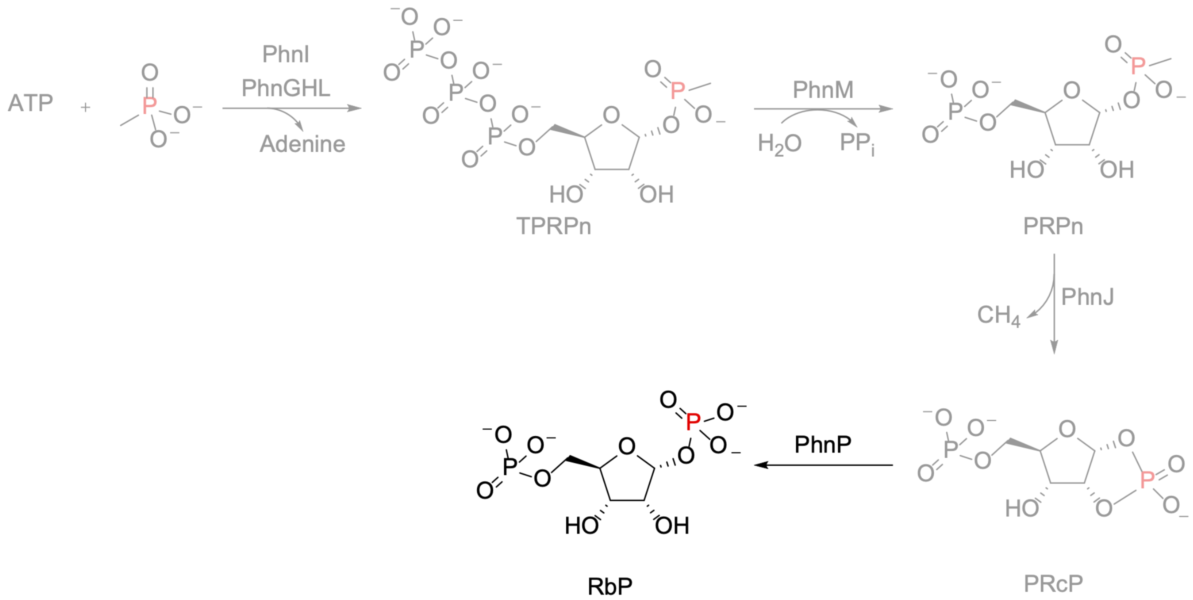
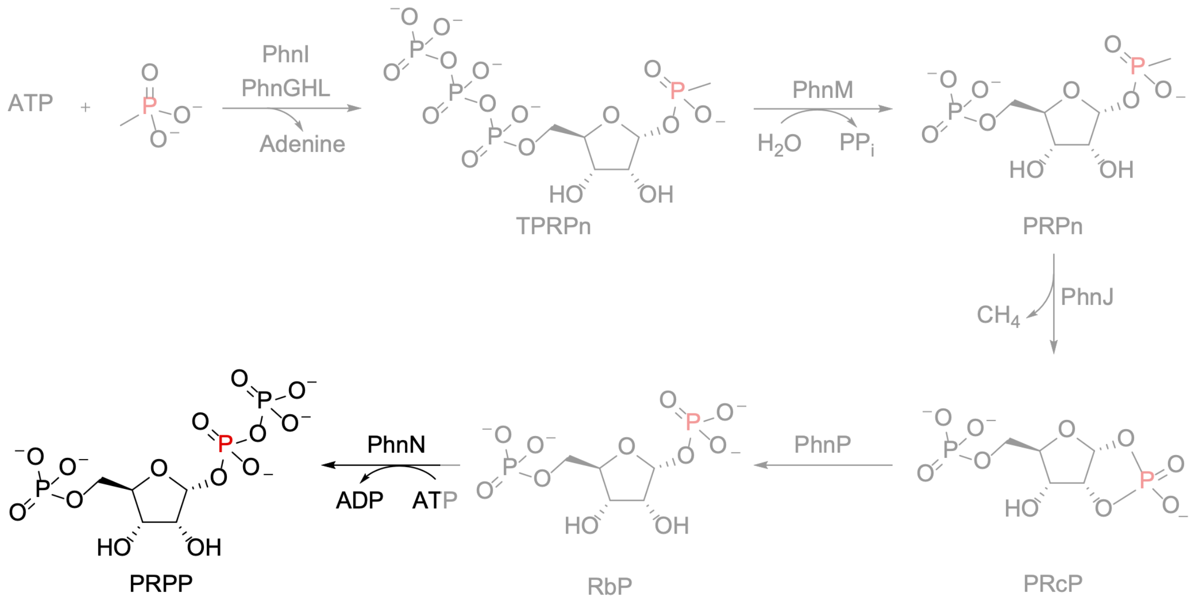


Radical C-P bond cleavage by carbon-phosphorus lyase

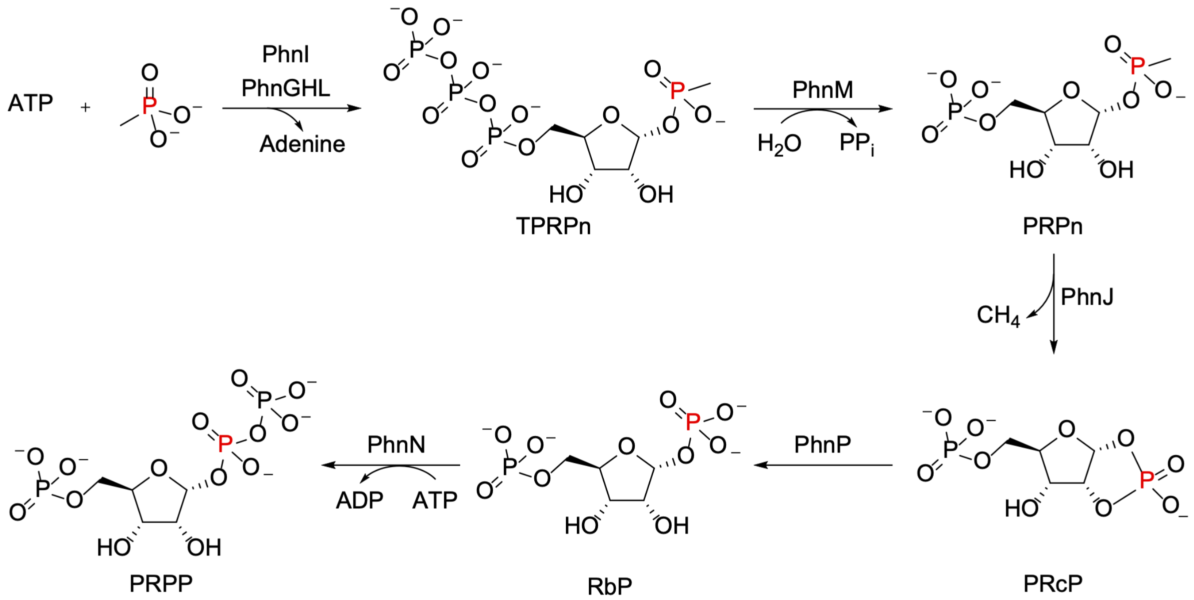
Ruffolo et al. (2023) *Molecules* 28, 6863



Radical C-P bond cleavage by carbon-phosphorus lyase

Ruffolo et al. (2023) *Molecules* 28, 6863

5 steps mediated
by 8 enzymes

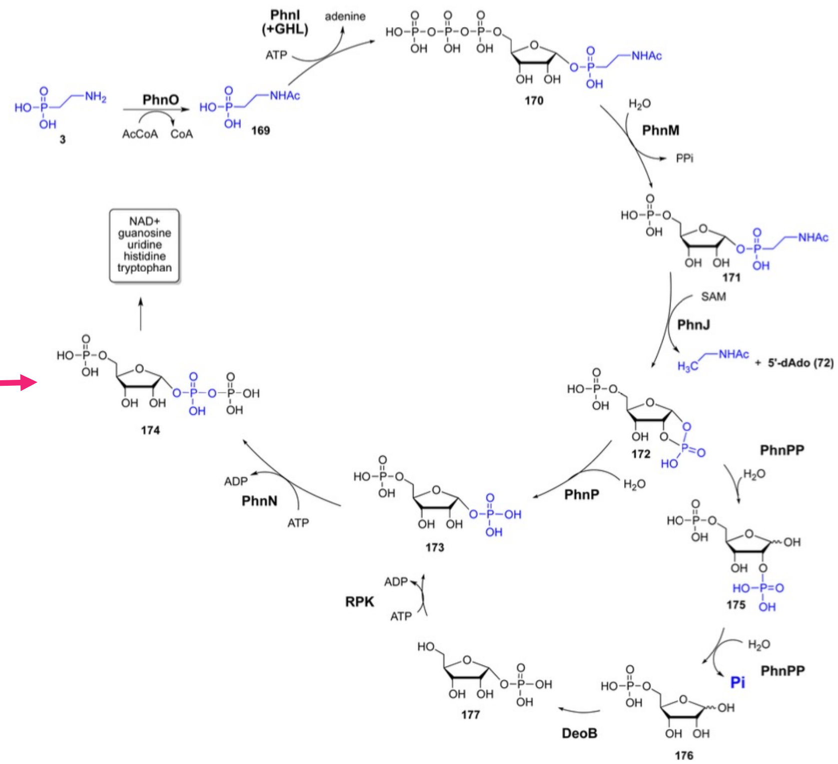


Radical C-P bond cleavage by carbon-phosphorus lyase

Horsman & Zechel (2017), Chemical Reviews 117, 5704-5783.

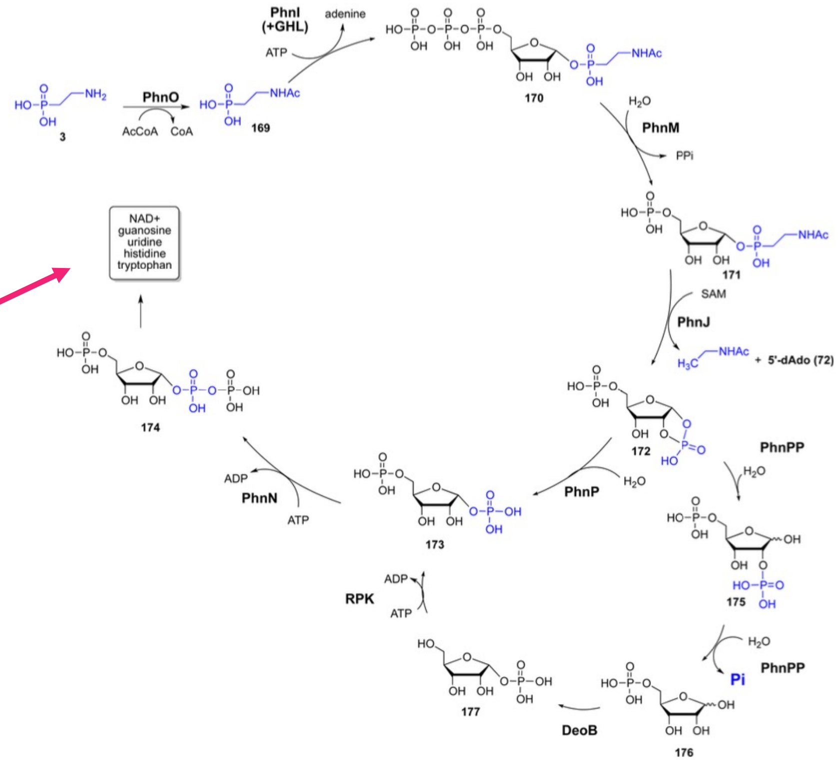
Acetylation of aminoalkylphosphonic acid

5´-phospho-α-d-ribosyl-bisphophate (PRPP)



Radical C-P bond cleavage by carbon-phosphorus lyase

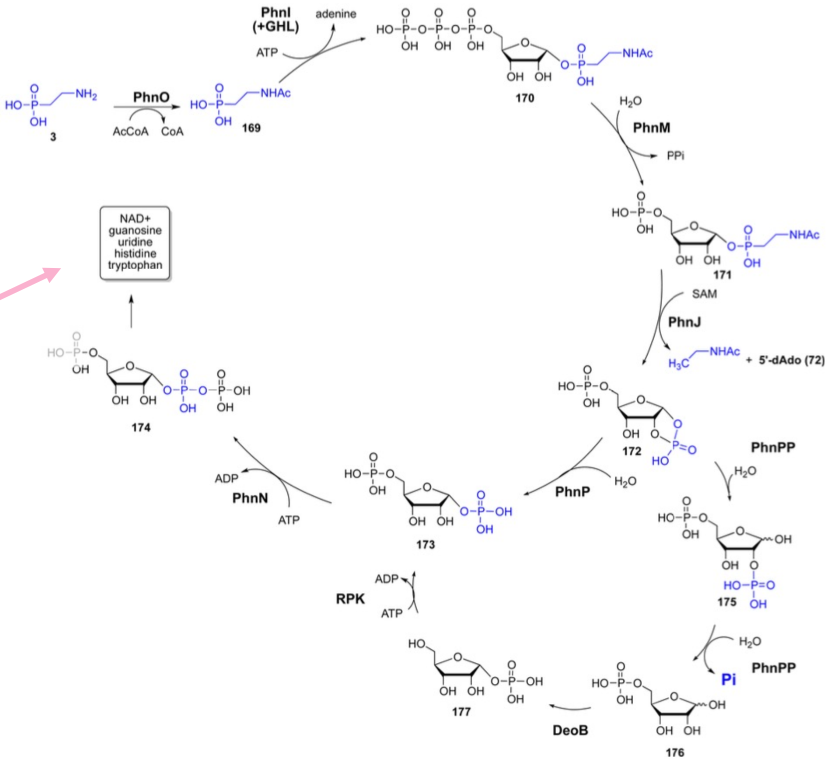
Horsman & Zechel (2017), Chemical Reviews 117, 5704-5783.



PRPP is a general donor for further biosynthesis of nucleotides and amino acids

Radical C-P bond cleavage by carbon-phosphorus lyase

Horsman & Zechel (2017), Chemical Reviews 117, 5704-5783.

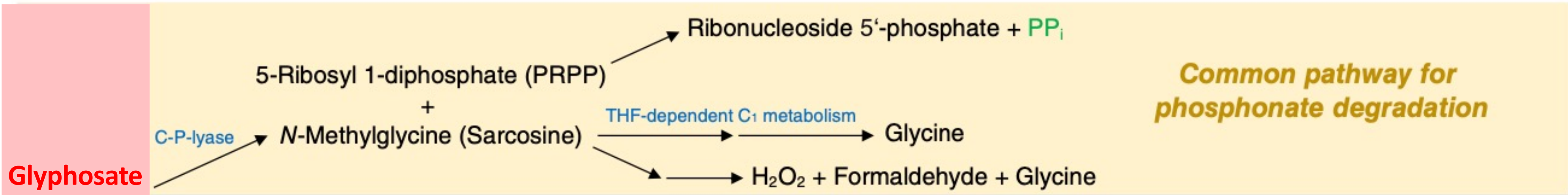


PRPP is a general donor for further biosynthesis of nucleotides and amino acids

Is this the same pathway for Glyphosate degradation?

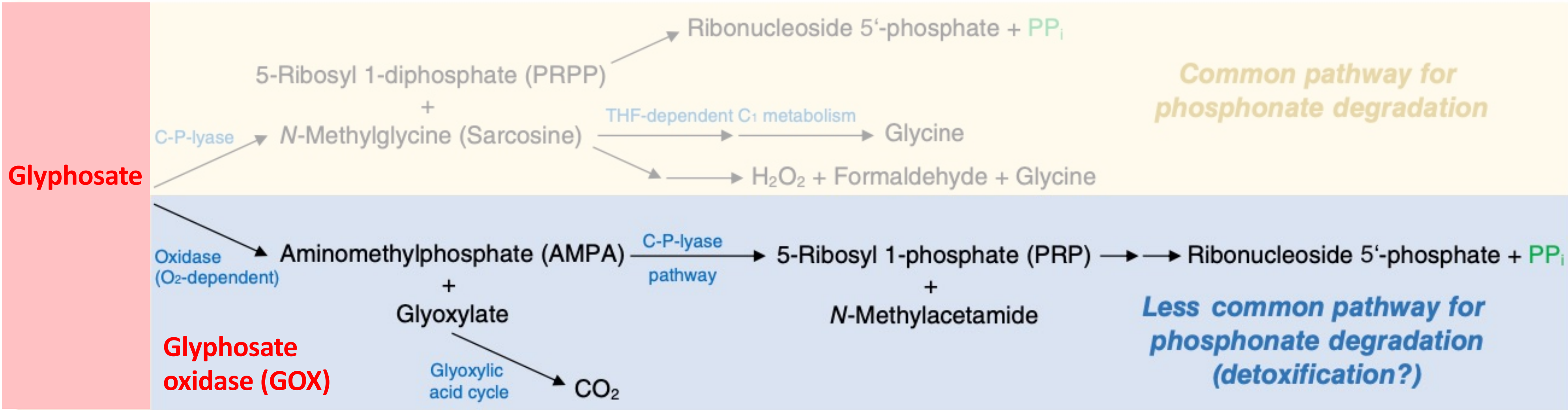
Radical C-P bond cleavage by carbon-phosphorus lyase

Hertel et al. (2021), Environ. Microbiol. 23, 2891-2905



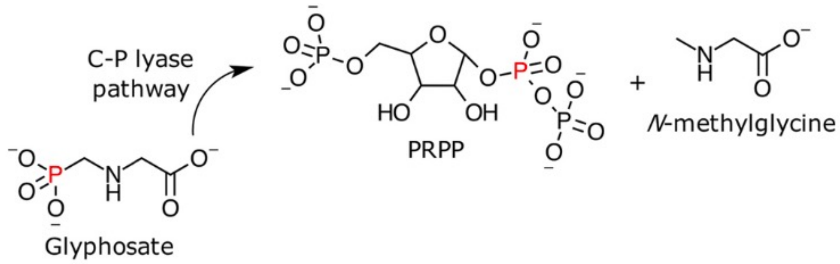
Radical C-P bond cleavage by carbon-phosphorus lyase

Hertel et al. (2021), Environ. Microbiol. 23, 2891-2905



Radical C-P bond cleavage by carbon-phosphorus lyase

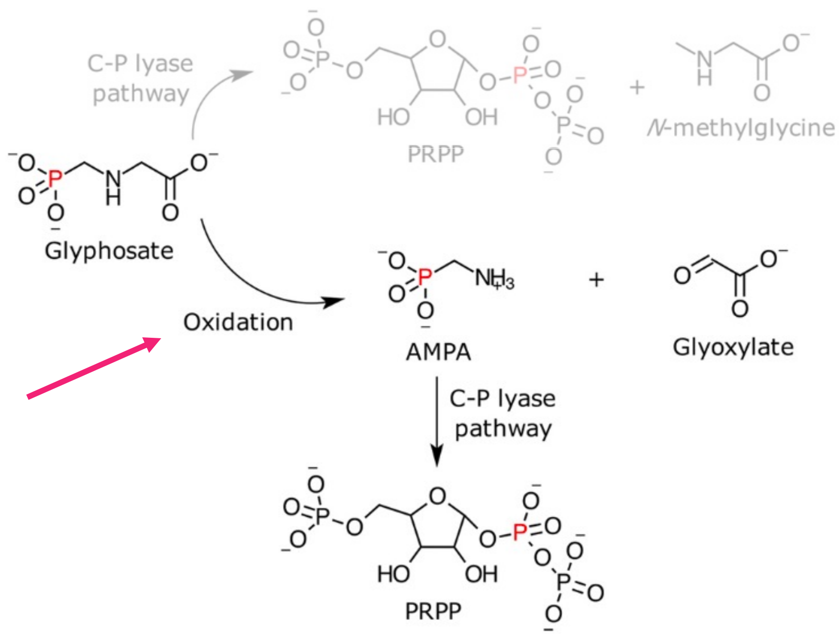
Hove-Jensen et al. (2014), MMBR 78, 176-197.



Sarcosine is further oxidased by SOX

Radical C-P bond cleavage by carbon-phosphorus lyase

Hove-Jensen et al. (2014), MMBR 78, 176-197.

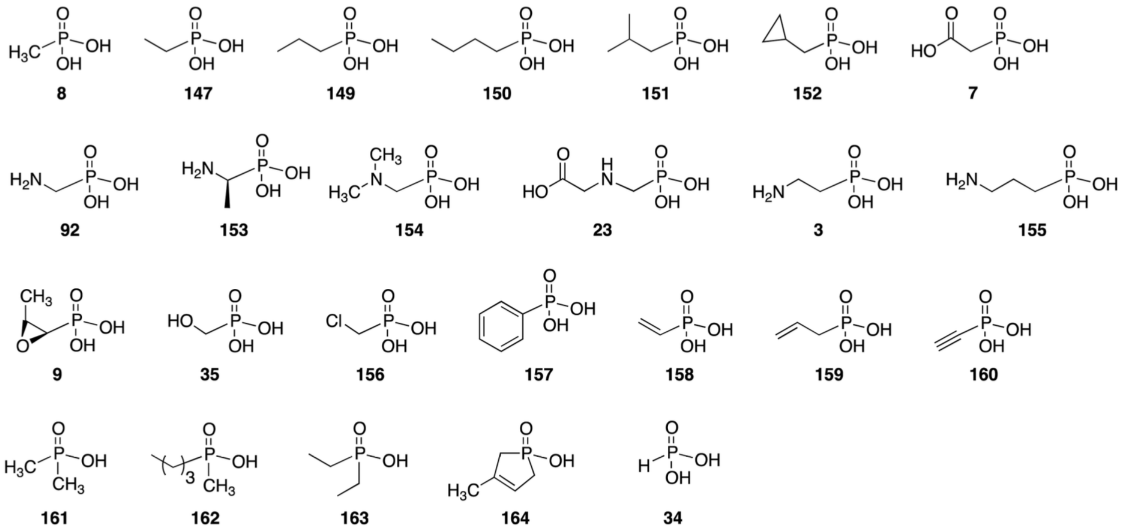


Sarcosine is further oxidased by SOX

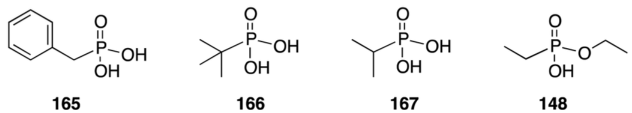
Mediated by GOX

Radical C-P bond cleavage by carbon-phosphorus lyase

Substrates



Not Substrates



Which pathway may be used to breakdown industrial phosphonates?

Which pathway may be used to breakdown industrial phosphonates?

2017 | 2019 | 2022

First Stage of development

- Choice of C & N source
- Buffer system
- Colourmetric test
- LC/MS

Done

First applications

- KH_2PO_4
- Phosphonates
- Pure strain

Done

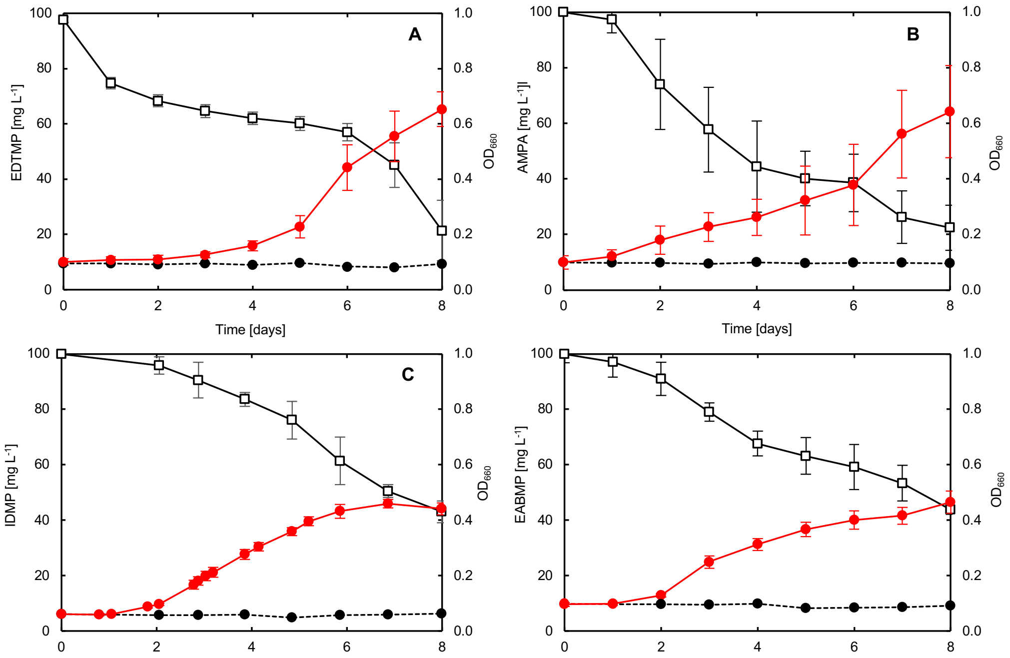
Further optimisation for standardisation

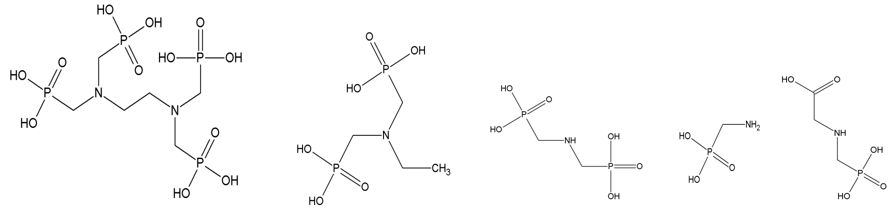
- Alternative Test for OECD Tests 301A-F
- Different inocula

Done

Biodegradation of selected aminophosphonates by *Ochrobactrum* sp. BTU1

Riedel et al. (2024), Microbiol. Res. 280, 127600.

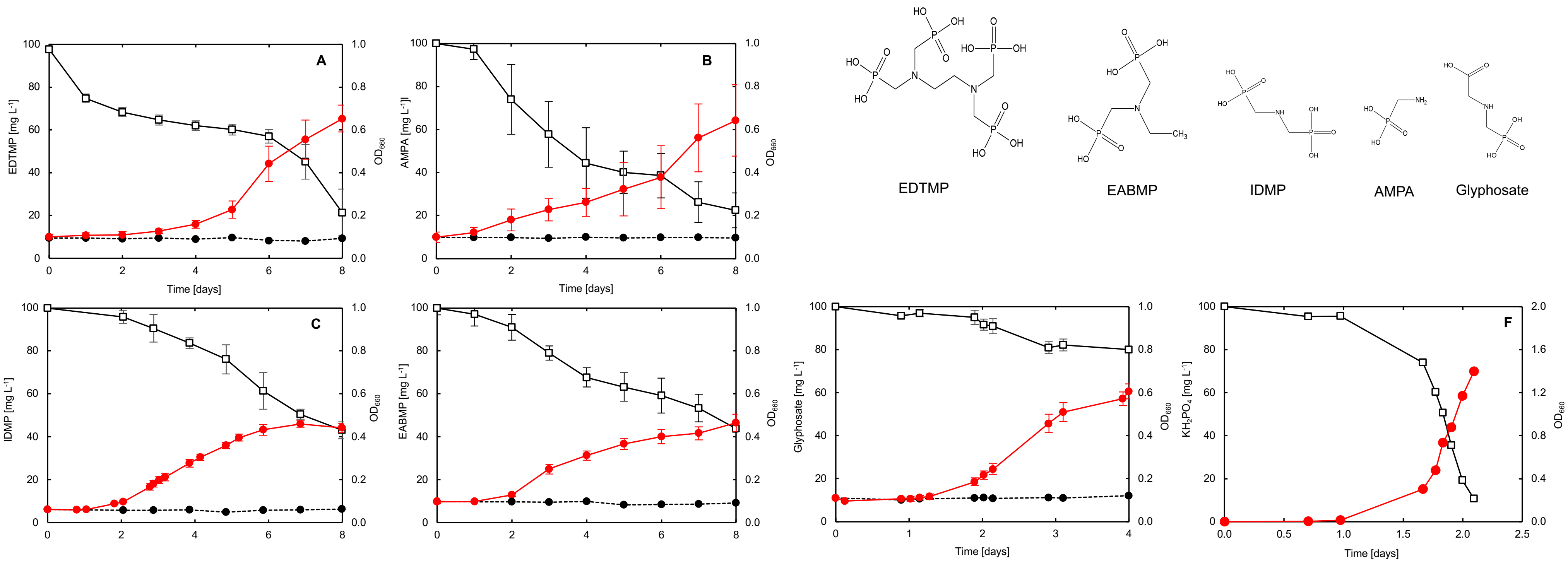




EDTMP EABMP IDMP AMPA Glyphosate

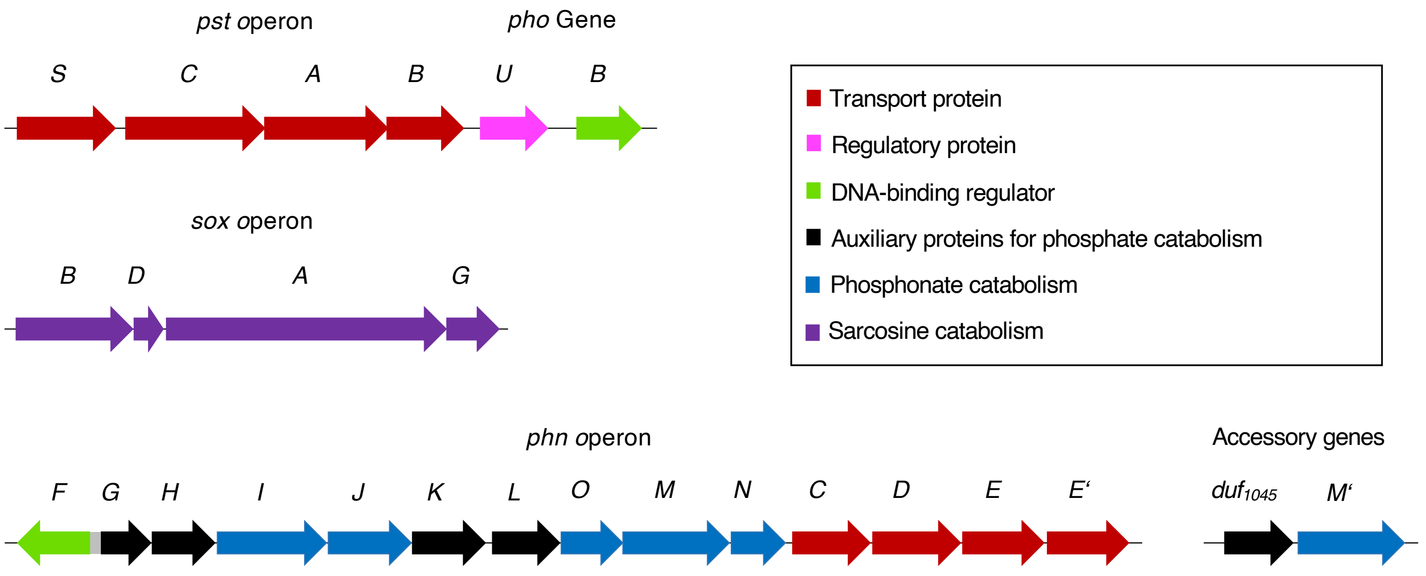
Biodegradation of selected aminophosphonates by *Ochrobactrum* sp. BTU1

Riedel et al. (2024), Microbiol. Res. 280, 127600.



Biodegradation of selected aminophosphonates by *Ochrobactrum* sp. BTU1

Riedel et al. (2024), Microbiol. Res. 280, 127600.



Biodegradation of selected aminophosphonates by *Ochrobactrum* sp. BTU1

R. Riedel et al. *Microbiological Research 280 (2024) 127600*

Table 3
Changes in the proteome of *Ochrobactrum* sp. BTU1 during growth with selected aminophosphonates. The volume of peptides was normalized to the total volume of each sample after the background had been corrected. Samples in triplicates were used to calculate the induction ratios ($\log_2$). Samples that had phosphates were used as positive control for the growth tests. Significant changes were filtered by t-test (p-value < 0.05). '-' marks non-significant ratios.

				$\log_2$ [aminophosphonate/positive control]				
Protein	**Locus tag**	**Functional category**	**Function**	**AMPA**	**GS**	**IDMP**	**EABMP**	**EDTMP**
DNA polymerase subunit	KMS41_00100	Information Profession	DNA polymerase γ/τ subunit, DNA polymerization	-2.43	-1.89	-3.39	-1.53	-2.71
CysQ	KMS41_00945	Metabolism	3'(2'),5'-bisphosphate nucleotidase, sulfate assimilation	-1.50	-1.15	-2.02	-2.69	-1.53
Sarcosine oxidase subunit	KMS41_01085	Metabolism	Sarcosine oxidase β subunit, sarcosine degradation	-1.56	2,59	-3.03	-	-3.12
Sarcosine oxidase subunit	KMS41_01090	Metabolism	Sarcosine oxidase δ subunit, sarcosine degradation	-	2.60	-	-	1.74
Sarcosine oxidase subunit	KMS41_01095	Metabolism	Sarcosine oxidase α subunit, sarcosine degradation	4.39	4.50	3.02	4.96	-
Sarcosine oxidase subunit	KMS41_01100	Metabolism	Sarcosine oxidase γ subunit, sarcosine degradation	1.50	2.99	1.65	-	2.42
Pgi	KMS41_01400	Metabolism	Glycolysis	2.41	1.22	3.16	2.85	2.60
Uncharacterized	KMS41_01590	Unknown	Unknown	1.98	3.00	2.43	2.95	1.68
Ppk	KMS41_03530	Metabolism	Polyphosphate kinase, polyphosphate synthesis	4.30	2.76	4.98	4.23	4.78
RibE	KMS41_03620	Transport	6,7-Dimethyl-8-ribityllumazine synthase, riboflavin biosynthesis	4.30	2.76	4.98	4.23	4.78
duf_{1045}	KMS41_04135	Metabolism	DUF1045 domain-containing protein	1.30	-	1.92	2.13	2.07
PhnM'	KMS41_04140	Metabolism	Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase, phosphonate degradation					
PhnE	KMS41_05445	Transport	ABC transporter permease subunit, phosphonate transport	-	-	-	-	-
PhnE'	KMS41_05450	Transport	ABC transporter permease subunit, phosphonate transport	-	-	-	-	-
PhnD	KMS41_05455	Transport	ABC transporter periplasmic binding protein, phosphonate transport	5.29	4.39	4.59	6.91	5.04
PhnC	KMS41_05460	Transport	ABC transporter nucleotide binding protein, phosphonate transport	3.63	2.39	4.29	6.21	4.20
PhnN	KMS41_05465	Metabolism	Ribosyl bisphosphate phosphokinase, phosphonate degradation	-	3.39	-	-	-
PhnM	KMS41_05470	Metabolism	5-Triphosphoribosyl 1-phosphonate diphosphohydrolase, phosphonate degradation	5.08	5.95	6.04	5.69	4.59
PhnO	KMS41_05475	Metabolism	Aminoalkylphosphonate *N*-acetyl-transferase, phosphonate degradation	7.58	8.56	8.58	7.92	8.12
PhnL	KMS41_05480	Metabolism	Phosphonate C-P lyase system protein, phosphonate degradation	7.07	8.77	8.34	7.85	7.54

Outcome

1. More than 5.000 proteins detected
2. Significant difference in the regulation

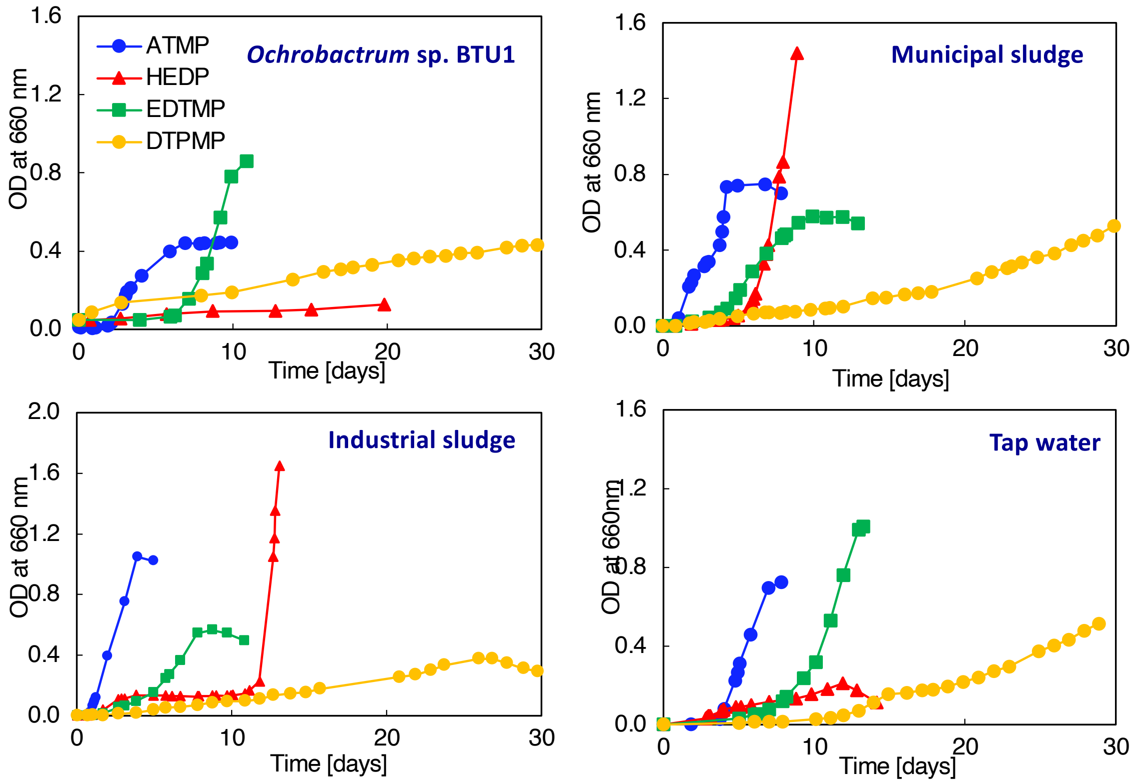
   GS vs. other phosphonates
3. Smallest phosphonate best degradable

→ **C-P lyase pathway preferred**

Can also other industrial phosphonates be catabolized?

Influence of different chemical structure

Riedel et al. (2023), Microbiol. Methods 212, 106793.



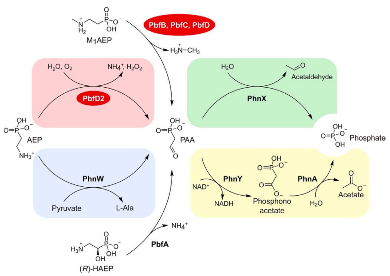
Outcome

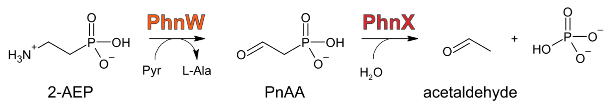
1. Suitable with different inoculums
2. Chemical structure has influence
3. First response for ATMP
4. Lowest response for DTPMP

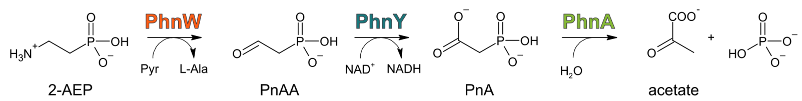
Summary

1. Hydrolytic C-P bond cleavage: PalA, PhnX and PhnA

- highly substrate specific and metal depending
- Encompased by Pbfb, PbfC, $PbfD_1$ and $PbfD_2$ (only four?)
- PhnX is often found as PhnWX pathway, very narrow substrate scope for 2-AEP
- PhnW can also occur in PhnWAY pathway, also specific for 2-AEP



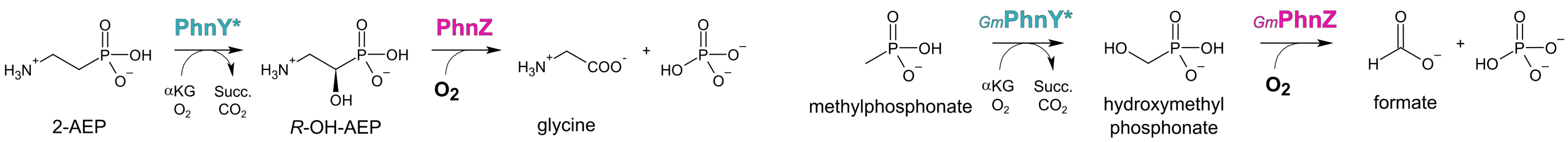




Summary

2. Oxidative C-P bond cleavage: PhnY*/PhnZ

- Highly depending on molecular oxygen and ferrous iron
- Commonly found associated to break down 2-AEP
- Yield P_i and different organic products depending on substrate



Summary

3. Radical C-P bond cleavage by carbon-phosphorus lyase

- Broad substrate spectrum
- Multi enzyme complex, requires P starvation for induction
- Yield 5’-phospho-ribosyl-bisphosphate (PRPP) rather than P_i
- Common pathway to break down glyphosate beside the GOX pathway