Insights into the previously undescribed bacterial trigonelline pathway reveal new enzymes for the aerobic breakdown of the pyridine ring

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Abstract

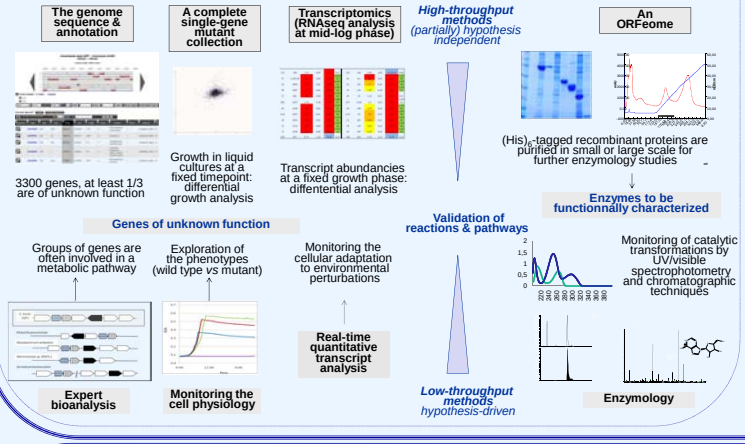
Many soil bacteria can **degrade** natural and human-made (and sometimes harmful) **pyridine compounds**. The nicotinic acid pathway is the only one to be genetically and biochemically elucidated.

We use post genomic resources implemented in our laboratory for the soil bacterium *A. baylyi* ADP1 **to investigate the (enzymatic) function of genes of unknown or imprecise function** and **identify new metabolic pathways.**
We focused on a cluster of genes conserved among many dozen bacterial species which proved to be responsible for **trigonelline (N-methylnicotinate) dissimilation**; the bacterial catabolism of this common plant osmoprotectant is not documented.

A degradation pathway for the solvent **pyridine**, and for a by-product of the paraquat herbicide, **N-methylisonicotinate** (an isomer of trigonelline) has been proposed more than 40 years ago, but still the enzymes and genes are unknown. Our work highlights **new enzymes for the degradation of the pyridine ring** that are encoded in many bacterial genomes. A comprehensive screening of their catalytic properties against a range of pyridines is planned.

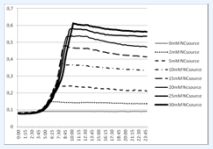
Discovering enzymes for the metabolism in *A. baylyi* sp. ADP1

***In silico and in vivo* tools provide candidates for metabolic reactions**

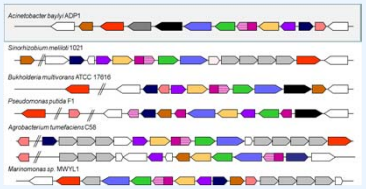


A conserved gene cluster for trigonelline catabolism

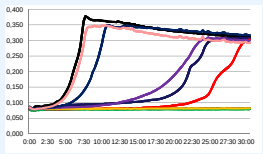
- ✓ Trigonelline is a **naturally occuring pyridine alkaloid**:
  - Produced by plants in response to draught
  - Osmoprotectant & niacin (vitamin B3) reservoir
  - **Antiproliferative & antidiabetic properties**
  - Abundant in coffee
- ✓ Trigonelline is used by *A. baylyi* ADP1 as a nitrogen and carbon source

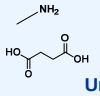


- ✓ We identified a **cluster of conserved genes** responsible for trigonelline catabolism.



- ✓ Five essential genes coding for enzymes were revealed by analyzing growth phenotypes of wild-type and mutant strains of *A. baylyi* ADP1 on trigonelline





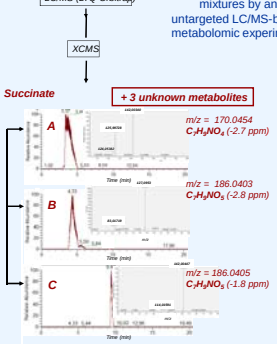
- ✓ Trigonelline is split into methylamine and succinate:
  - Mutant strains blocked in methylamine catabolism cannot grow on trigonelline as a nitrogen source
  - A succinate semi-aldehyde dehydrogenase ensures the entry of carbons into the tricarboxylic acid cycle

Until now, the catabolic genes were not known

Still, their annotation do not fully describe the pathway

Elucidating the essential steps : finding metabolites, assigning a functional role to enzymes

Untargeted metabolomic to recover new metabolites



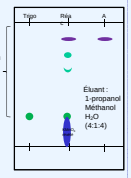
We identified catabolic intermediates of unknown identity in reaction mixtures by an untargeted LC/MS-based metabolomic experiment.

Metabolite A ($C_7H_9NO_4$)

NADH consumption occurred upon addition of a reductase and Trigonelline, but reduced Trigonelline could not be isolated. Addition of a second enzyme yielded Metabolite A, which was produced on a preparative scale and purified.

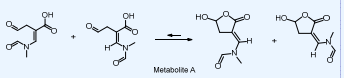
■ Production

- ✓ Trigonelline ($C_7H_7NO_2$) + *'reductase'* + *'oxidoreductase'*
- ✓ Detection: spectrophotometer (340 nm) + TLC
- ✓ Optimisations of reaction parameters: temperature, aeration, enzymes, substrates, ions, volume



■ Structural elucidation

- ✓ Purification: preparative TLC
- ✓ RMN: RMN-1D and 2D (APT, COSY, HMQC et HMBC)



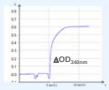
- ✓ ESI-MS and MS/MS: concordant fragmentation pattern

Metabolite A harbors a rare **cyclic 5-hydroxy-butyrolactone structure** instead of the pyridinium ring. An aldehyde-hemiacetal equilibrium involving a **4-oxobutyrate open-form** occurs.

Metabolite C ($C_7H_9NO_5$)

■ Production

- ✓ trigonelline + *'reductase'* + *'oxidoreductase'* or A + *'aldehyde dehydrogenase'*
- ✓ Detection: spectrophotometer (NADH production)



- ✓ Purification: preparative TLC

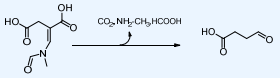
■ Structural elucidation

A cascade reaction using three enzymes and trigonelline led to the production of the next metabolite (Metabolite C) whose structure was assigned to a dicarboxylic acid derivative.

From C to succinate-semi-aldehyde and succinate

■ Reaction

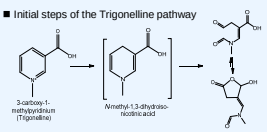
- ✓ Metabolite C + *'hydrolase'*
- ✓ Detection: spectrophotometer
  - 253 nm, consumption of metabolite C
  - 340 nm, coupled with succinate semi-aldehyde dehydrogenase



Metabolite C is directly broken down into succinate semi-aldehyde This step is reminiscent of the last step of vitamine B6 degradation, but the enzymes are not identical.

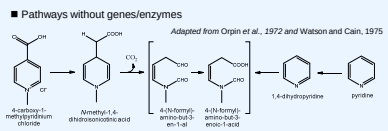
Specificity of the first step : toward other pyridine derivatives ?

■ Initial steps of the Trigonelline pathway

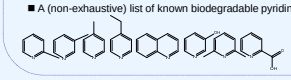


■ Pathways without genes/enzymes

Adapted from Orpin *et al., 1972 and* Watson and Cain, 1975



■ A (non-exhaustive) list of known biodegradable pyridines



Many pathways in many bacteria are proposed in the literature, without enzymes. Could the trigonelline enzymes, or homologous enzymes of unknown function found either in A. baylyi *ADP1 or in known pyridine degraders act on these compounds ?*

Conclusions and Perspectives

We have **solved the catabolic pathway of trigonelline**, a natural compound with a pyridine ring. Interestingly, the **reductive-oxidative pyridine ring** attack in trigonelline resembles the initial steps postulated for N-methylisonicotinate or (methyl)pyridine biodegradation and differs from the nicotinic acid pathway present in some bacteria.

In *A. baylyi* ADP1 and other bacteria, beside the **cluster of genes responsible for trigonelline degradation**, the presence of **other homologous genes** for the key steps raises the question of the biotransformation of pyridine derivatives. As a spin-off of the initial trigonelline project, we are now intending to investigate the **catalytic activities of a set of bacterial enzymes toward selected pyridine compounds** to search for valuable candidates for biotechnological applications in the field of bioremediation.

References

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