Study of the structure and inhibition of Mycobacterium avium phosphoserine phosphatase

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Study of the structure and the inhibition of the *Mycobacterium avium* phosphoserine phosphatase

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I. *M. tuberculosis*: need for a new therapeutic target

Tuberculosis is currently one of the top ten causes of death worldwide. The pathogen responsible for this disease, *Mycobacterium tuberculosis* (*M.tb*), spreads quickly through saliva droplets in air[1]. Moreover, *M.tb* is becoming more and more multidrug-resistant[2].

→ **New effective drugs need to be found.**

Our research group focuses on *M.tb* phosphoserine phosphatase (SerB2) as new perfect therapeutic target, for three main reasons:

1) SerB2 inhibition kills bacteria through interruption of the L-serine pathway[3] (Figure 1).
2) SerB2 has an intrinsic pathogenic effect and expedites the infection[4].
3) The sequence of phosphoserine phosphatase is highly conserved among organisms. The study of its inhibition could lead to an efficient drug against various pathogens.

→ **Effective SerB2 inhibitors have been discovered.**

→ **The mechanism of action remains unclear: SerB2 has never been crystallized.**

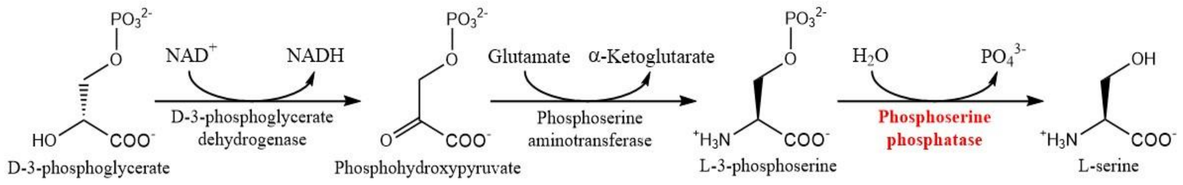

Figure 1 – Biosynthetic pathway of L-serine (adapted from G.A. Grant[3]).

II. Study of *M. avium* phosphoserine phosphatase

The aim of this research project is the study of the closest counterpart of SerB2 (83.7% of identity) that can be crystallized: the *Mycobacterium avium* phosphoserine phosphatase (SerB)[5]. This study is performed through three different ways, summarized in Figure 2.

1) The study of SerB enzymatic kinetics in presence of SerB2 inhibitors.
2) The study of oligomerization states in presence of some compounds.
3) The final outcome of this project is a crystallographic study of SerB complexed with the aforementioned inhibitors in order to provide more information about SerB2 inhibition.

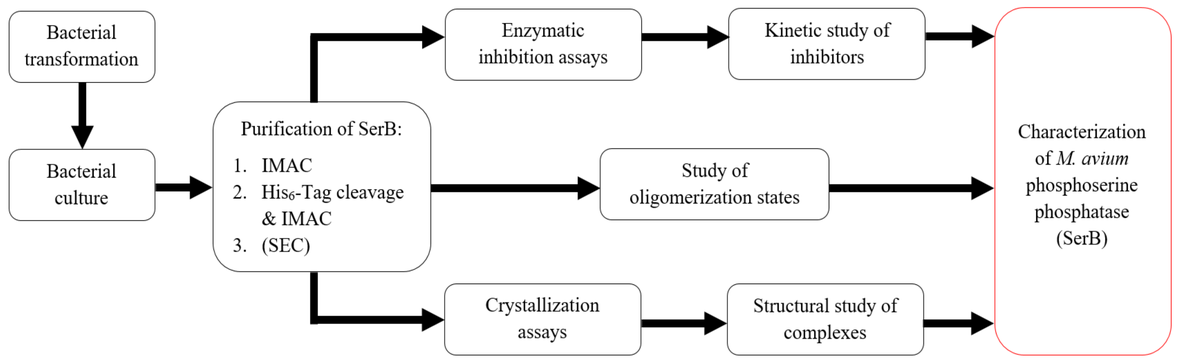

Figure 2 – Overview about the adopted strategy.

III. Results

Overexpression and purification of SerB

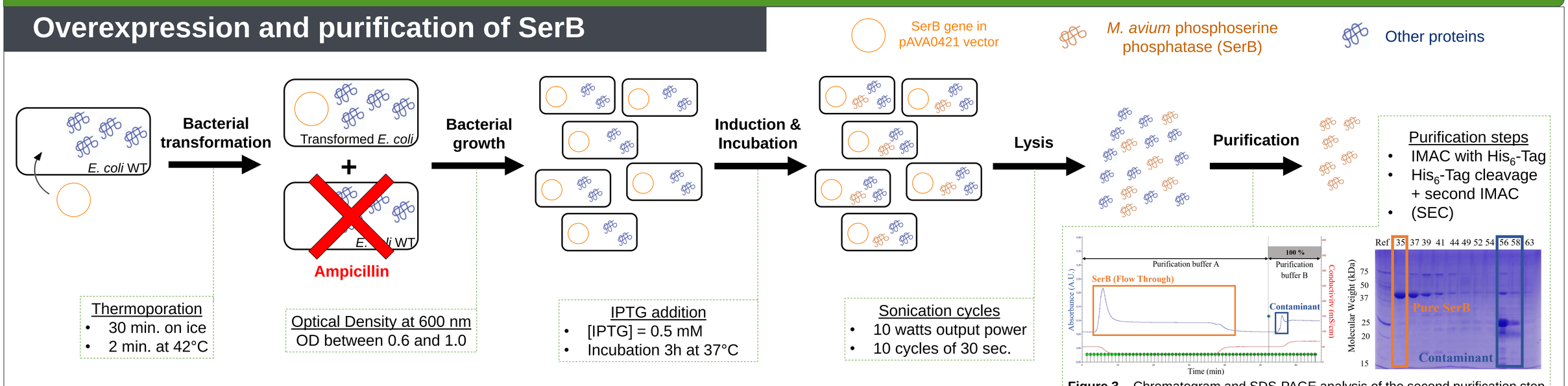

Figure 3 – Chromatogram and SDS-PAGE analysis of the second purification step.

Kinetic study of SerB

Michaelis-Menten kinetics

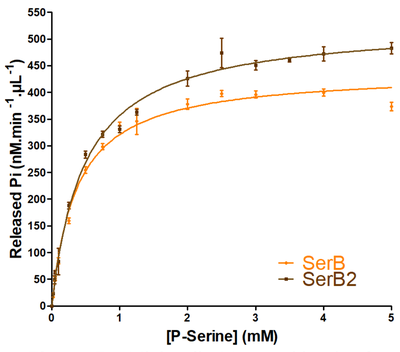

Figure 5 – Michaelis-Menten kinetics for SerB (this research) and SerB2[7].

Table 1 – Michaelis-Menten parameters of SerB and SerB2 (determination through the Lineweaver-Burk plot).

	SerB (*M. avium*)	SerB2[7] (*M. tuberculosis*)
Km (mM)	0.3797 (± 0.0342)	0.4082 (± 0.0358)
V_{max} (nM.min^{-1}.µL^{-1})	441.3 (± 9.1)	487.4 (± 9.6)
R^2	0.9997	0.9983

→ **SerB & SerB2 follow similar enzymatic kinetics.**

Crystallization of SerB

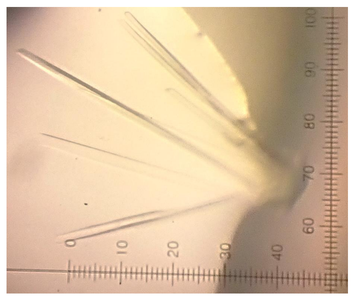

Figure 4 – SerB crystals ($MgCl_2$ 0.3 M, MES 0.1 M, PEG 6000 20%, pH 6.5).

- Sample is concentrated after purification (~27 mg/mL).
- Vapor diffusion sitting drop is performed.
- Tested conditions (based on SSGCID research[6]):
  - $MgCl_2$ 0.1 to 0.4 M.
  - MES 0.1 M.
  - PEG 6000 20%.
  - pH 6.0 to 6.5.

→ **SerB crystals have been obtained.**

→ **Diffraction with good resolution (2.07 Å).**

Enzymatic inhibition assays

- Four efficient SerB2 inhibitors are tested on SerB:
  - They are harmine derivatives (Figure 6).
  - Inhibitors are referenced as 88, 91, 95 & 124.

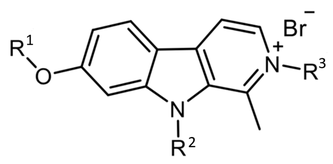

Figure 6 – Harmine derivatives.

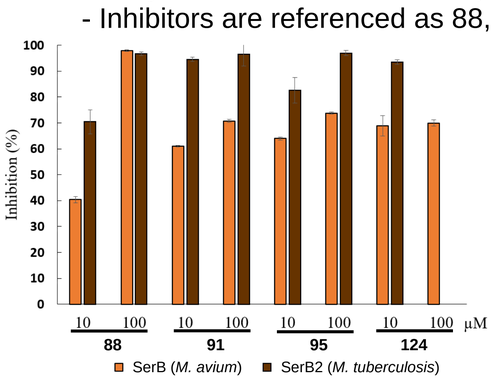

Figure 7 – Inhibition percentages of inhibitors on SerB (this research) and SerB2[7].

→ **SerB2 inhibitors are efficient against SerB!**

→ **They are slightly more effective on SerB2.**

Inhibition mechanism seems similar!

- Inhibitors are reversible and competitive on both SerB and SerB2.
- Comparison between SerB and SerB2 are performed through **Ki** determination.

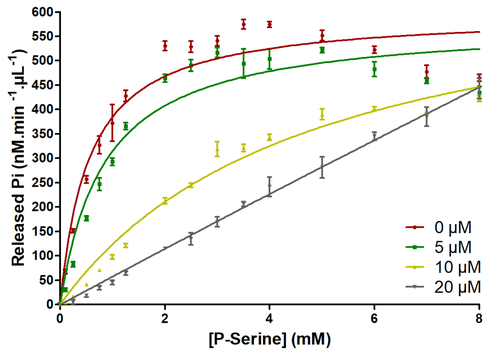

Figure 8 – Evolution of Michaelis-Menten kinetics of SerB in function of inhibitor 95 concentration.

Table 2 – Determination of the inhibition constant (Ki) for each selected inhibitor on SerB (this research) and SerB2[7].

	SerB (*M. avium*)	SerB2[7] (*M. tuberculosis*)
Ki 88	14.43 (± 1.33)	1.75
Ki 91	1.35 (± 0.16)	0.18
Ki 95	11.38 (± 2.50)	-
Ki 124	3.57 (± 0.07)	-

→ **91 is the best inhibitor of SerB.**

→ **Inhibitors are more effective on SerB2.**

→ **Is the difference really significant ?**

IV. Conclusion & outlooks

After bacterial transformation, optimal SerB overexpression and purification have been established and completed. A kinetic study of SerB has been performed and shows that SerB and SerB2 follow similar enzymatic kinetics. Moreover, SerB2 inhibitors remain efficient against SerB. Crystals of SerB have finally been obtained. In a near future, crystallization assays of SerB complexed with the aforementioned inhibitors and a study of the oligomerization state will be performed to provide more information about SerB2 inhibition.

V. Aknowledgments & references

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