Yttrium removal by alpha-proteobacteria

Carina Coimbra[1], **Rita Branco**[1] and **Paula V. Morais**[1]

[1] Department of Life Sciences, CEMMPRE, University of Coimbra, 3000-456 Coimbra, Portugal.

cgcoimbra1992@gmail.com

Abstract

Yttrium (Y) is a metallic element belonging to the group of rare earth elements (REE) that are mainly extracted in China. This element has considerable industrial and economic importance due to its unique properties as luminescence, magnetism and strength. Face on its large application in several areas, Y is considered a critical element since the supply cannot meet the growing demand in the near future.

Although Y is considered as non-essential for living organisms, its role as an essential or toxic element is not well characterized, as well as the Y-organism interaction is also not completely elucidated.

In the present study, bacteria–metal interaction was explored using a vast bacterial collection from the University of Coimbra Bacterial Culture Collection (UCBCC), isolated from different environments contaminated with metals, in terms of their resistance to Y. The selected strains were also tested for their Y-accumulation capability as well as to study the subcellular distribution of the respective metal in bacteria.

With this, the main objective of this work was to select the bacterial strain able to resist to high concentrations of Y and to accumulate the highest amount of Y in cells.

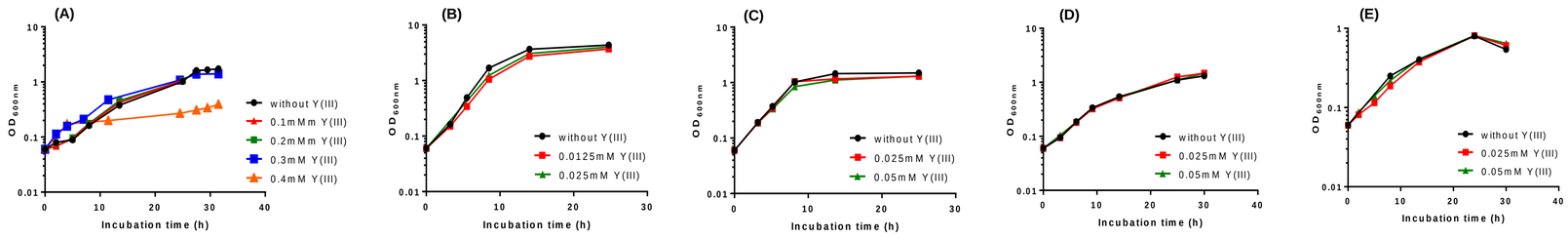
Yttrium resistance and growth curve

Table I. Minimal inhibitory concentration (MIC) to Y in Reasoner's 2A (R2A) agar plates of selected strains from 207 strains belonging to alpha-proteobacteria from UCCCB.

		0.5mM Y	1mM Y	2mM Y	2.5mM Y	3mM Y	4mM Y
Rhizobium massilae	**HST12bk**	+	+	+	+	+	-
Methylobacterium radiotolerans	**Lc. 37**	+	+	+	+	+	-
Mesorhizobium qingshengii	**J19**	+	+	+	+	+	+
Allorhizobium oryziradicis	**7A-195**	+	+	+	+	-	-
Mesorhizobium norvegicum	**J20**	+	+	-	-	-	-

Growth evaluation: (+) positive growth; (-) negative growth

Out of 207 tested strains, J19 is the strain with ability to resist to the highest [Y].
Moreover, strain J20 is highly sensitive to Y, resisting just to 1mM Y.



Only the growth of strain J19 was affected at higher [Y], 0.4mM.

Figure 1. Growth curves of selected strains in R2A/LB medium supplemented with different concentrations of Y, separatelly: **(A)** J19, **(B)** HST12bk, **(C)** 7A-195, **(D)** Lc. 37 and **(E)** J20. A control is present for each bacteria strain (without Y).

Yttrium accumulation by alpha-proteobacteria

Similar accumulation pattern:
Low accumulation -> low [Y]
High accumulation -> high [Y]

Strain J19 was the best Y-accumulator, showing the highest accumulation of Y.

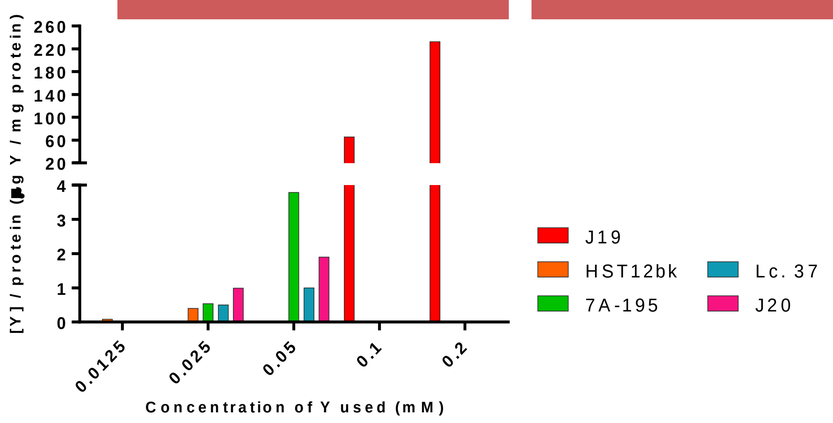


Figure 2. Accumulation of yttrium at the middle of exponential phase of the growth curve of seleted strains: J19, HST12bk, 7A-195, Lc. 37 and J20. Accumulation results are the concentration of metal after normalization for protein content.

Jales 19 > 7A-195 > Jales 20 > HST12bk

Yttrium is eliminated from the growth media.

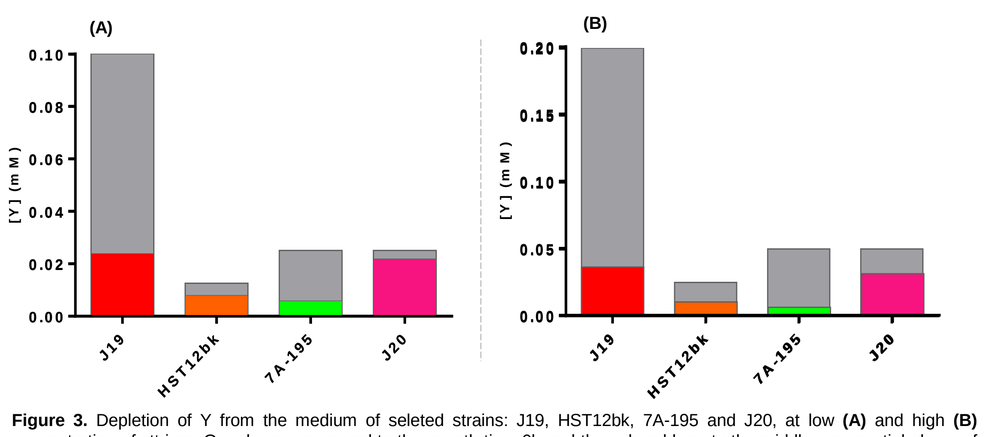
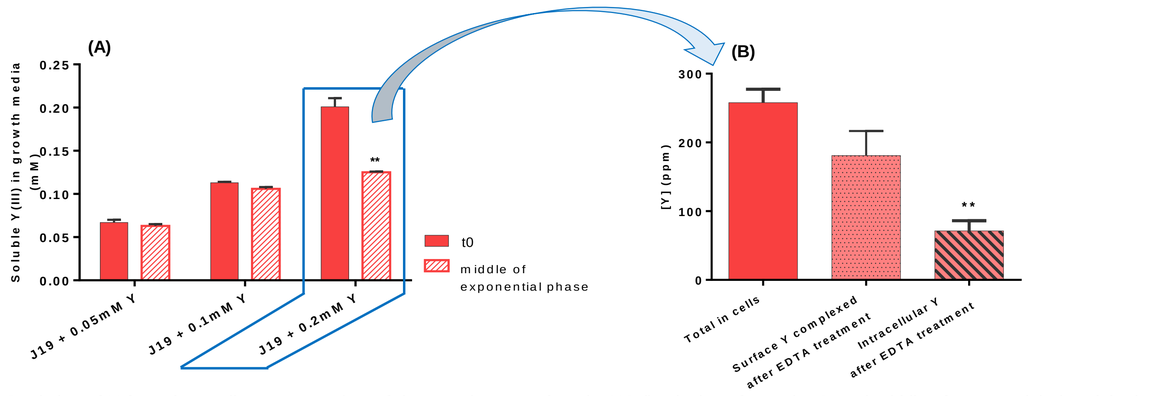


Figure 3. Depletion of Y from the medium of seleted strains: J19, HST12bk, 7A-195 and J20, at low **(A)** and high **(B)** concentration of yttrium. Gray bars correspond to the growth time 0h and the colored bars to the middle exponential phase of the growth curve of the respective strain.

Subcellular distribution of yttrium

No difference at low [Y], 0.05 and 0.1mM.

Removal of Y from the growth medium by strain J19 cells is clearer visible at high [Y], 0.2mM.



Y is mostly present in celular surface binding.

Figure 4. (A) Depletion of Y from the medium at two points of the growth curve of strain J19 (beginning of growth, t0, and middle of exponential phase) in the presence of different concentrations of Y: 0.05, 0.1 and 0.2mM (means ± standard deviations – error bars – from two independent experiments; * - means significant difference compared to the control, t0). **(B)** Accumulation of Y by strain J19. Yttrium amounts in the three fractions resulted from cells collected at 14h of growth in the presence of 0.2mM of Y: without EDTA treatment – total Y in cells (red), cellular fraction after EDTA treatment – intracellular Y (light red with dots) and supernatant fraction after EDTA treatment – surface Y complexed (light red with streaks) (means ± standard deviations – error bars – from two independent experiments; * - means significant difference compared to the control, total in cells).

Main conclusion

The findings indicate that strain J19 is a great Y-bioaccumulator, making it a promising strain for Y- recovery from mixed metal solutions or environmental samples from sites contaminated with metals.

Acknowledgments

The work is financed by the projects Biorecover from the European Union's Horizon 2020 research and innovation programme under grant agreement No 821096, MicroMiner PTDC/CTAAMB/31820/2017 funded by Fundação para a Ciência e Tecnologia (FCT) and Programa Operacional da Região, ERA-MIN 2 REVIVING ERA-MIN-2019_67 from the Fundação para a Ciência e Tecnologia (FCT) and sponsored by FEDER founds through the program COMPETE – Programa Operacional Factores de Competitividade – and by national funds through FCT – Fundação para a Ciência e a Tecnologia -, under the project UIDB/00285/2020.

Carina Coimbra was supported by POCh – "Programa Operacional Capital Humano" (cofounded by the European Social Fund and national funding by MCTES), through a PhD research grant (SFRH/BD/147292/2019) from FCT and Rita Branco from SFRH/BPD/ 110807 2015.