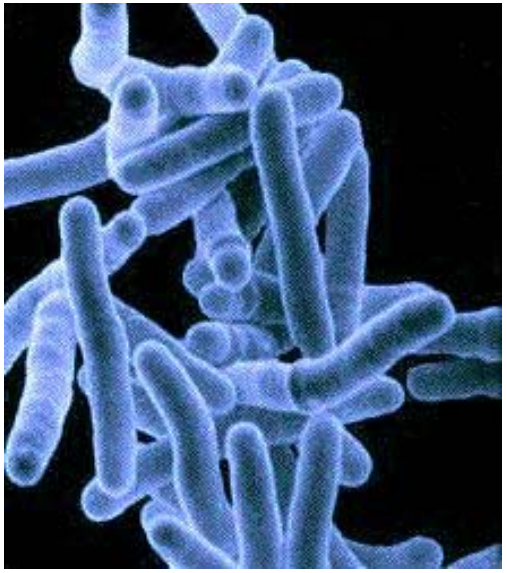
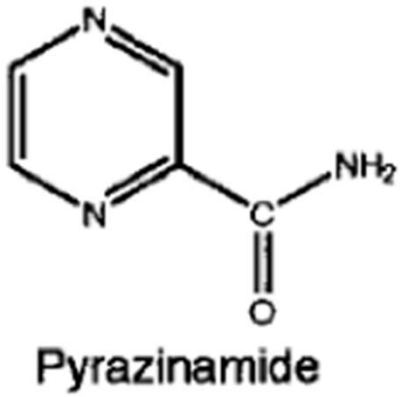
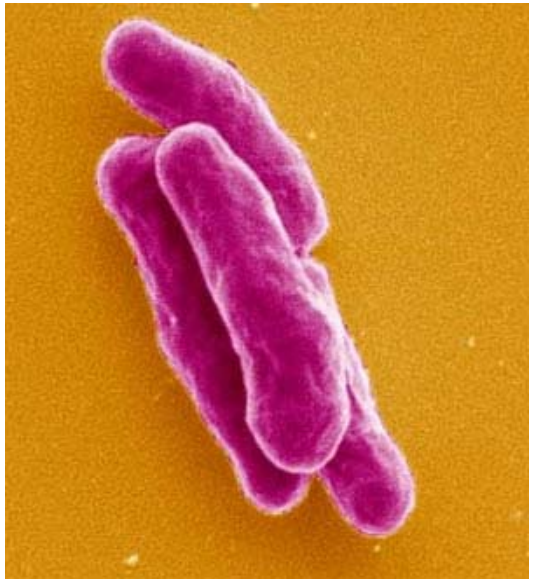
WORKSHOP
Demystifying Pyrazinamide – Challenges and Opportunities

Novel Factors Involved in PZA Mechanism of Action/Resistance



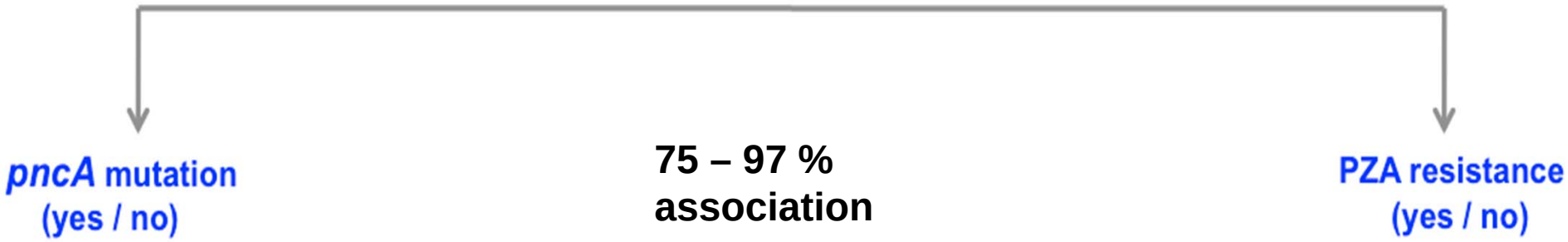


Pyrazinamide



Mirko Zimic, PhD
Unidad de Bioinformática y Biología Molecular
Facultad de Ciencias y Filosofía
Universidad Peruana Cayetano Heredia

Association between PZAse mutations and PZA resistance

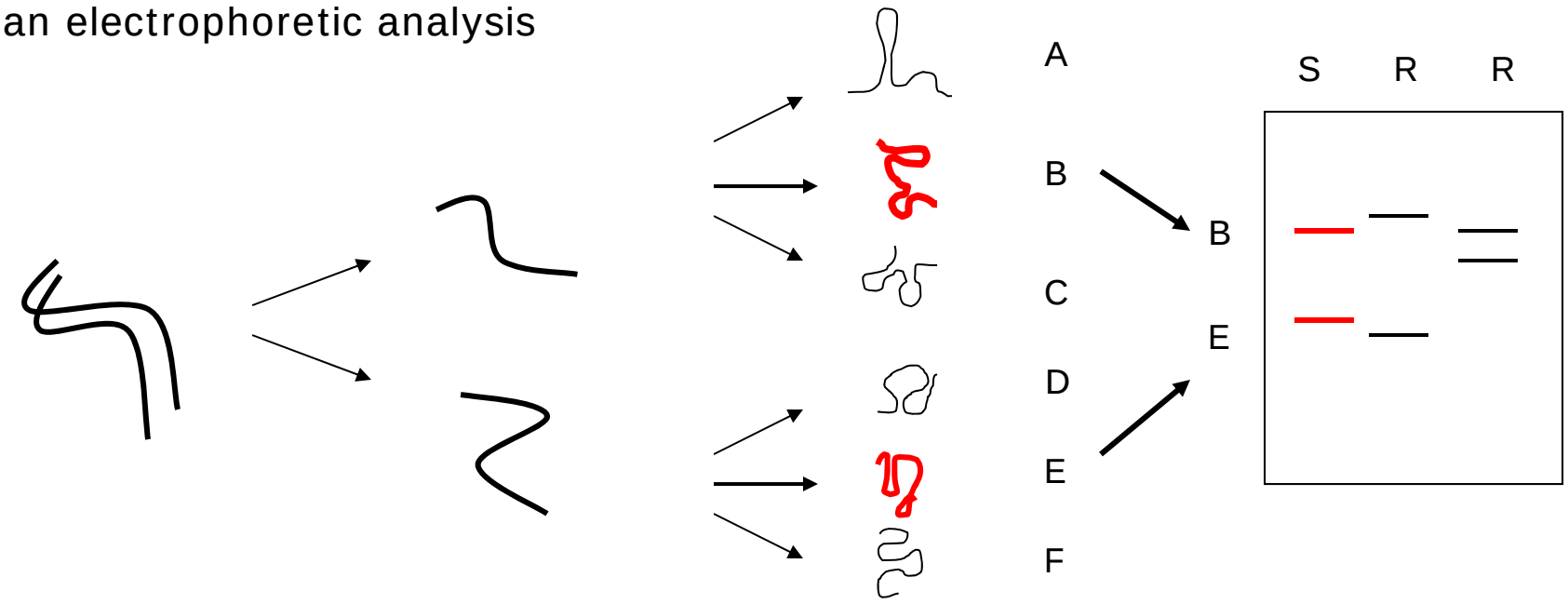


pncA mutations → predict resistance

Single strand conformational polymorphism (SSCP)

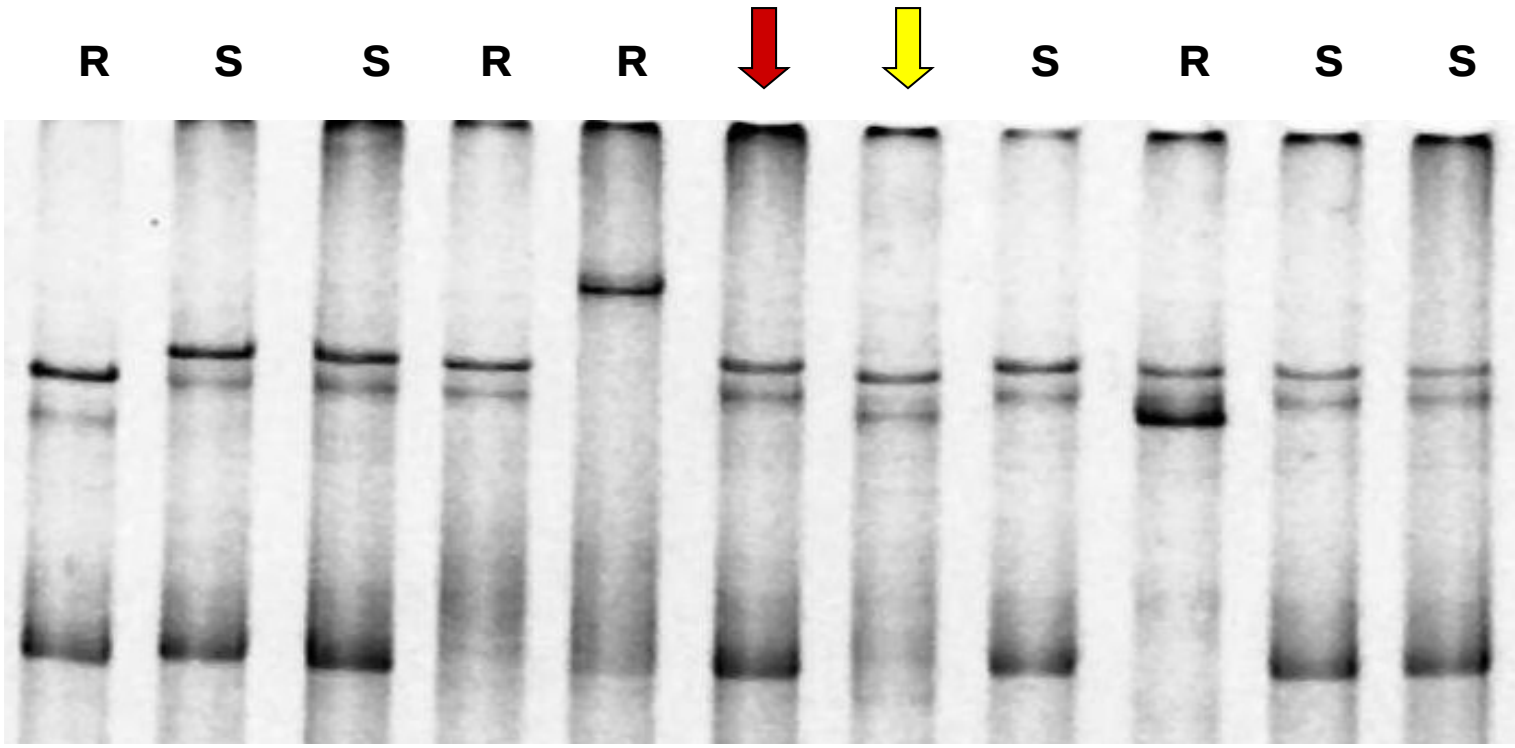
Fundament: A mutation generate a characteristic folding that is visualized in an electrophoretic analysis

1. Silent mutations are rare
2. Mutations→ resistance



Amplification product of the entire gene (559bp) → Separation of DNA strands by boiling for 10 min → Folding of each strand by rapid cooling → Electrophoresis on MDE-polyacrylamide gel at 10˚C for 20 hours

SSCP typical patterns of *pncA*



H37Rv (PZA sensible)

***M. bovis* (PZA Resistente)**

Sensitivity 85%
Specificity 91%

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2009, p. 2937–2943 Vol. 47, No. 9
0095-1137/09/$08.00+0 doi:10.1128/JCM.01594-08

Sputum PCR–Single-Strand Conformational Polymorphism Test for Same-Day Detection of Pyrazinamide Resistance in Tuberculosis Patients▽

Patricia Sheen,[1] Melissa Méndez,[1] Robert H. Gilman,[1,2] Lizeth Peña,[1] Luz Caviedes,[1] Mirko J. Zimic,[3] Ying Zhang,[4] David A. J. Moore,[1,2,5] and Carlton A. Evans[1,2,5]*

Laboratory of Infectious Diseases Research, Department of Microbiology, Faculty of Sciences, Universidad Peruana Cayetano Heredia, Lima, Peru[1]; Department of International Health, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland[2]; Laboratory of Bioinformatics, Department of Biochemistry and Molecular Biology, Faculty of Sciences, Universidad Peruana Cayetano Heredia, Lima, Peru[3]; Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland[4]; and Wellcome Trust Centre for Clinical Tropical Medicine, Department of Infectious Diseases & Immunity, Imperial College London Hammersmith Hospital Campus, London, United Kingdom[5]

Received 16 August 2008/Returned for modification 27 October 2008/Accepted 8 June 2009

Pyrazinamide is a first-line drug for treating tuberculosis, but pyrazinamide resistance testing is usually too slow to guide initial therapy, so some patients receive inappropriate therapy. We therefore aimed to optimize and evaluate a rapid molecular test for tuberculosis drug resistance to pyrazinamide. Tuberculosis PCR–single-strand conformational polymorphism (PCR-SSCP) was optimized to test for mutations causing pyrazinamide resistance directly from sputum samples and *Mycobacterium tuberculosis* isolates. The reliability of PCR-SSCP tests for sputum samples ($n = 65$) and *Mycobacterium tuberculosis* isolates ($n = 185$) from 147 patients was compared with four tests for pyrazinamide resistance: Bactec-460 automated culture, the Wayne biochemical test, DNA sequencing for *pncA* mutations, and traditional microbiological broth culture. PCR-SSCP provided interpretable results for 96% (46/48) of microscopy-positive sputum samples, 76% (13/17) of microscopy-negative sputum samples, and 100% of *Mycobacterium tuberculosis* isolates. There was 100% agreement between PCR-SSCP results from sputum samples and *Mycobacterium tuberculosis* isolates and 100% concordance between 50 blinded PCR-SSCP rereadings by three observers. PCR-SSCP agreement with the four other tests for pyrazinamide resistance varied from 89 to 97%. This was similar to how frequently the four other tests for pyrazinamide resistance agreed with each other: 90 to 94% for Bactec-460, 90 to 95% for Wayne, 92 to 95% for sequencing, and 91 to 95% for broth culture. PCR-SSCP took less than 24 hours and cost approximately $3 to $6, in contrast with the other assays, which took 3 to 14 weeks and cost $7 to $47. In conclusion, PCR-SSCP is a relatively reliable, rapid, and inexpensive test for pyrazinamide resistance that indicates which patients should receive pyrazinamide from the start of therapy, potentially preventing months of inappropriate treatment.

Distribution of PZAse missense mutations associated to PZA resistance

Infection, Genetics and Evolution 10 (2010) 346–349

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Peruvian and globally reported amino acid substitutions on the *Mycobacterium tuberculosis* pyrazinamidase suggest a conserved pattern of mutations associated to pyrazinamide resistance

Mirko Zimic [a,1,*], Patricia Sheen [b,1], Miguel Quiliano [a,1], Andrés Gutierrez [a,1], Robert H. Gilman [b,c,1]

[a] *Unidad de Bioinformática, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, SMP, Lima, Peru*

[b] *Laboratorio de Enfermedades Infecciosas, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, SMP, Lima, Peru*

[c] *Department of International Health, Bloomberg School of Public Health, The Johns Hopkins University, 615N. Wolfe St., Room W5515, Baltimore, MD, 21205, USA*

ARTICLE INFO

Article history:
Received 18 October 2009
Received in revised form 28 November 2009
Accepted 30 November 2009
Available online 4 December 2009

Keywords:
Tuberculosis
Pyrazinamide
Resistance
Mutations
Pyrazinamidase
pncA

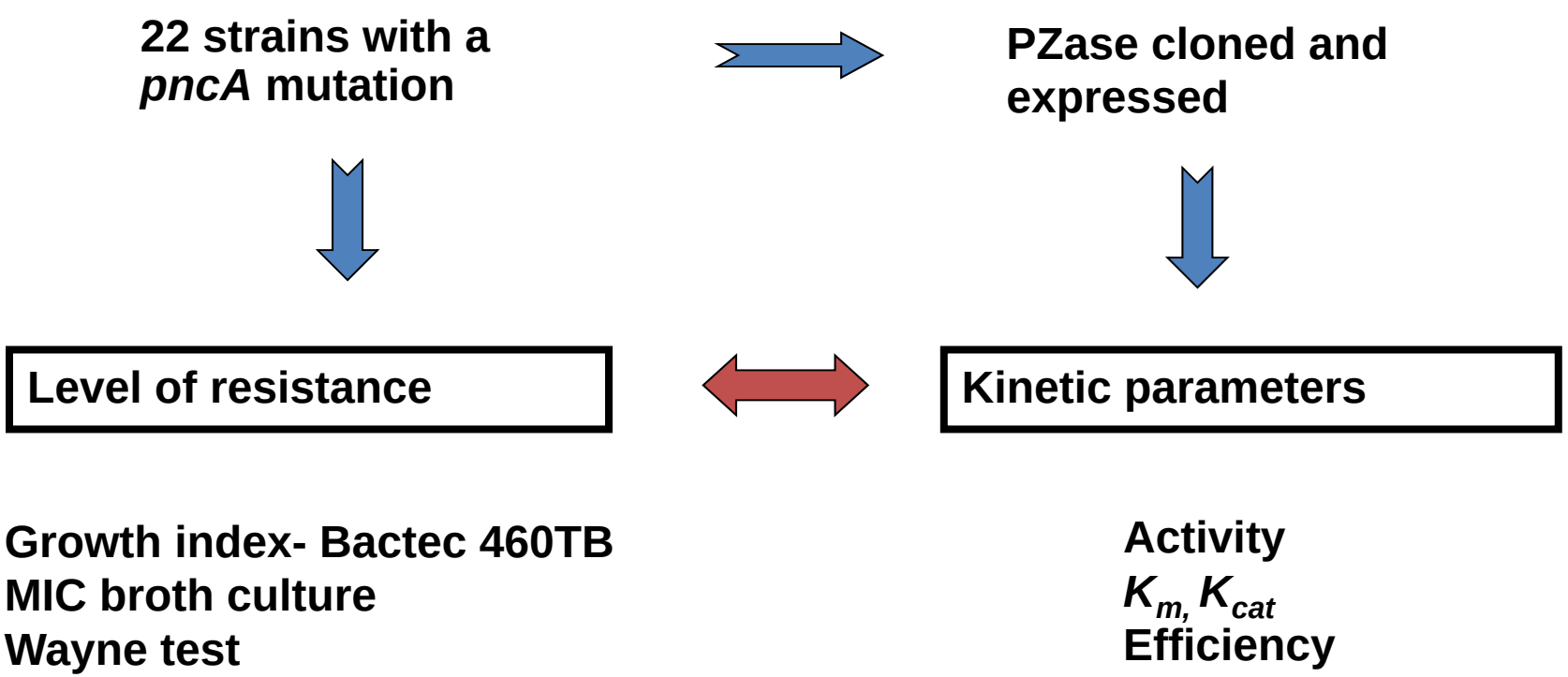
ABSTRACT

Resistance to pyrazinamide in *Mycobacterium tuberculosis* is usually associated with a reduction of pyrazinamidase activity caused by mutations in *pncA*, the pyrazinamidase coding gene. Pyrazinamidase is a hydrolase that converts pyrazinamide, the antituberculous drug against the latent stage, to the active compound, pyrazinoic acid. To better understand the relationship between *pncA* mutations and pyrazinamide resistance, it is necessary to analyze the distribution of *pncA* mutations from pyrazinamide resistant strains.

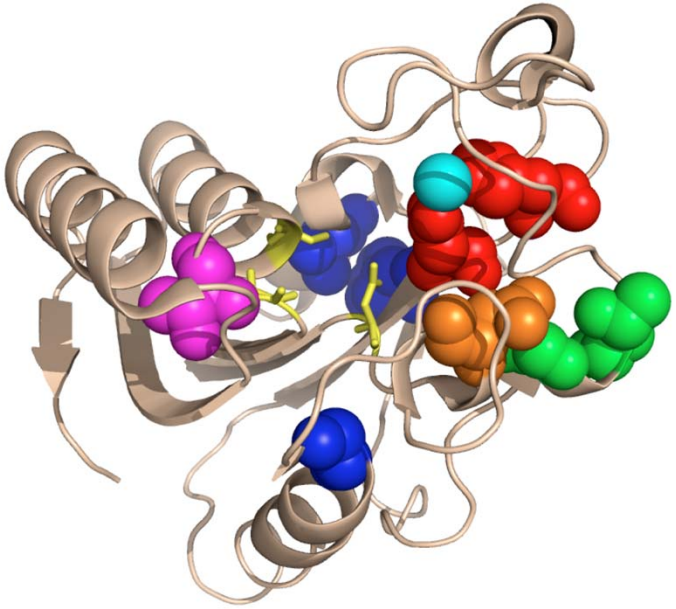
We determined the distribution of Peruvian and globally reported *pncA* missense mutations from *M. tuberculosis* clinical isolates resistant to pyrazinamide. The distributions of the single amino acid substitutions were compared at the secondary structure domains level. The distribution of the Peruvian mutations followed a similar pattern as the mutations reported globally. A consensus clustering of mutations was observed in hot-spot regions located in the metal coordination site and to a lesser extent in the active site of the enzyme.

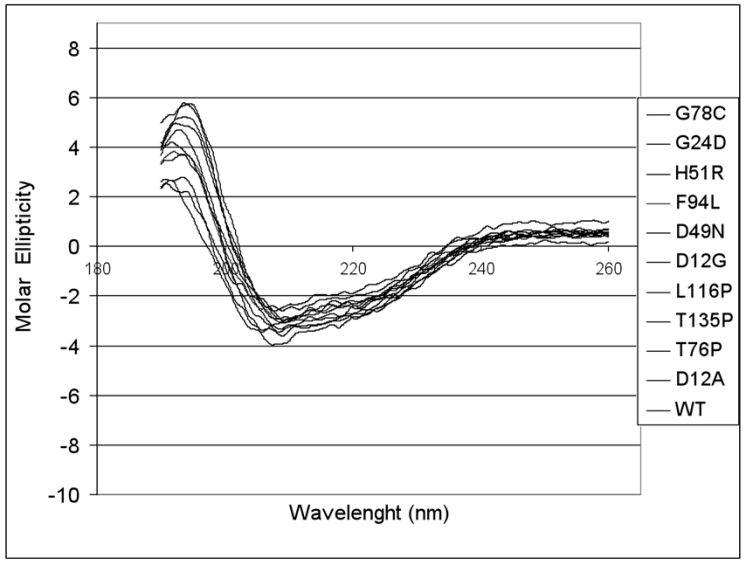
The data was not able to reject the null hypothesis that both distributions are similar, suggesting that *pncA* mutations associated to pyrazinamide resistance in *M. tuberculosis*, follow a conserved pattern responsible to impair the pyrazinamidase activity.

Causal pathway


22 strains with a *pncA* mutation
PZase cloned and expressed
Level of resistance
Kinetic parameters
Growth index- Bactec 460TB
MIC broth culture
Wayne test
Activity
K_m, K_{cat}
Efficiency

Mutated PZAse	α-helix (%)	β-sheet(%)	Turn(%)	Random coil(%)	Mutation type
WT	19	30	20	30	
D12G	23	33	21	22	Close to the catalytic site and the metal binding site
D12A	24	26	22	29	Close to the catalytic site and the metal binding site
G24D	16	32	19	34	Distant from the catalytic site and the metal binding site
D49N	34	22	18	25	Metal binding site
H51R	33	23	18	26	Metal binding site
T76P	33	29	16	22	Close to the metal binding site
G78C	35	19	18	28	Close to the metal binding site
F94L	22	25	20	32	Distant from the catalytic site and the metal binding site
L116P	21	21	17	41	Distant from the catalytic site and the metal binding site
T135P	29	17	17	38	Close to the catalytic site





Other factors are required to explain the remaining variability of PZA resistance

30% of the variability of PZA resistance level was explained by the PZAse activity

Tuberculosis 89 (2009) 109–113

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

Effect of pyrazinamidase activity on pyrazinamide resistance in *Mycobacterium tuberculosis*

Patricia Sheen [a], Patricia Ferrer [a], Robert H. Gilman [a,b,*], Jon López-Llano [a], Patricia Fuentes [a], Eddy Valencia [c], Mirko J. Zimic [d]

[a] *Laboratorio de Enfermedades Infecciosas, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430 San Martín de Porres, Lima, Peru*
[b] *Department of International Health, School of Public Health, Johns Hopkins University, Baltimore, MD, USA*
[c] *Laboratorio de Tuberculosis, Instituto Nacional de Salud, Capac Yupanqui 1400 Jesús María, Lima 11, Peru*
[d] *Laboratorio de Bioinformática, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430 San Martín de Porres, Lima, Peru*

ARTICLE INFO

Article history:
Received 10 October 2008
Received in revised form
12 January 2009
Accepted 14 January 2009

Keywords:
Tuberculosis
Pyrazinamidase
pncA
Enzymatic activity
Resistance

SUMMARY

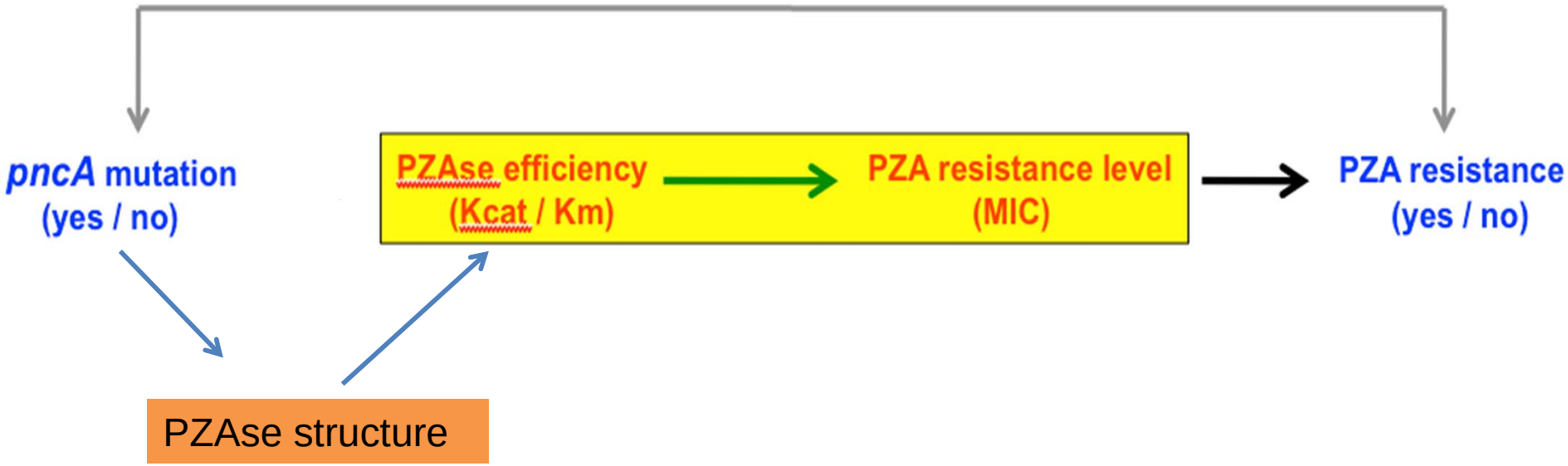
Resistance of *Mycobacterium tuberculosis* to pyrazinamide is associated with mutations in the *pncA* gene, which codes for pyrazinamidase. The association between the enzymatic activity of mutated pyrazinamidases and the level of pyrazinamide resistance remains poorly understood.

Twelve *M. tuberculosis* clinical isolates resistant to pyrazinamide were selected based on Wayne activity and localization of pyrazinamidase mutation. Recombinant pyrazinamidases were expressed and tested for their kinetic parameters (activity, k_{cat}, K_m, and efficiency). Pyrazinamide resistance level was measured by Bactec-460TB and 7H9 culture. The linear correlation between the resistance level and the kinetic parameters of the corresponding mutated pyrazinamidase was calculated.

The enzymatic activity and efficiency of the mutated pyrazinamidases varied with the site of mutation and ranged widely from low to high levels close to the corresponding of the wild type enzyme. The level of resistance was significantly associated with pyrazinamidase activity and efficiency, but only 27.3% of its statistical variability was explained.

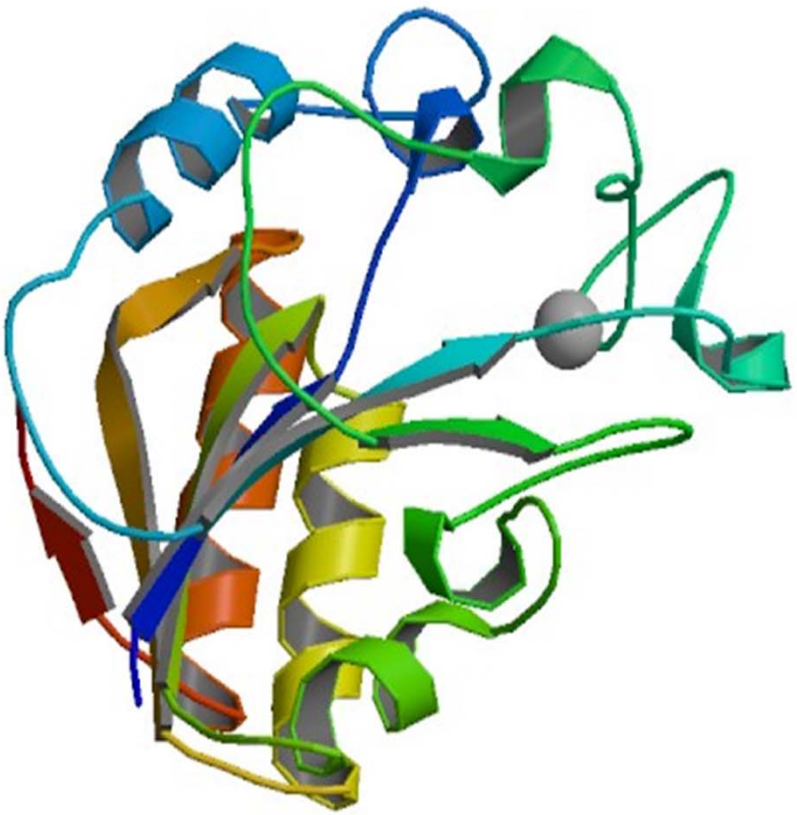
Although pyrazinamidase mutations are indeed associated with resistance, the loss of pyrazinamidase activity and efficiency as assessed in the recombinant mutated enzymes is not sufficient to explain a high variability of the level of pyrazinamide resistance, suggesting that complementary mechanisms for pyrazinamide resistance in *M. tuberculosis* with mutations in *pncA* are more important than currently thought.

PZAse structural change is an intermediate step



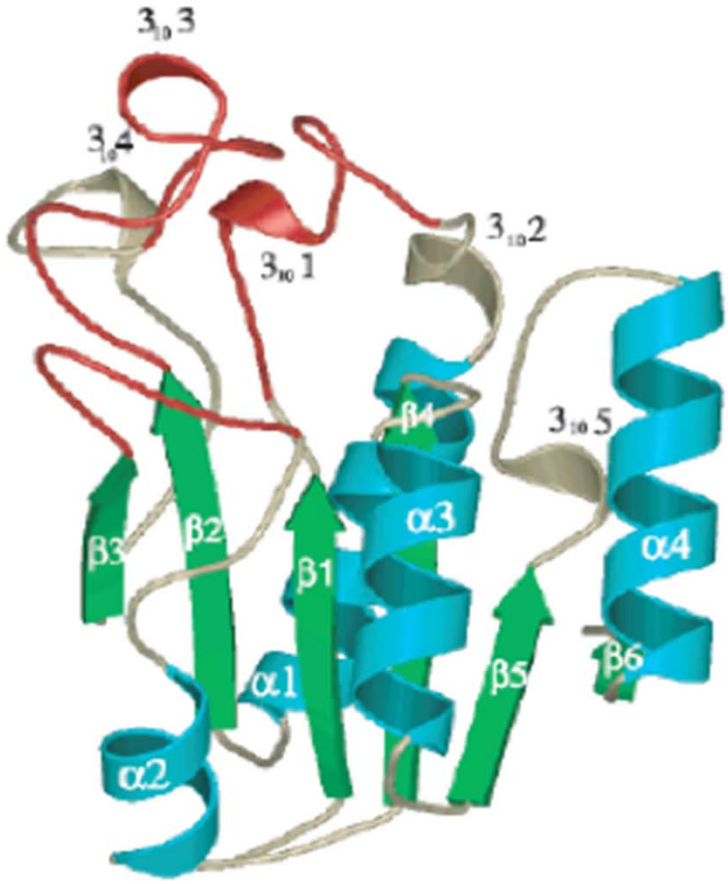
PZAse structural modeling

M. Tuberculosis Pyrazinamidase



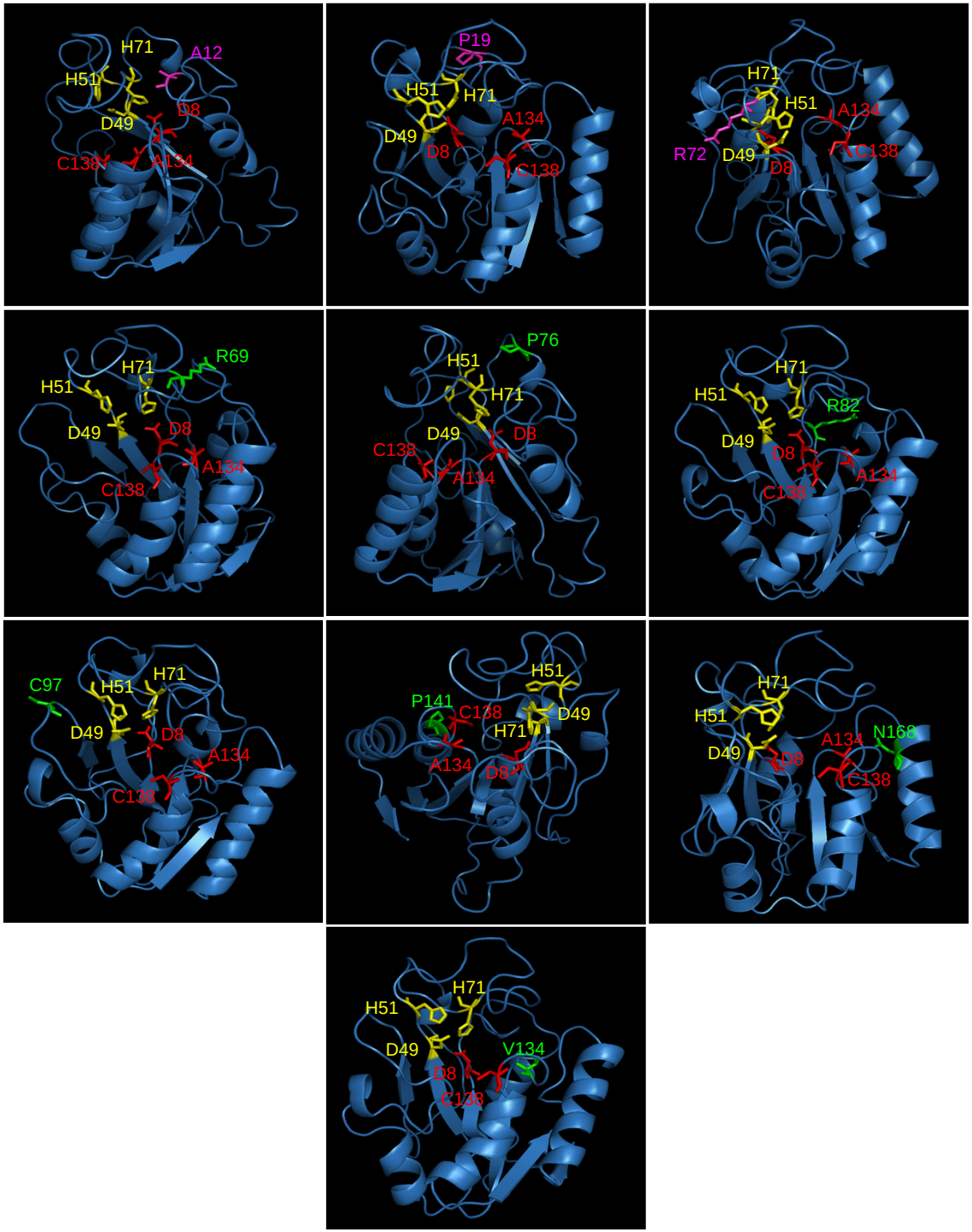
Petrella S, et al., 2011

P. horikoshii Pyrazinamidase

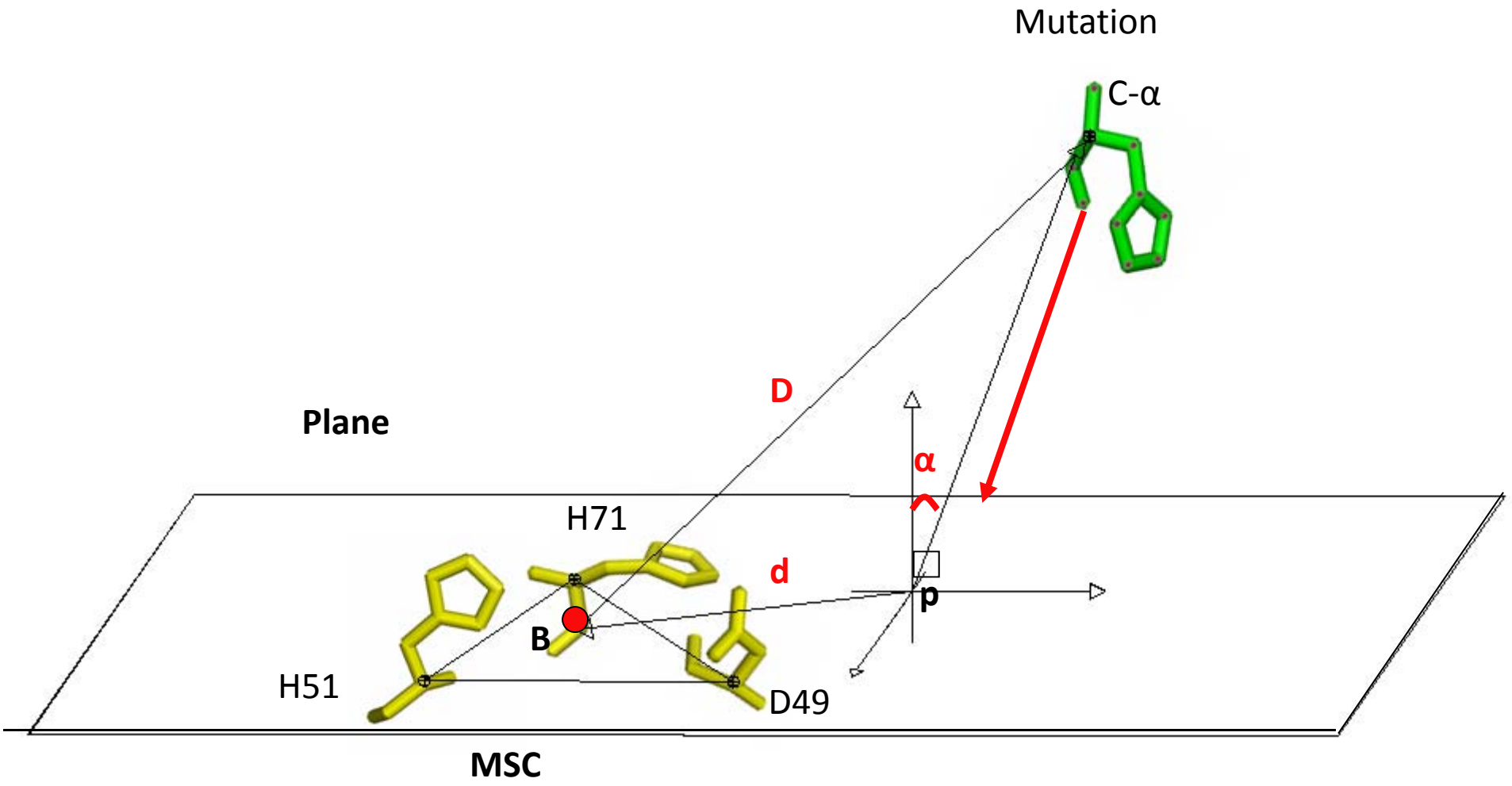


Du, et al., 2002

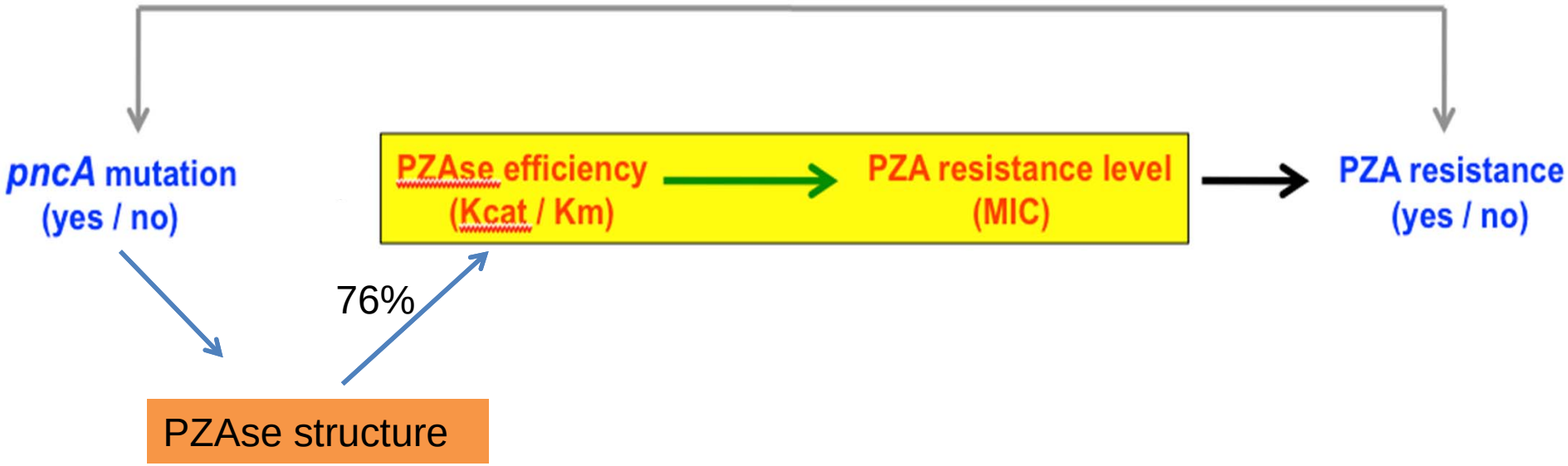
37% identical


H71
A12
H51
D8
D49
C138
A134
P19
H51
H71
A134
D49
D8
C138
H71
H51
A134
R72
D49
D8
C138
R69
H71
H51
D49
D8
A134
C138
P76
H51
H71
D49
D8
C138
A134
H71
H51
R82
D49
D8
A134
C138
C97
H51
H71
D49
D8
A134
C138
H51
P141
C138
D49
H71
A134
D8
H71
H51
D49
A134
N168
D8
C138
H71
H51
D49
V134
D8
C138

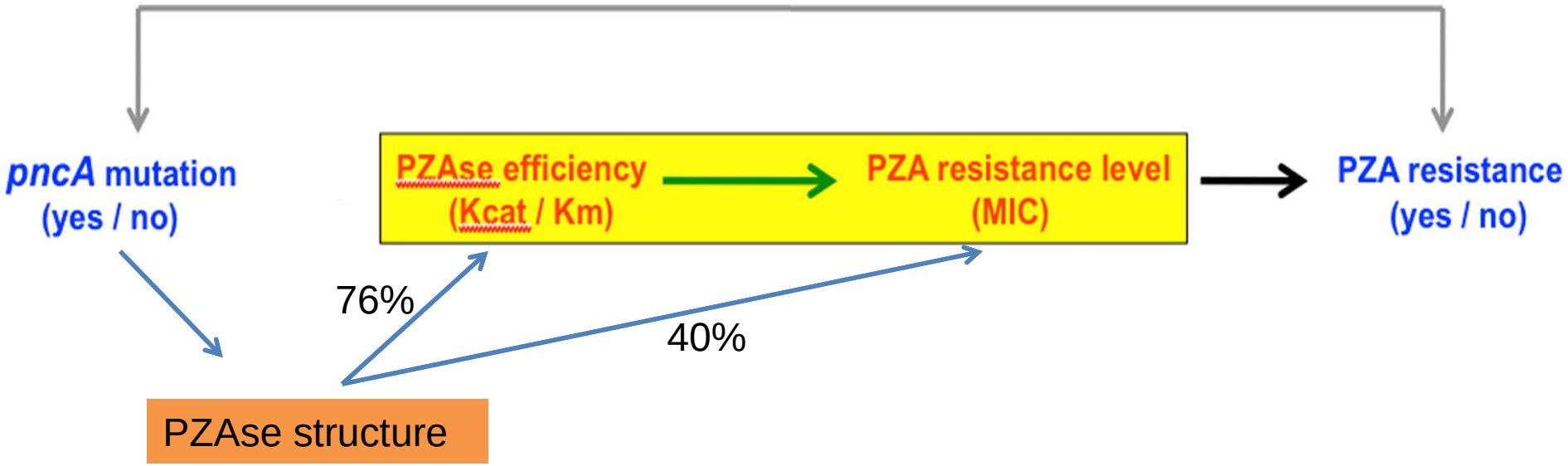
Structural and physical-chemical parameters of mutated PZAses



High association between structural variability and PZAse activity



Low association between PZAse structural variability and PZA resistance level



BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 6(9)

Structure-Activity relationship in mutated pyrazinamidases from *Mycobacterium tuberculosis*

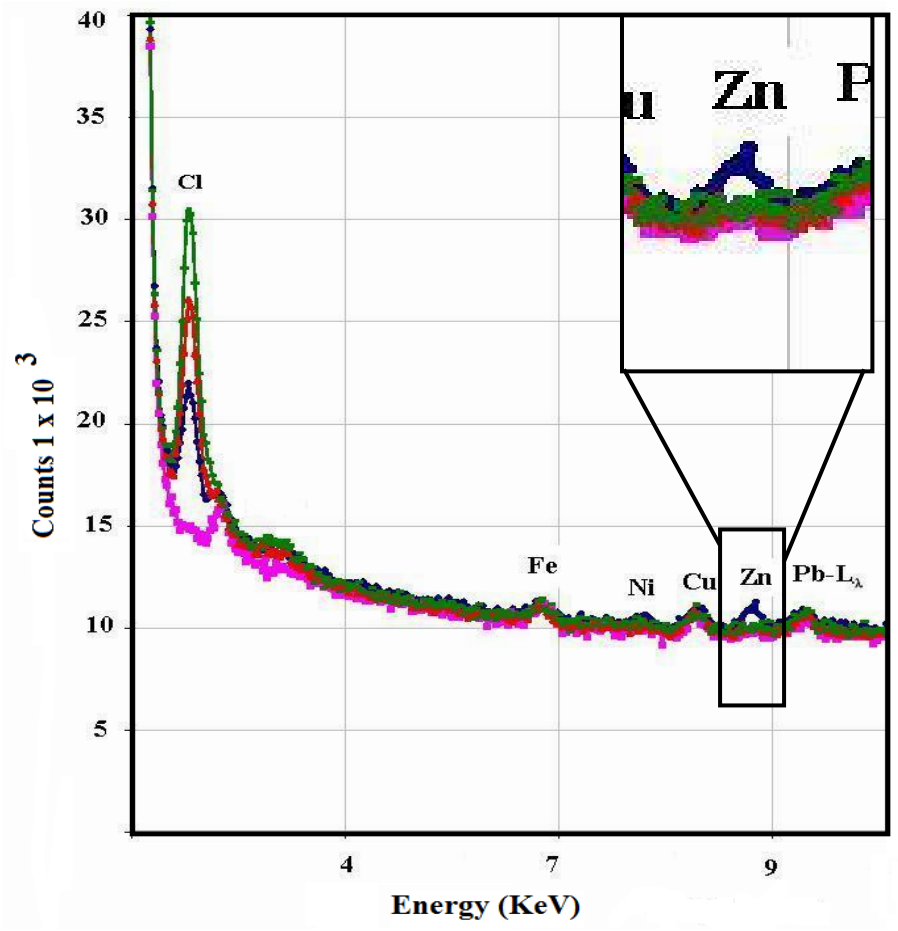
Miguel Quiliano[1], Andres Hazaet Gutierrez[1], Robert Hugh Gilman[1,2], César López[1], Wilfredo Evangelista[1], Jun Sotelo[1], Patricia Sheen[1], Mirko Zimic[1]*

[1]Unidad de Bioinformática y Biología Molecular, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia; [2]Department of International Health, Bloomberg School of Public Health, The Johns Hopkins University; Mirko Zimic - Email: mzimic@jhsph.edu; Phone: (511) 483-2942; Fax: (511) 483-2942; *Corresponding author

Received June 22, 2011; Accepted June 28, 2011; Published July 19, 2011

Abstract:
The *pncA* gene codes the pyrazinamidase of *Mycobacterium tuberculosis*, which converts pyrazinamide to ammonia and pyrazinoic-acid, the active anti-tuberculous compound. Pyrazinamidase mutations are associated to pyrazinamide-resistant phenotype, however how mutations affect the structure of the pyrazinamidase, and how structural changes affect the enzymatic function and the level of pyrazinamide-resistance is unknown. The structures of mutated pyrazinamidases from twelve *Mycobacterium tuberculosis* strains and the pyrazinamide-susceptible H37Rv reference strain were modelled using homology modelling and single amino acid replacement. Physical-chemical and structural parameters of each pyrazinamidase were calculated. These parameters were: The change of electrical charge of the mutated amino acid, the change of volume of the mutated amino acid, the change of a special amino acid, the distance of the mutated amino acid to the active site, the distance of the mutated amino acid to the metal-coordination site, and the orientation of the side-chain of the mutated amino acid. The variability of the enzymatic activity of the recombinant pyrazinamidases, and the microbiological susceptibility to pyrazinamide determined by BACTEC 460TB, were modelled in multiple linear regressions. Physical-chemical and structural parameters of the mutated pyrazinamidases were tested as predictors. Structural and physical-chemical variations of the pyrazinamidase explained 75% of the variability of the enzymatic activity, 87% of the variability of the kinetic constant and 40% of the variability of the pyrazinamide-resistance level. Based on computer models of mutated pyrazinamidases, the structural parameters explained a high variability of the enzymatic function, and to a lesser extent the resistance level.

X-ray fluorescence spectroscopy of H37Rv PZase



Purified and concentrated *E. coli* extract without plasmid

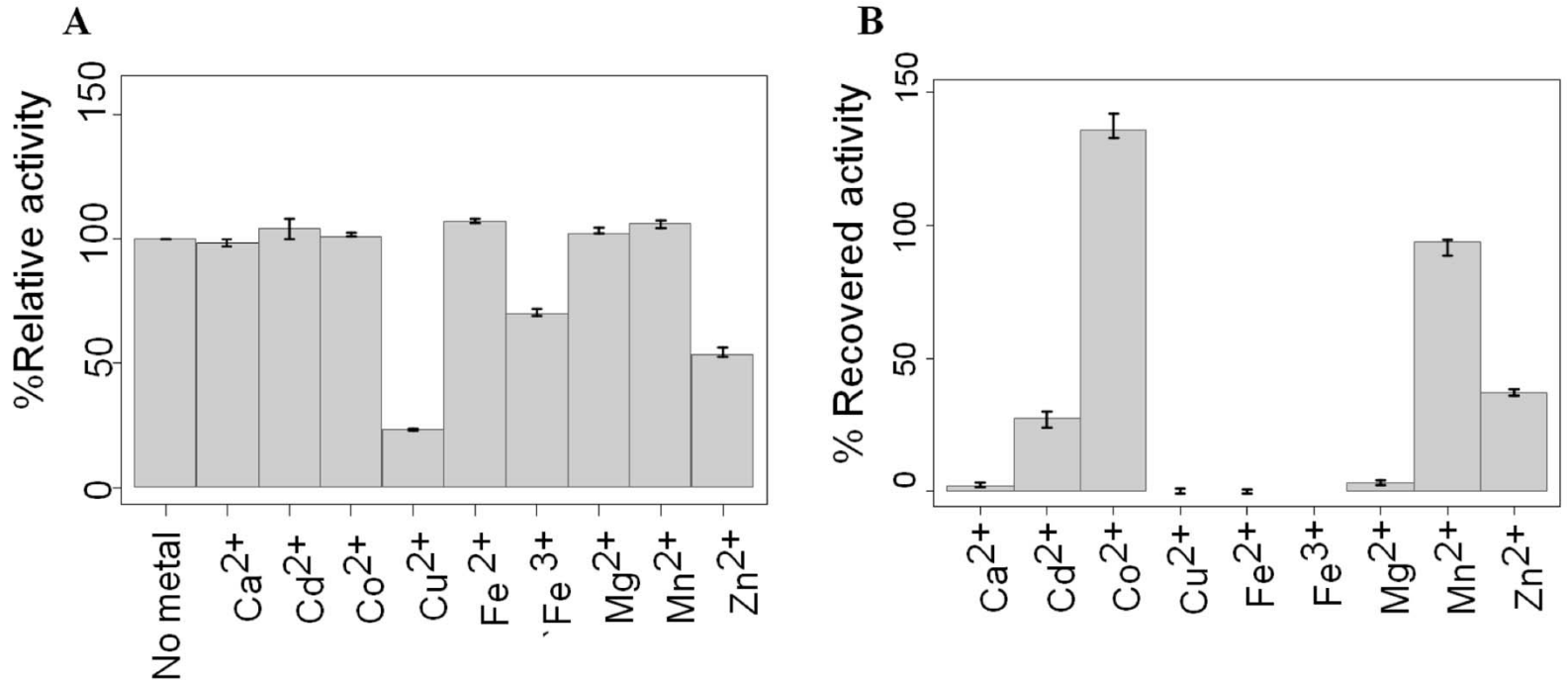
TRIS

Purification buffer

PZAse in TRIS

0.3 Zn ions per PZase molecule

Effect of metal ions in the PZAse activity



Am. J. Trop. Med. Hyg., 00(0), 2012, pp. 000–000
doi:10.4269/ajtmh.2012.10-0565

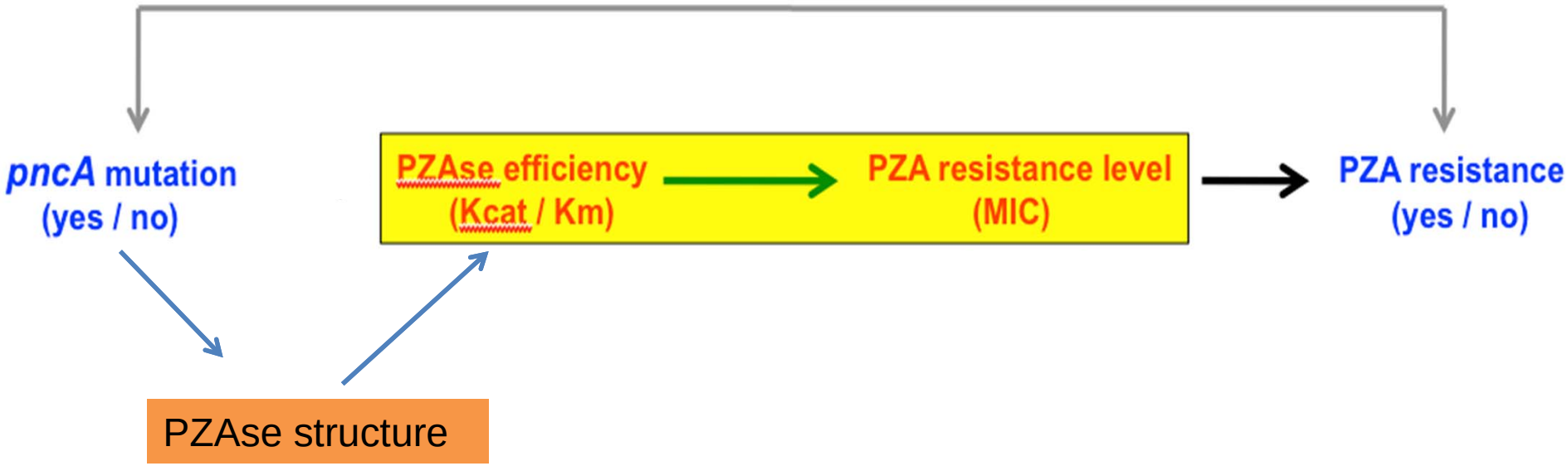
Role of Metal Ions on the Activity of *Mycobacterium tuberculosis* Pyrazinamidase

Patricia Sheen, Patricia Ferrer, Robert H. Gilman, Gina Christiansen, Paola Moreno-Román, Andrés H. Gutiérrez, Jun Sotelo, Wilfredo Evangelista, Patricia Fuentes, Daniel Rueda, Myra Flores, Paula Olivera, José Solis, Alessandro Pesaresi, Doriano Lamba, and Mirko Zimic*

Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú; Department of International Health, School of Public Health, Johns Hopkins University, Baltimore, Maryland; Instituto Peruano de Energía Nuclear, Lima, Perú; Istituto di Cristallografia, Consiglio Nazionale delle Ricerche, Area Science Park—Basovizza, Trieste, Italy

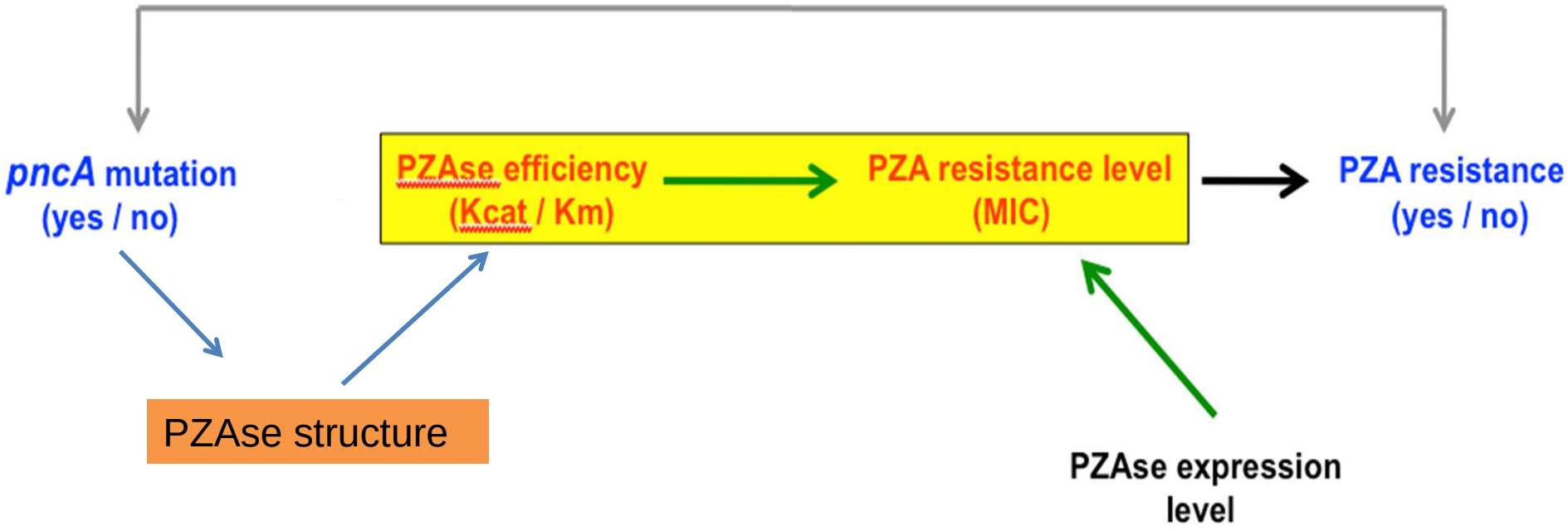
Abstract. Pyrazinamidase of *Mycobacterium tuberculosis* catalyzes the conversion of pyrazinamide to the active molecule pyrazinoic acid. Reduction of pyrazinamidase activity results in a level of pyrazinamide resistance. Previous studies have suggested that pyrazinamidase has a metal-binding site and that a divalent metal cofactor is required for activity. To determine the effect of divalent metals on the pyrazinamidase, the recombinant wild-type pyrazinamidase corresponding to the H37Rv pyrazinamide-susceptible reference strain was expressed in *Escherichia coli* with and without a carboxy terminal. His-tag was inactivated by metal depletion and reactivated by titration with divalent metals. Although Co^{2+}, Mn^{2+}, and Zn^{2+} restored pyrazinamidase activity, only Co^{2+} enhanced the enzymatic activity to levels higher than the wild-type pyrazinamidase. Cu^{2+}, Fe^{2+}, Fe^{3+}, and Mg^{2+} did not restore the activity under the conditions tested. Various recombinant mutated pyrazinamidases with appropriate folding but different enzymatic activities showed a differential pattern of recovered activity. X-ray fluorescence and atomic absorbance spectroscopy showed that recombinant wild-type pyrazinamidase expressed in *E. coli* most likely contained Zn. In conclusion, this study suggests that *M. tuberculosis* pyrazinamidase is a metalloenzyme that is able to coordinate several ions, but *in vivo*, it is more likely to coordinate Zn^{2+}. However, *in vitro*, the metal-depleted enzyme could be reactivated by several divalent metals with higher efficiency than Zn.

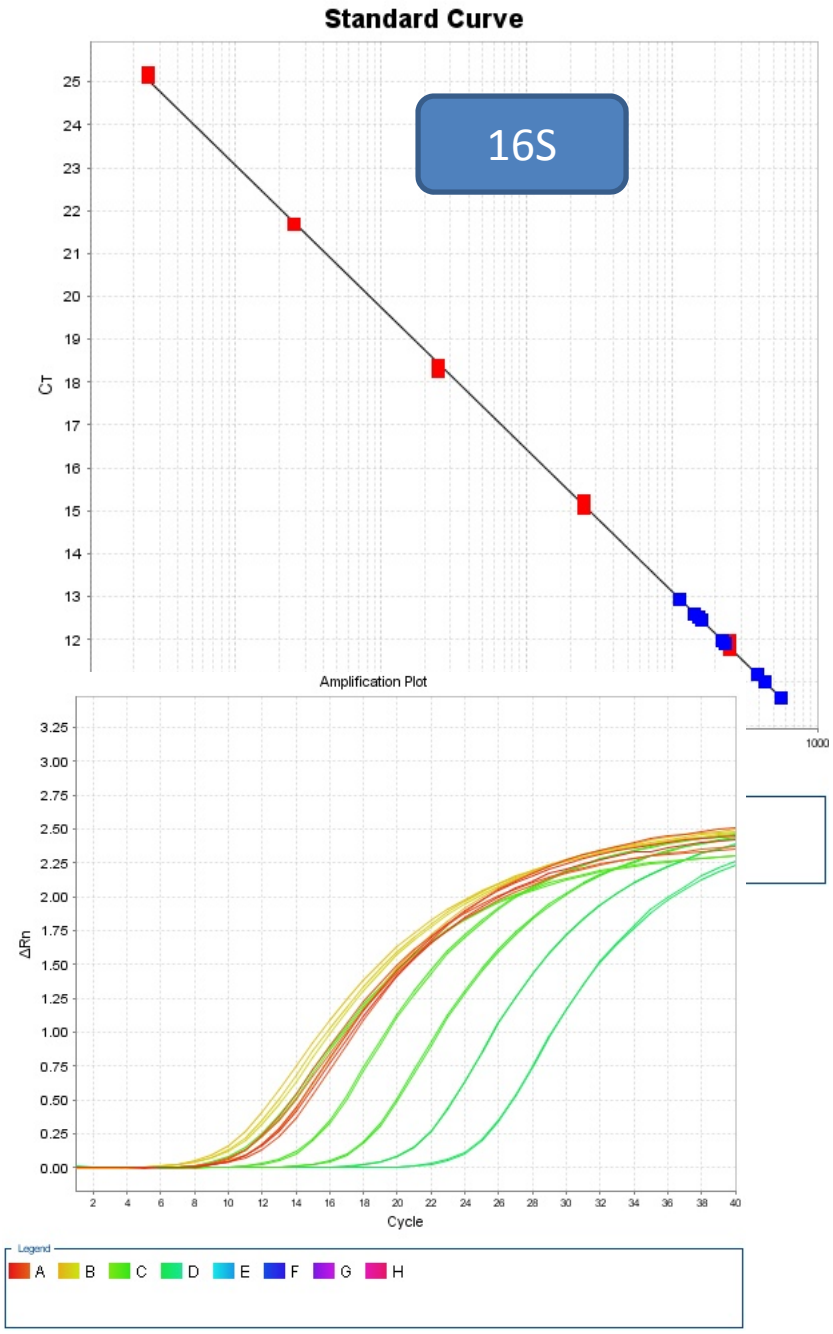
This causal pathway is not complete
What other factors affect PZA resistance level?

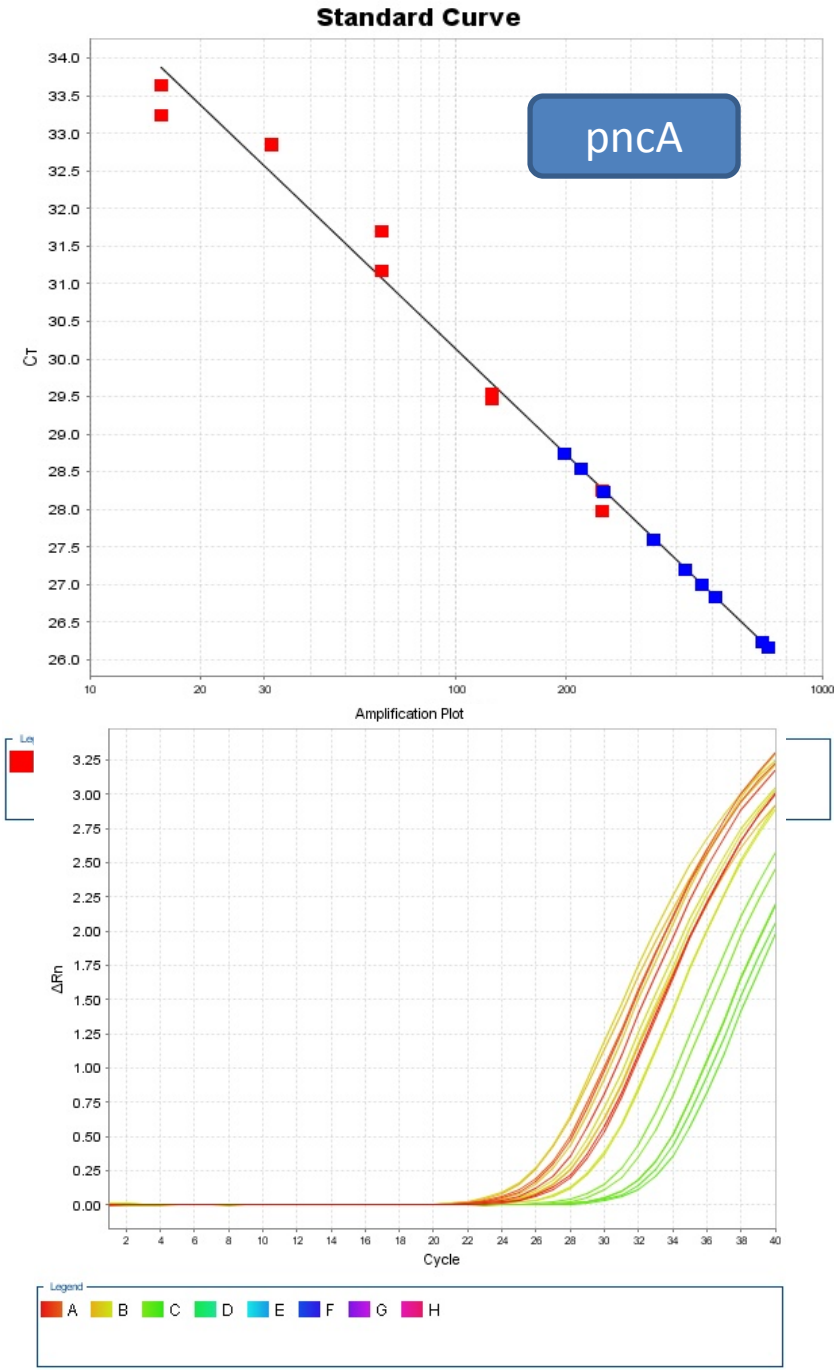


What other factors affect PZA resistance level?

(1) PZAse (pncA) expression level

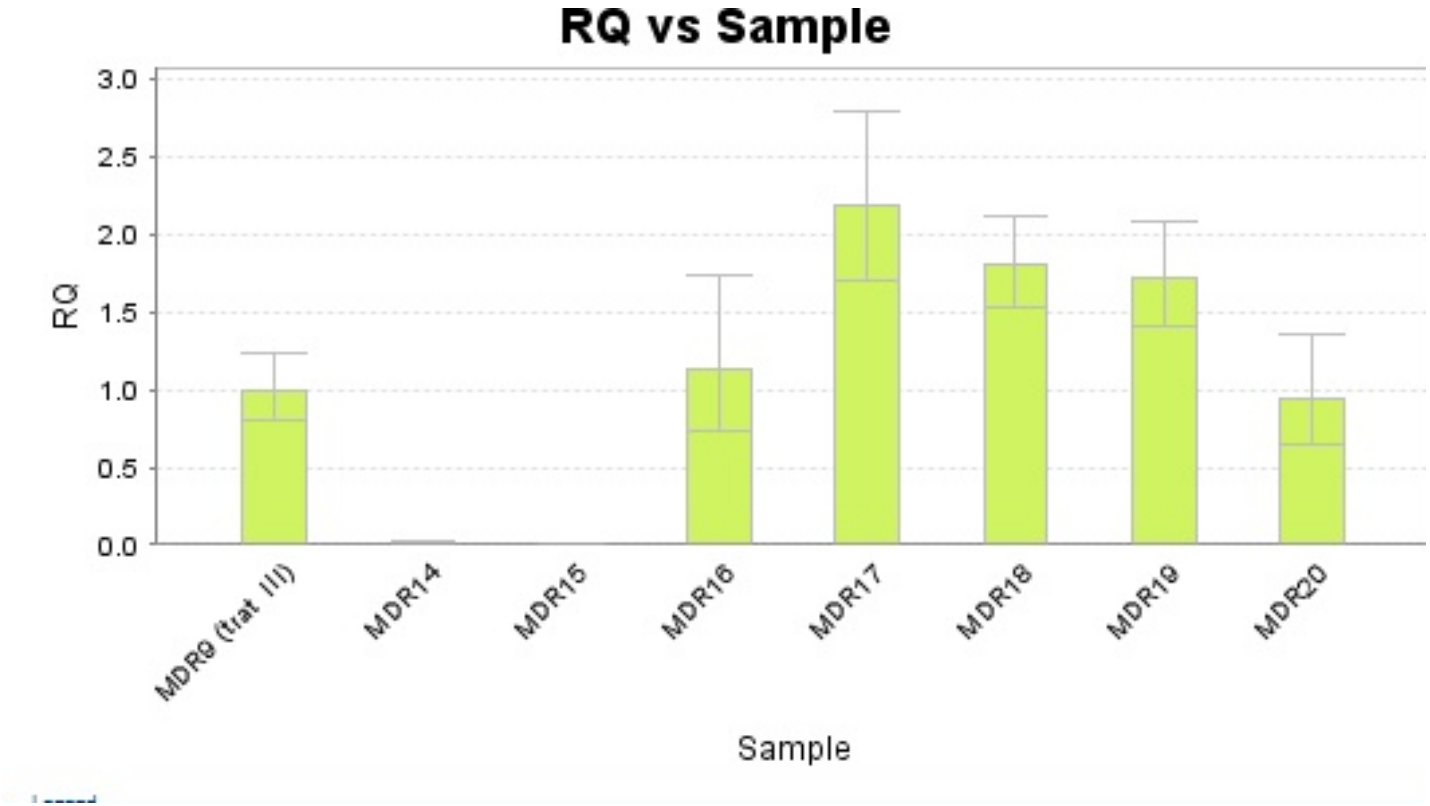



Standard Curve
16S
CT
Amplification Plot
ΔRn
Cycle
Legend
A B C D E F G H


Standard Curve
pncA
CT
Amplification Plot
ΔRn
Cycle
Legend
A B C D E F G H

High variability of pncA expression level

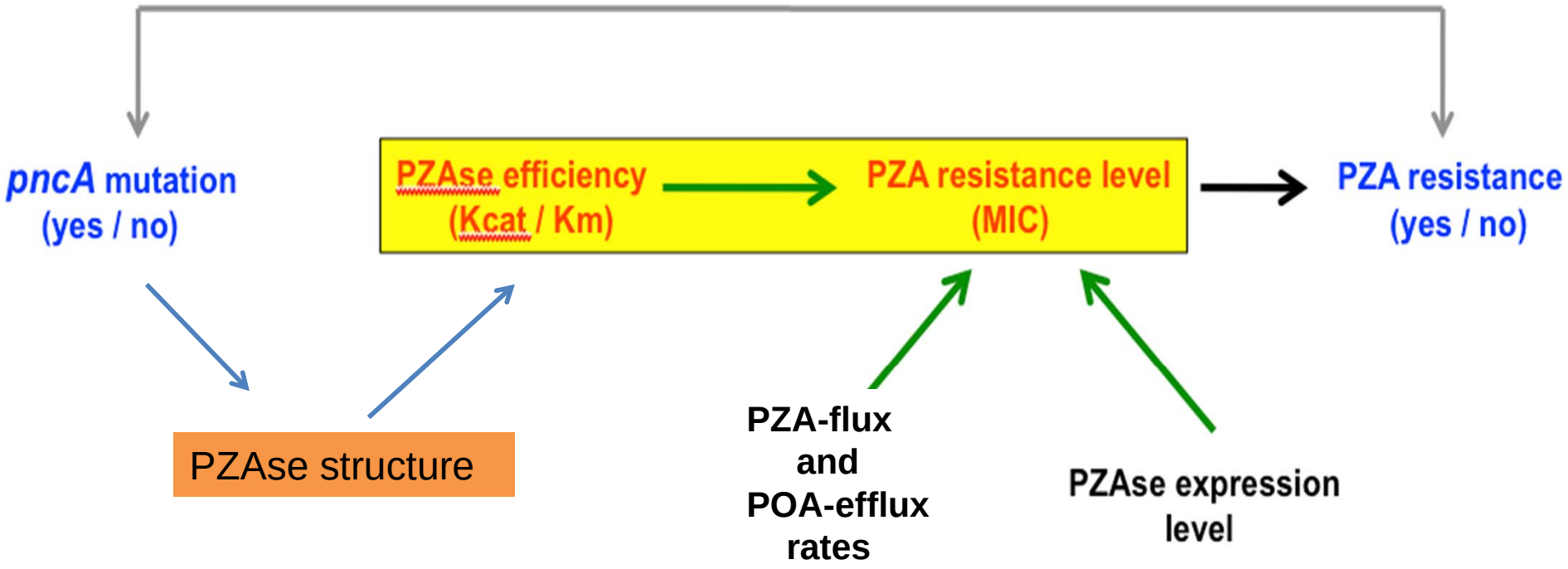
Lack of pncA expression associated to mutations in the pncA promoter and associated to PZA-resistance



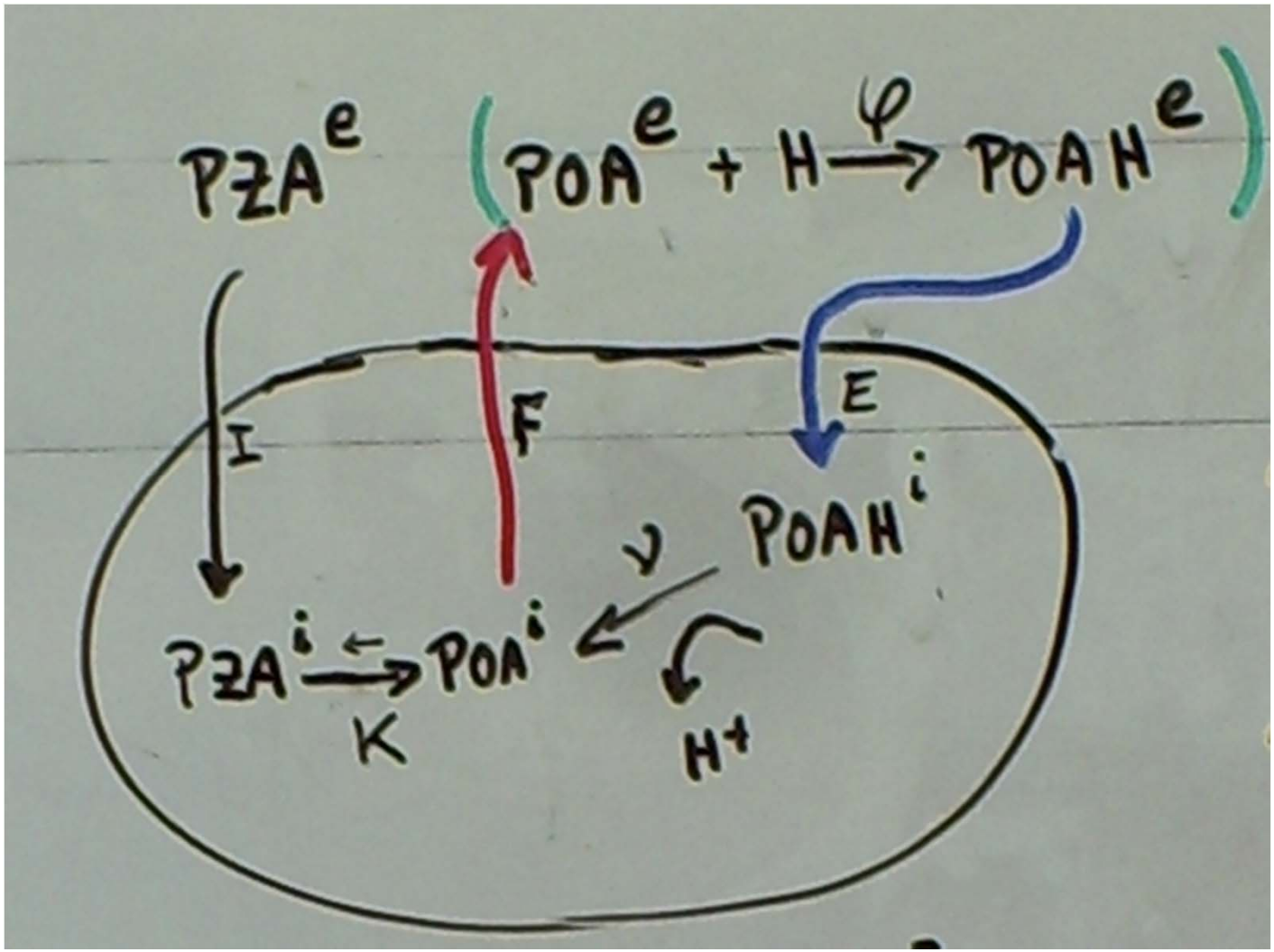
What other factors affect PZA resistance level?

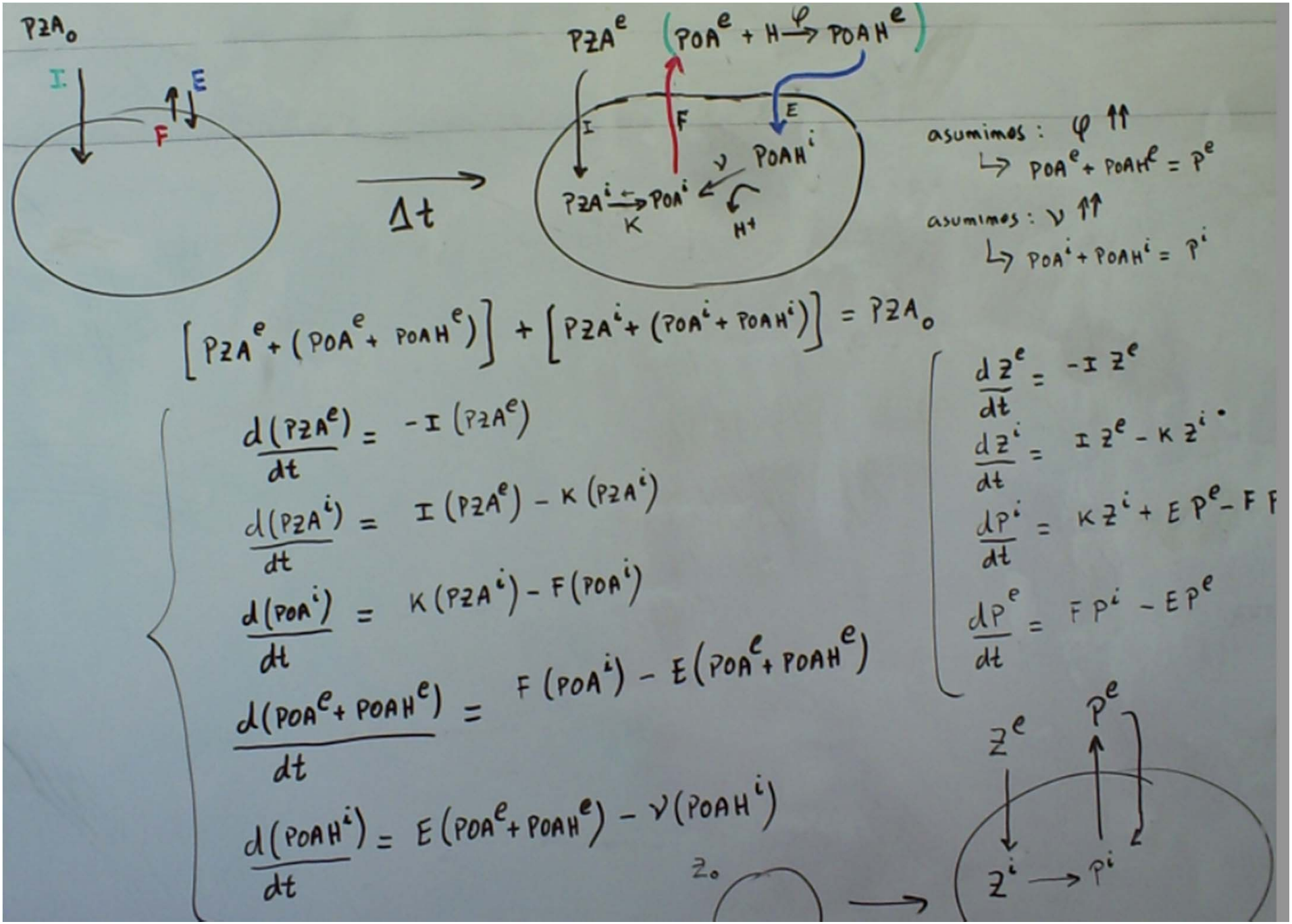
(1) PZAse (pncA) expression level

(2) POA-efflux rate

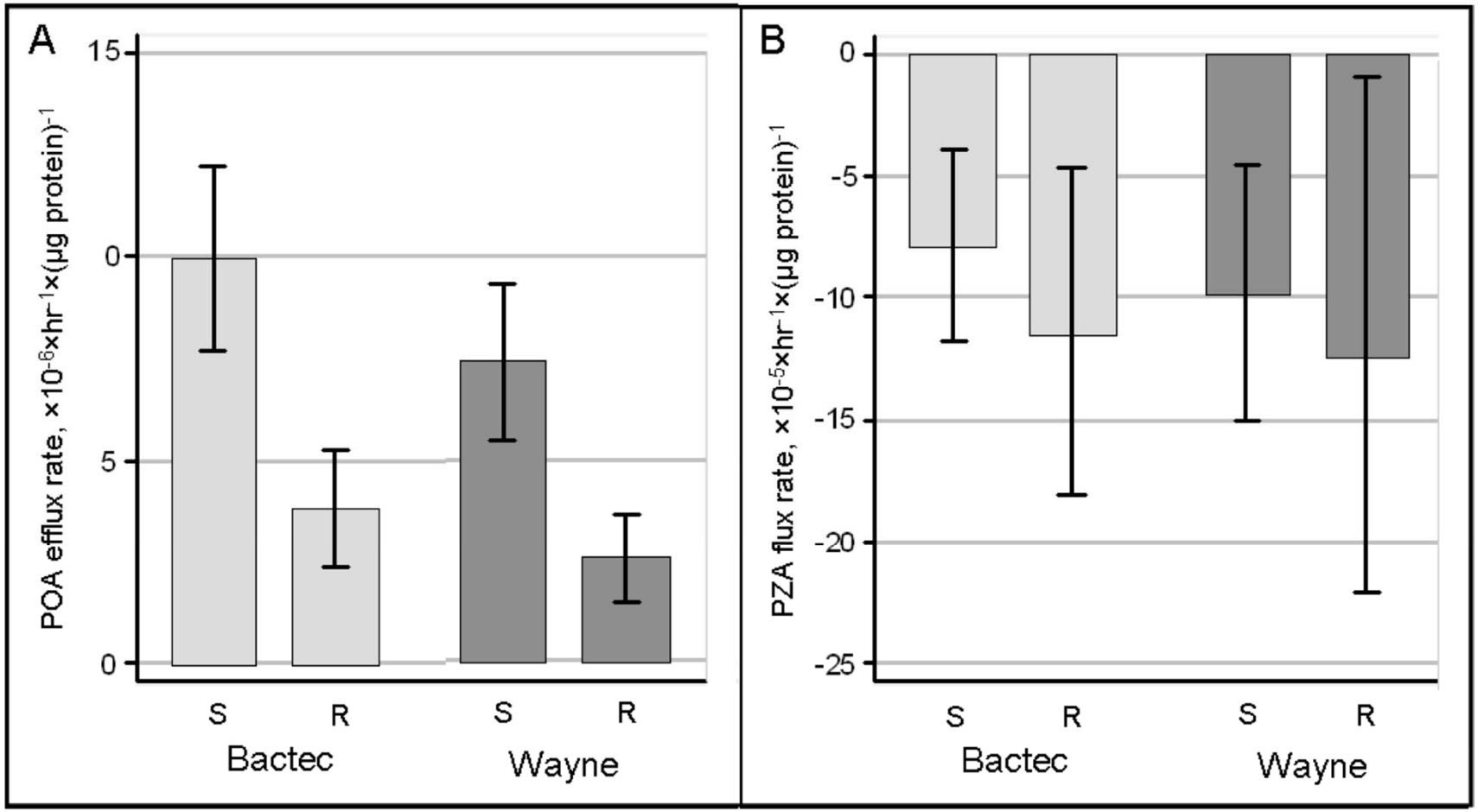


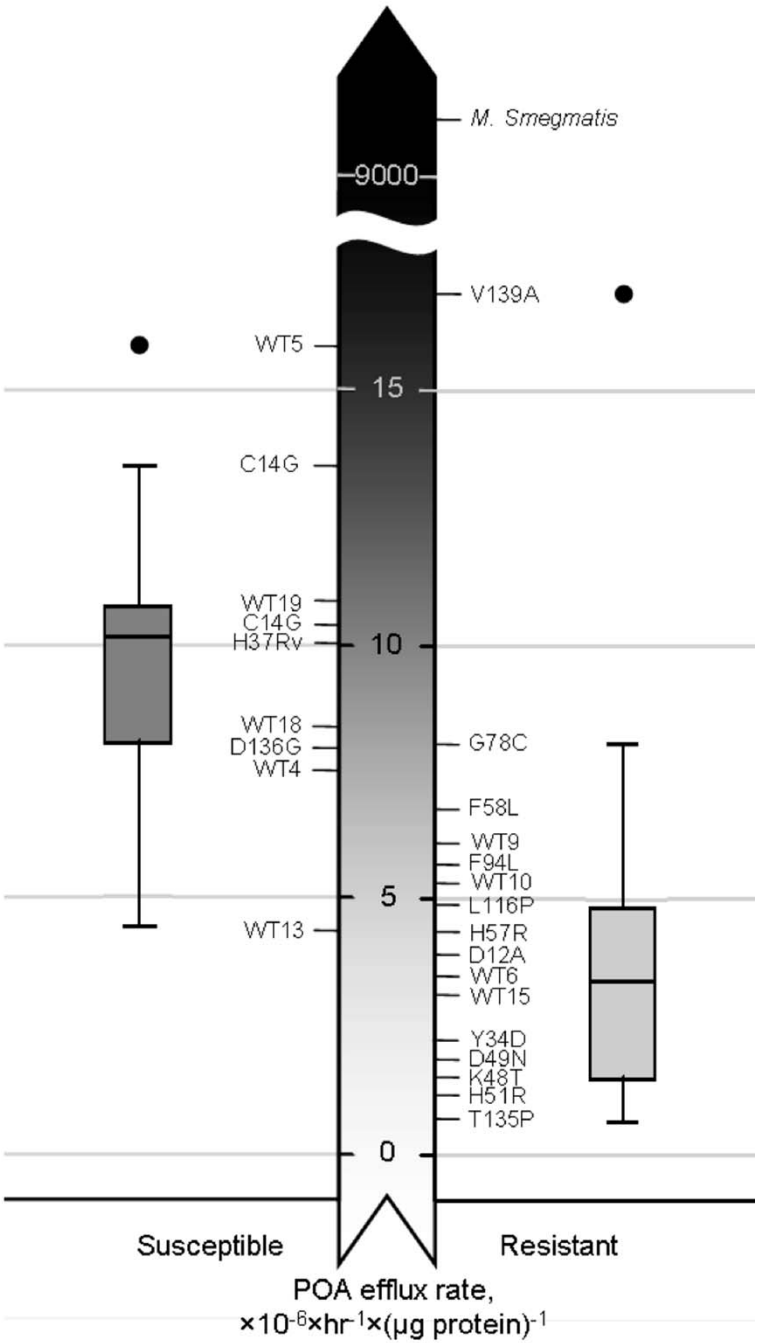
Estimation of the PZA-flux rate and the POA-efflux rate in multiple strains




PZA0
I
E
F
Δt
PZA^e
(POA^e + H → POAH^e)
I
F
E
PZA^i ⇄ POA^i
K
ν
POAH^i
H+
asumimos : φ ↑↑
↳ POA^e + POAH^e = P^e
asumimos : ν ↑↑
↳ POA^i + POAH^i = P^i
[PZA^e + (POA^e + POAH^e)] + [PZA^i + (POA^i + POAH^i)] = PZA0
d(PZA^e)/dt = -I (PZA^e)
d(PZA^i)/dt = I (PZA^e) - K (PZA^i)
d(POA^i)/dt = K (PZA^i) - F (POA^i)
d(POA^e + POAH^e)/dt = F (POA^i) - E (POA^e + POAH^e)
d(POAH^i)/dt = E (POA^e + POAH^e) - ν (POAH^i)
dz^e/dt = -I z^e
dz^i/dt = I z^e - K z^i
dP^i/dt = K z^i + E P^e - F P^i
dP^e/dt = F P^i - E P^e
z0
z^e
P^e
z^i
P^i

Low variability of PZA-flux rate
High variability of the POA-efflux rate




M. Smegmatis
9000
V139A
WT5
15
C14G
WT19
C14G
H37Rv
10
WT18
D136G
WT4
G78C
F58L
WT9
F94L
WT10
5
L116P
WT13
H57R
D12A
WT6
WT15
Y34D
D49N
K48T
H51R
T135P
0
Susceptible
Resistant
POA efflux rate,
×10-6×hr-1×(µg protein)-1

Tuberculosis 92 (2012) 84–91

Contents lists available at SciVerse ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

DRUG DISCOVERY AND RESISTANCE

Pyrazinoic acid efflux rate in *Mycobacterium tuberculosis* is a better proxy of pyrazinamide resistance

Mirko Zimic[a,*], Patricia Fuentes[a], Robert H. Gilman[a,b], Andrés H. Gutiérrez[a], Daniela Kirwan[c], Patricia Sheen[a]

[a] *Laboratorio de Bioinformática y Biología Molecular, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martín de Porres, Lima, Perú*
[b] *Department of International Health, School of Public Health, Johns Hopkins University, Baltimore, USA*
[c] *Department of Infectious Diseases and Immunity, Imperial College London, London W6 0DT, UK*

ARTICLE INFO

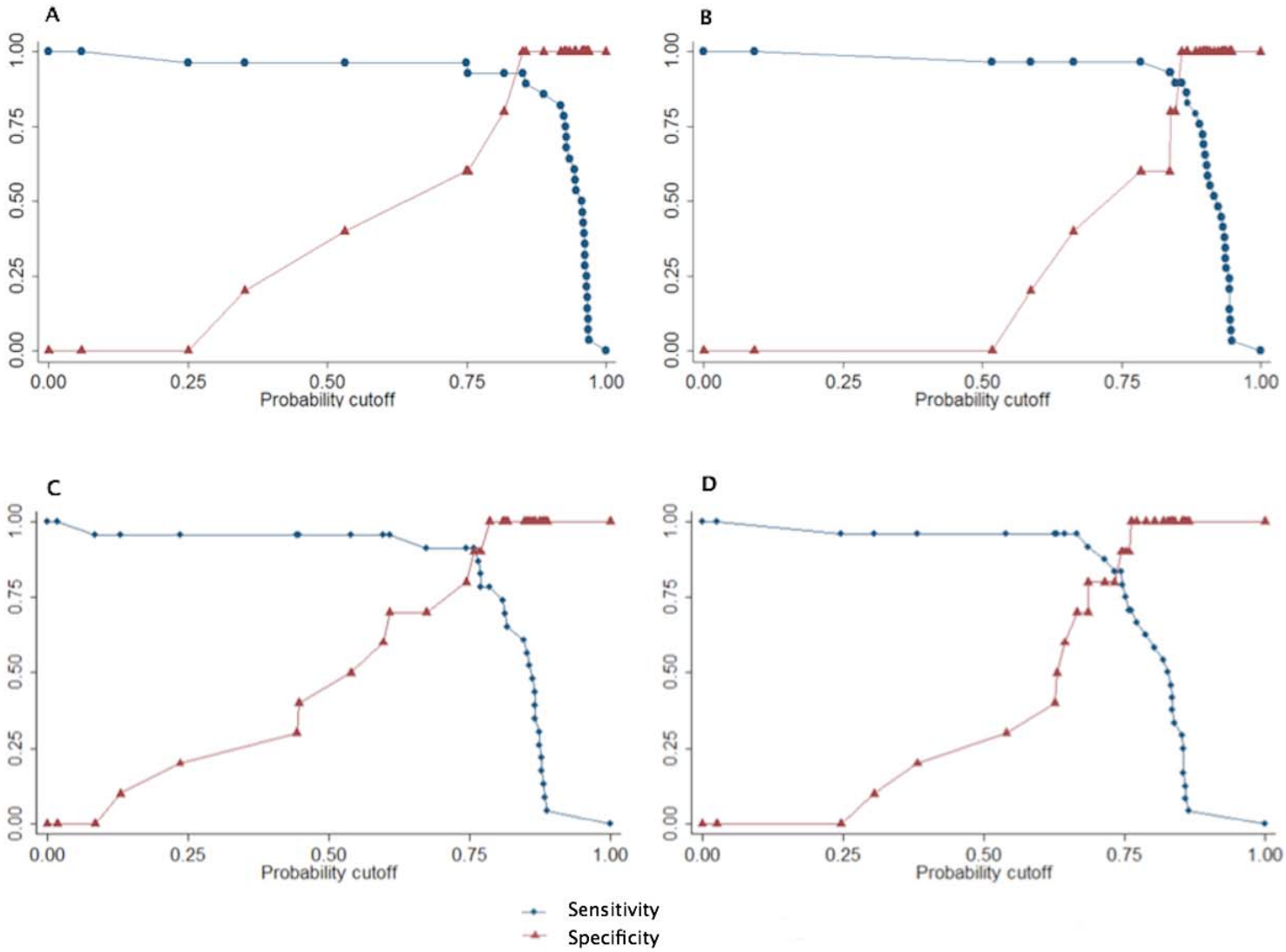
Article history:
Received 14 June 2011
Received in revised form
19 August 2011
Accepted 11 September 2011

Keywords:
Mycobacterium tuberculosis
POA efflux rate
PZA flux rate
PZA resistance

SUMMARY

Pyrazinamide is one of the most important drugs in the treatment of latent *Mycobacterium tuberculosis* infection. The emergence of strains resistant to pyrazinamide represents an important public health problem, as both first- and second-line treatment regimens include pyrazinamide. The accepted mechanism of action states that after the conversion of pyrazinamide into pyrazinoic acid by the bacterial pyrazinamidase enzyme, the drug is expelled from the bacteria by an efflux pump. The pyrazinoic acid is protonated in the extracellular environment and then re-enters the mycobacterium, releasing the proton and causing a lethal disruption of the membrane. Although it has been shown that mutations causing significant loss of pyrazinamidase activity significantly contribute to pyrazinamide resistance, the mechanism of resistance is not completely understood.

The pyrazinoic acid efflux rate may depend on multiple factors, including pyrazinamidase activity, intracellular pyrazinamidase concentration, and the efficiency of the efflux pump. Whilst the importance of the pyrazinoic acid efflux rate to the susceptibility to pyrazinamide is recognized, its quantitative effect remains unknown.


A
B
C
D
0.00
0.25
0.50
0.75
1.00
Probability cutoff
Sensitivity
Specificity

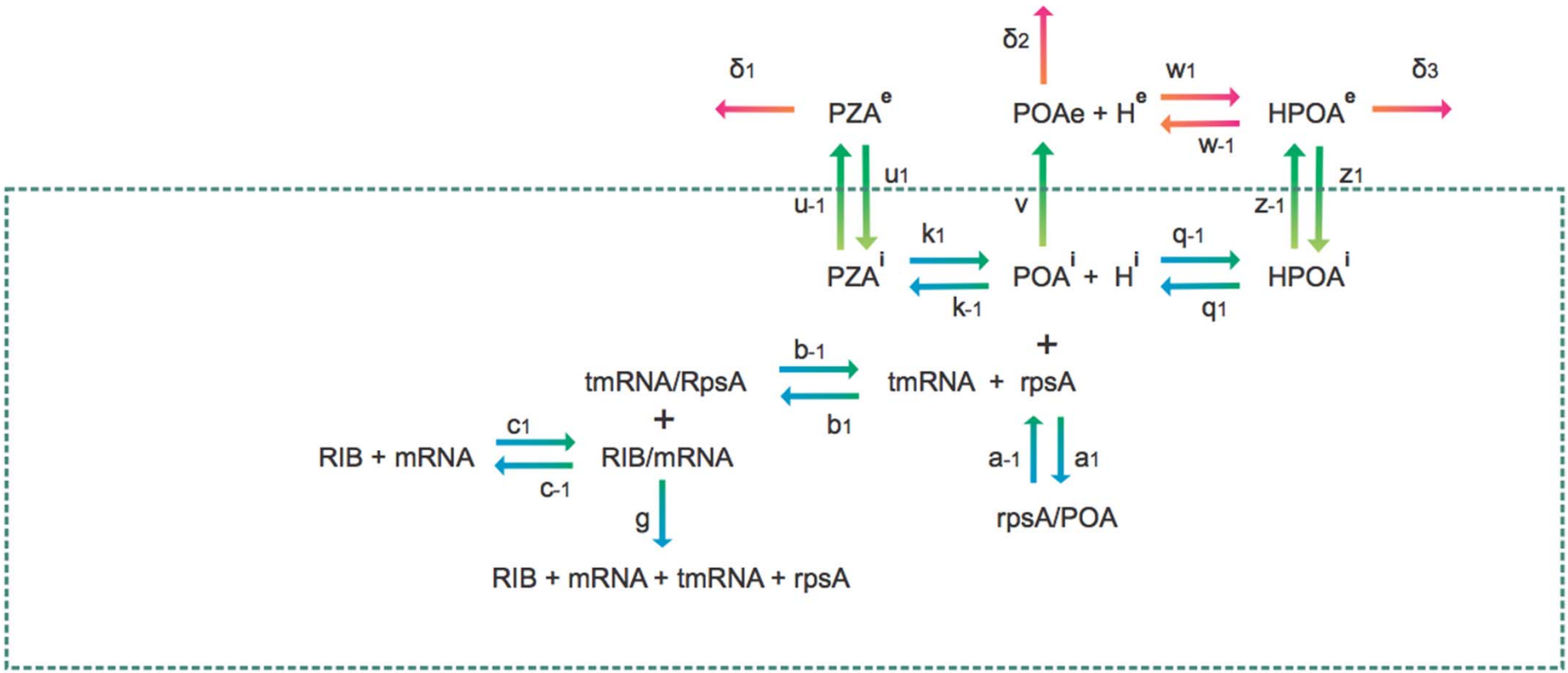
MICROBIAL DRUG RESISTANCE
Volume 00, Number 0, 2012

DOI: 10.1089/mdr.2011.0207

A New Approach for Pyrazinamide Susceptibility Testing in *Mycobacterium tuberculosis*

Mirko Zimic,[1] Sebastian Loli,[1] Robert H. Gilman,[1,2] Andrés Gutierrez,[1] Patricia Fuentes,[1] Milagros Cotrina,[1] Daniela Kirwan,[3] and Patricia Sheen[1]

Background: Pyrazinamide (PZA) is an important drug in the treatment of tuberculosis. Microbiological methods of PZA susceptibility testing are controversial and have low reproducibility. After conversion of PZA into pyrazinoic acid (POA) by the bacterial pyrazinamidase enzyme, the drug is expelled from the bacteria by an efflux pump. ***Objective:*** To evaluate the rate of POA extrusion from *Mycobacterium tuberculosis* as a parameter to detect PZA resistance. ***Methods:*** The rate of POA extrusion and PZA susceptibility determined by BACTEC 460 were measured for 34 strains in a previous study. PZA resistance was modeled in a logistic regression with the pyrazinoic efflux rate. ***Result:*** POA efflux rate predicted PZA resistance with 70.83%–92.85% sensitivity and 100% specificity compared with BACTEC 460. ***Conclusion:*** POA efflux rate could be a useful tool for predicting PZA resistance in *M. tuberculosis*. Further exploration of this approach may lead to the development of new tools for diagnosing PZA resistance, which may be of public health importance.


δ2
δ1
w1
δ3
PZA^e
POAe + H^e
HPOA^e
w-1
u1
z1
u-1
v
z-1
k1
q-1
PZA^i
POA^i + H^i
HPOA^i
k-1
q1
+
b-1
tmRNA/RpsA
tmRNA + rpsA
+
b1
c1
RIB + mRNA
RIB/mRNA
a-1
a1
c-1
g
rpsA/POA
RIB + mRNA + tmRNA + rpsA

B System of equations

$$\frac{d[PZA^e]}{dt} = -\left(u_1+\delta_1\right).[PZA^e]+u_{-1}.[PZA^i] \quad (1)$$

$$\frac{d[PZA^i]}{dt} = u_1.[PZA^e]+k_{-1}.[POA^i] - \left(u_{-1}+k_1.\left(\frac{Act_m^{PZAse}}{Act_w^{PZAse}}\right).\left(\frac{[mRNA]_m^{pncA}}{[mRNA]_w^{pncA}}\right)\right).[PZA^i] \quad (2)$$

$$\frac{d[POA^i]}{dt} = k_1.\left(\frac{Act_m^{PZAse}}{Act_w^{PZAse}}\right).\left(\frac{[mRNA]_m^{pncA}}{[mRNA]_w^{pncA}}\right).[PZA^i] - \left(k_{-1}+v\right).[POA^i]+q_1.[HPOA^i]-q_{-1}.[POA^i].[H^i] - a_1.[POA^i].[rpsA]+a_{-1}.[POA^i/rpsA] \quad (3)$$

$$\frac{d[POA^e]}{dt} = v.[POA^i]-w_1.[POA^e].[H^e]+w_{-1}.[HPOA^e]-\delta_2.[POA^e] \quad (4)$$

$$\frac{d[HPOA^e]}{dt} = w_1.[POA^e].[H^e]-\left(w_{-1}+z_1+\delta_3\right).[HPOA^e]+z_{-1}.[HPOA^i] \quad (5)$$

$$\frac{d[HPOA^i]}{dt} = z_1.[HPOA^e]-\left(z_{-1}+q_1\right).[HPOA^i]+q_{-1}.[POA^i].[H^i] \quad (6)$$

$$\frac{d[POA^i/rpsA]}{dt} = a_1.[POA^i].[rpsA]-a_{-1}.[POA^i/rpsA] \quad (7)$$

$$\frac{d[rpsA]}{dt} = -a_1.[POA^i].[rpsA]+a_{-1}.[POA^i/rpsA]-b_1.[rpsA].[tmRNA] + b_{-1}.[tmRNA/rpsA]+\gamma.[tmRNA/rpsA].[RIB/mRNA] \quad (8)$$

$$\frac{d[tmRNA/rpsA]}{dt} = b_1.[rpsA].[tmRNA]-b_{-1}.[tmRNA/rpsA] - \gamma.[tmRNA/rpsA].[RIB/mRNA] \quad (9)$$

$$\frac{d[tmRNA]}{dt} = -b_1.[rpsA].[tmRNA]+b_{-1}.[tmRNA/rpsA] + \gamma.[tmRNA/rpsA].[RIB/mRNA] \quad (10)$$

$$\frac{d[RIB/mRNA]}{dt} = c_1.[RIB].[mRNA]-c_{-1}.[RIB/mRNA] - \gamma.[tmRNA/rpsA].[RIB/mRNA] \quad (11)$$

$$\frac{d[RIB]}{dt} = -c_1.[RIB].[mRNA]+c_{-1}.[RIB/mRNA] + \gamma.[tmRNA/rpsA].[RIB/mRNA] \quad (12)$$

$$\frac{d[mRNA]}{dt} = -c_1.[RIB].[mRNA]+c_{-1}.[RIB/mRNA] + \gamma.[tmRNA/rpsA].[RIB/mRNA] \quad (13)$$

Acknowledgements

TUBERCULOSIS WORKING GROUP IN PERU

- Robert Gilman
- Patricia Sheen
- Carlton Evans
- David Moore
- Luz Caviedes
- Jorge Coronel

STUDENTS

- **Molecular Group**
  - Patricia Fuentes
  - Kathy Lozano
  - Rhosalyns Reynoso
  - Ricardo Antiparra
  - Patricia Durand
  - Juan Carlos Gaspar
  - Antonio Flores
  - Marco Santos
  - Rodolfo Huerta
  - Patricia Ferrer
- **Bioinformatics Group**
  - David Requena
  - Sebastian Loli
  - Carlos Bueno
  - Ana Dávila
  - Basilio Cieza
  - Bryan Lucero
  - Hugo Valdivia
  - Andrés Gutierrez
  - Miguel Quiliano