

Supporting Information

NMR insights on Nano silver post-surgical treatment of superficial caseous lymphadenitis in small ruminants

Danijela Stanisic^{1#}, Natália L. Fregonesi¹, Caio H. N. Barros¹, João G. M. Pontes¹, Stephanie F. Fulaz¹, Ulisses J. Menezes², Jorge L. Nicoleti², Thiago L. P. Castro³, Núbia Seyffert³, Vasco A. C. Azevedo³, Nelson Durán^{4,5}, Ricardo W. Portela², Ljubica Tasic^{1,4*}

¹Laboratório de Química Biológica, Departamento de Química Orgânica, Instituto de Química, Universidade Estadual de Campinas, Campinas-SP, Brazil.

²Laboratório de Imunologia e Biologia Molecular, Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador-BA, Brazil.

³Departamento de Biologia Geral, Instituto de Estudos Avançados Transdisciplinares, Universidade Federal de Minas Gerais, Belo Horizonte-MG, Brazil.

⁴NanoBioSS – Institute of Chemistry, University of Campinas, Campinas, SP, Brazil.

⁵UFABC, São Paulo, SP, Brazil.

Corresponding author: ljubica@iqm.unicamp.br

KEYWORDS: *Corynebacterium pseudotuberculosis*, Nuclear Magnetic Resonance, metabolomics, caseous lymphadenitis, biogenic nanosilver based cream, wound healing, antimicrobial activity.

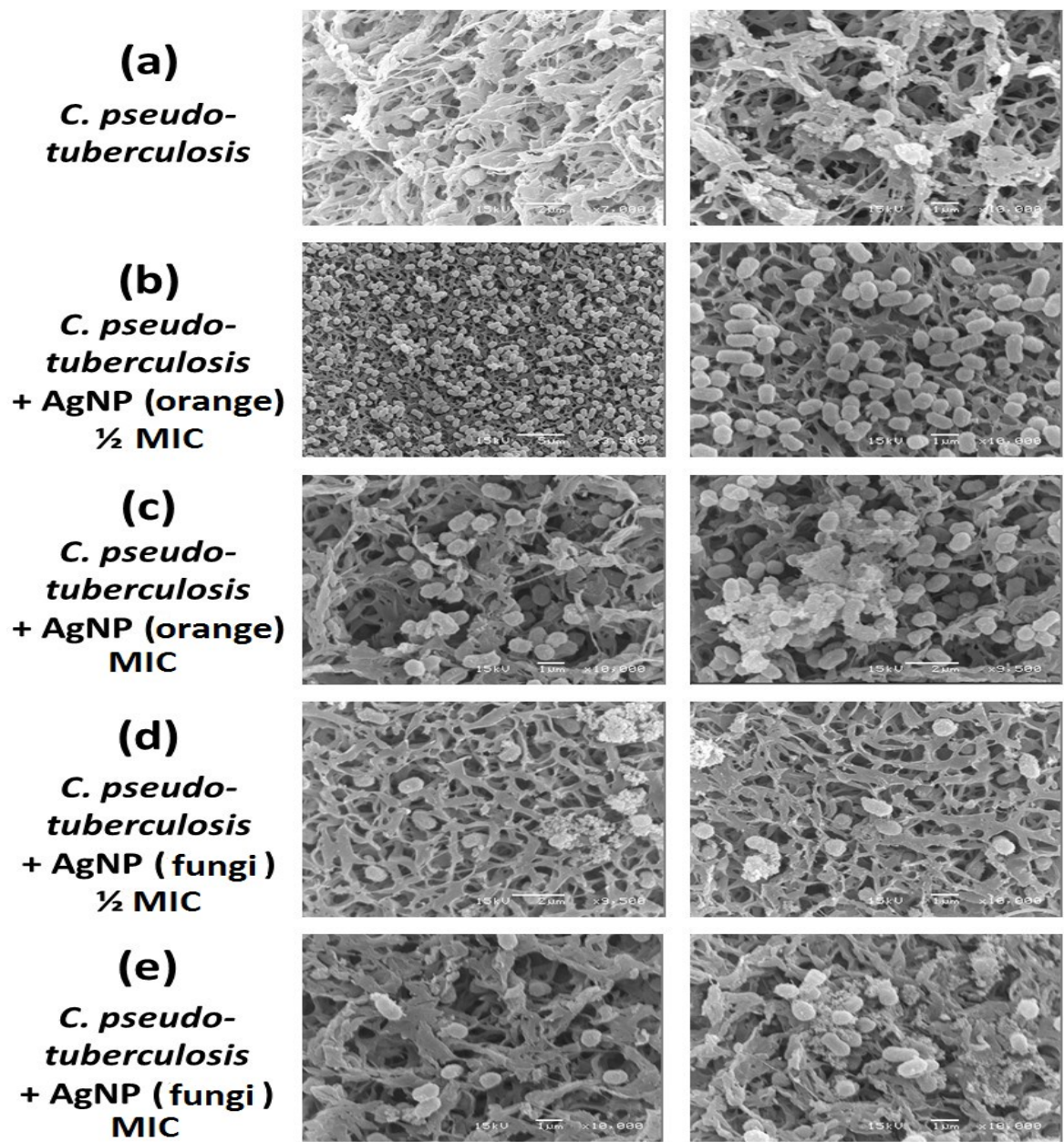


Figure S1. SEM Images of *Corynebacterium pseudotuberculosis*: (a) cells at a great dilution; (b) after addition of the biogenic AgNPs synthesized from the orange (*Citrus sinensis*) peel extract in the concentration of $11.0 \mu\text{g mL}^{-1}$ (half of the MIC); (c) at the concentration $22.0 \mu\text{g mL}^{-1}$ (MIC); (d) bacteria with biogenic AgNPs synthesized from *Fusarium oxysporum* in the concentration $16.8 \mu\text{g mL}^{-1}$ (half of the MIC); (e) at the concentration $33.5 \mu\text{g mL}^{-1}$ (MIC).

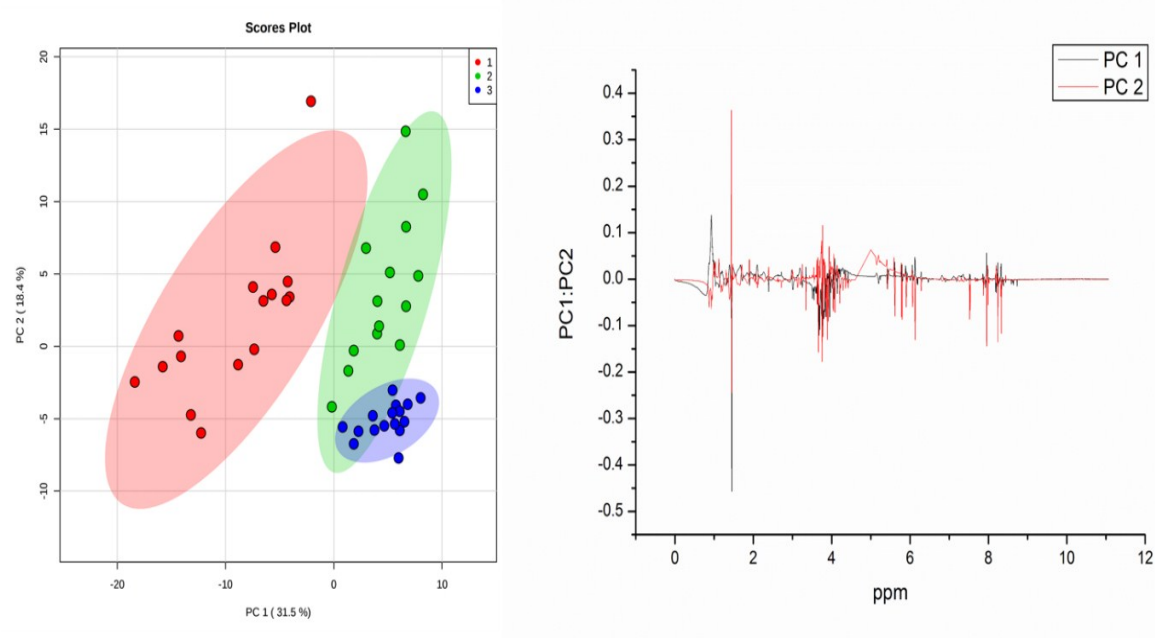
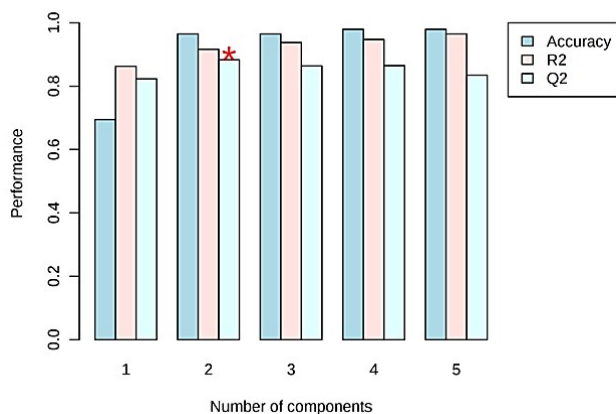


Figure S2. PCA on *C. pseudotuberculosis* NMR data: 2D score (left) and loading graphs (right). NMR data variances were 31.5% in PC 1 and 18.4% in PC 2. The NMR data were normalized by sum and mean centered previous to PCA. The red circles present the group of *C. pseudotuberculosis* extract, the green circles correspond to *C. pseudotuberculosis* extract under ampicillin effects and blue circles represent the *C. pseudotuberculosis* extract under AgNP effects.



PLS-DA cross validation details:

Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.89444	0.96528	0.96528	0.97917	0.97917
R2	0.8824	0.91588	0.93738	0.94758	0.96502
Q2	0.82281	0.88288	0.8837	0.86489	0.83498

Figure S3. Cross validation of *C. pseudotuberculosis* extract PLS-DA model using different number of components. The red star indicates the best classifier.

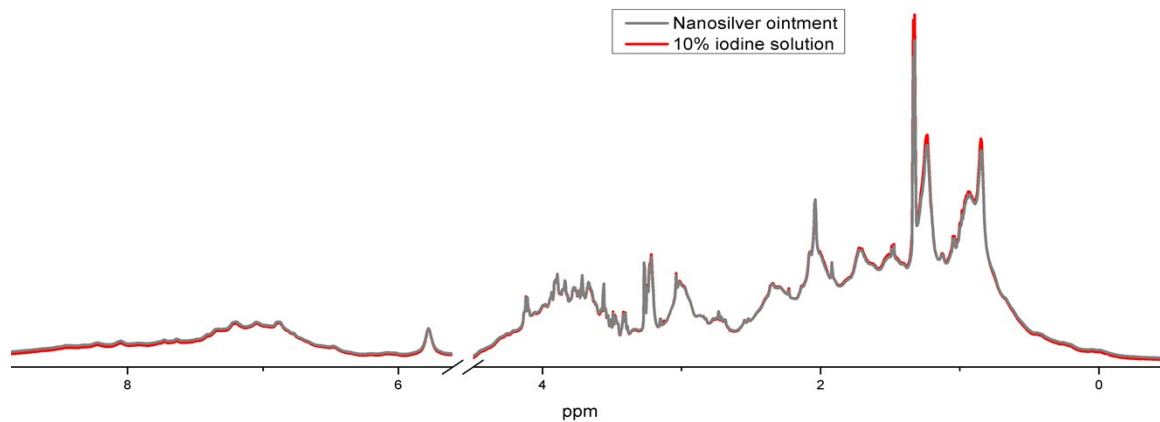


Figure S4. Mean ¹H NMR spectra of serum samples taken from goats treated with iodine solution (red line) and AgNP-based cream (gray line).

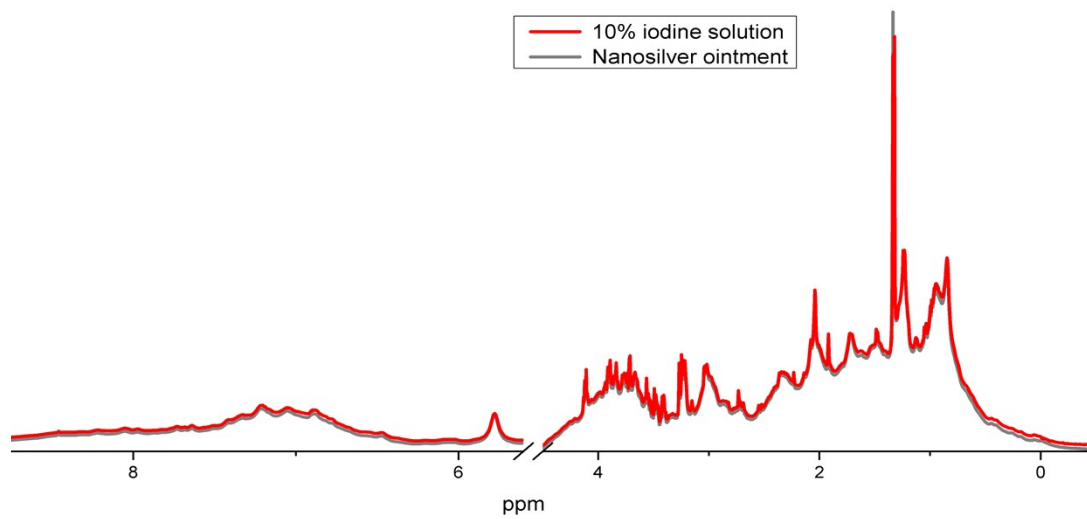


Figure S5. Mean ¹H NMR spectra of serum samples taken from sheep treated with iodine solution (red line) and AgNP-based cream (gray line).

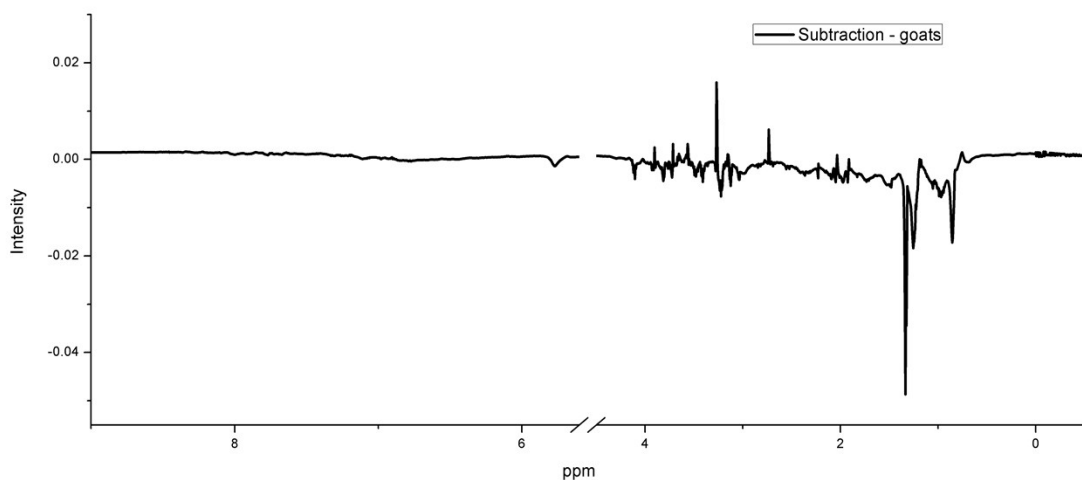


Figure S6. Subtraction of serum ^1H NMR mean spectra, shown in Figure S4, iodine solution versus AgNP-based cream (I-P). There are minimal differences in spectral data when compared serum samples from two treatments.

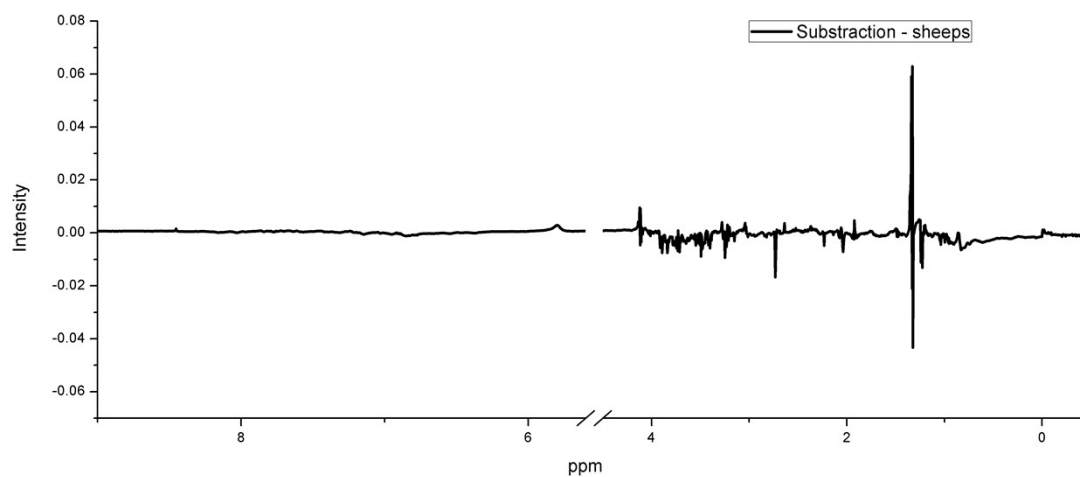


Figure S7. Subtraction of serum ^1H NMR mean spectra, shown in Figure S5, iodine solution versus AgNP-based cream (I-P). There are minimal differences in spectral data when compared serum samples from two treatments.

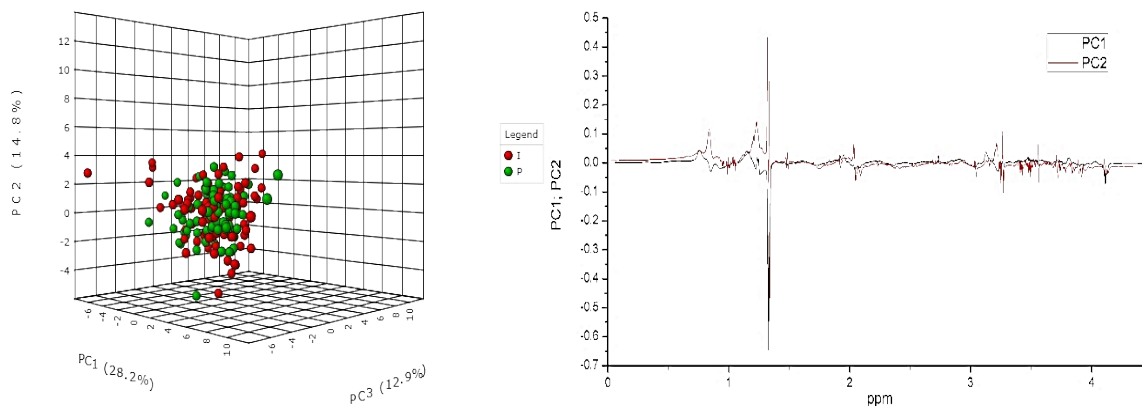


Figure S8. ^1H NMR PCA data on goat serum samples: 3D scores (left) and loadings (right) with variance of 28.2% in PC 1 and 14.8% in PC 2. The NMR data were normalized by sum and mean centered after the exclusion of outliers. The green circles correspond to animals treated with AgNP-based cream (P) and the red circles correspond to the NMR data of animals treated with iodine 10% (I).

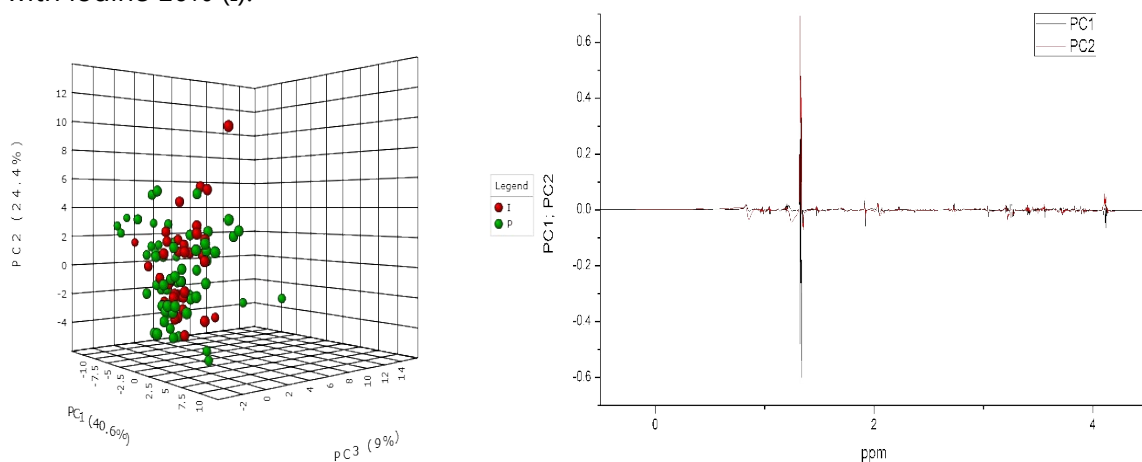


Figure S9. ^1H NMR PCA data on sheep serum samples: 3D scores (left) and loadings (right) with variance of 40.6% in PC 1 and 24.4% in PC 2. The NMR data were normalized by sum and mean centered after the exclusion of outliers. The green circles correspond to animals treated with AgNP-based cream (P) and the red circles correspond to the NMR data of animals treated with iodine 10% (I).

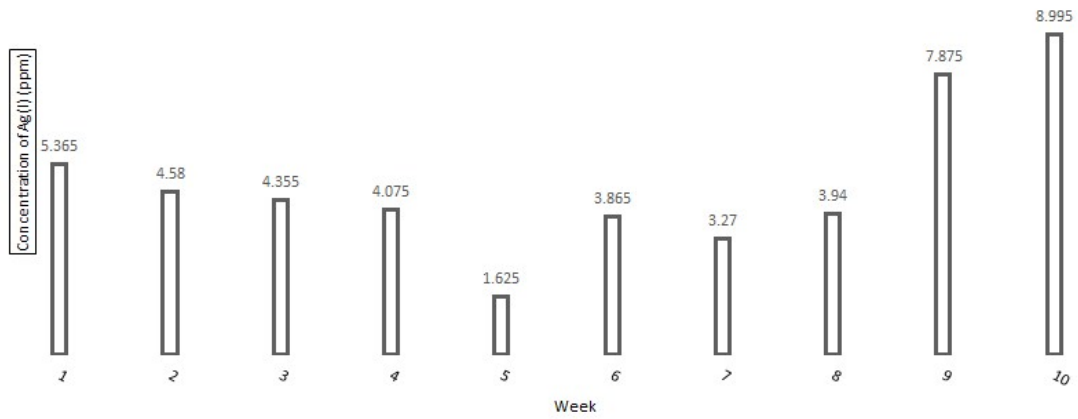


Figure S10. Concentration of silver in serum sample from sheep treated with AgNP-based cream during 10 weeks, numbers 1-10 mean week upon treatment had started.