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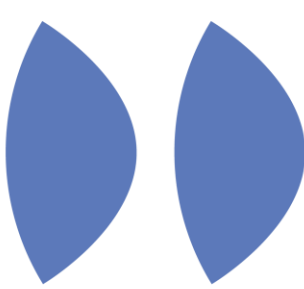
Resistance of *Salmonella* to heavy metals, a key element for dominance in the agro-food industry in Europe?



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For the last 50 years, the success of the epidemic clones of the *Salmonella* Typhimurium and its monophasic variant (S. 4,[5],12:i:-) (DT9, DT204, DT104 and DT193) was mostly attributed to their antimicrobial resistance profile. The recent discovery of a genetic island SGI-4 carrying resistance genes to copper (*pco*), arsenic (*ars*) and silver (*sil*) and its role in the emergence of the European clone (DT193 or ST34) suggested that other factors, such as heavy metal resistance, can favorite the selection of epidemic clones enabling them to survive along the agro-food production chain until humans [1, 2].

1. Genomic approach

In this study, the heavy metal resistance genes from the BacMet database were screened in 168 genomes belonging to the 11 *Salmonella* serovars prevalent in agro-food sectors in France.

- 8,725 identified sequences
- 52 metals resistances genes (Fig. 1)

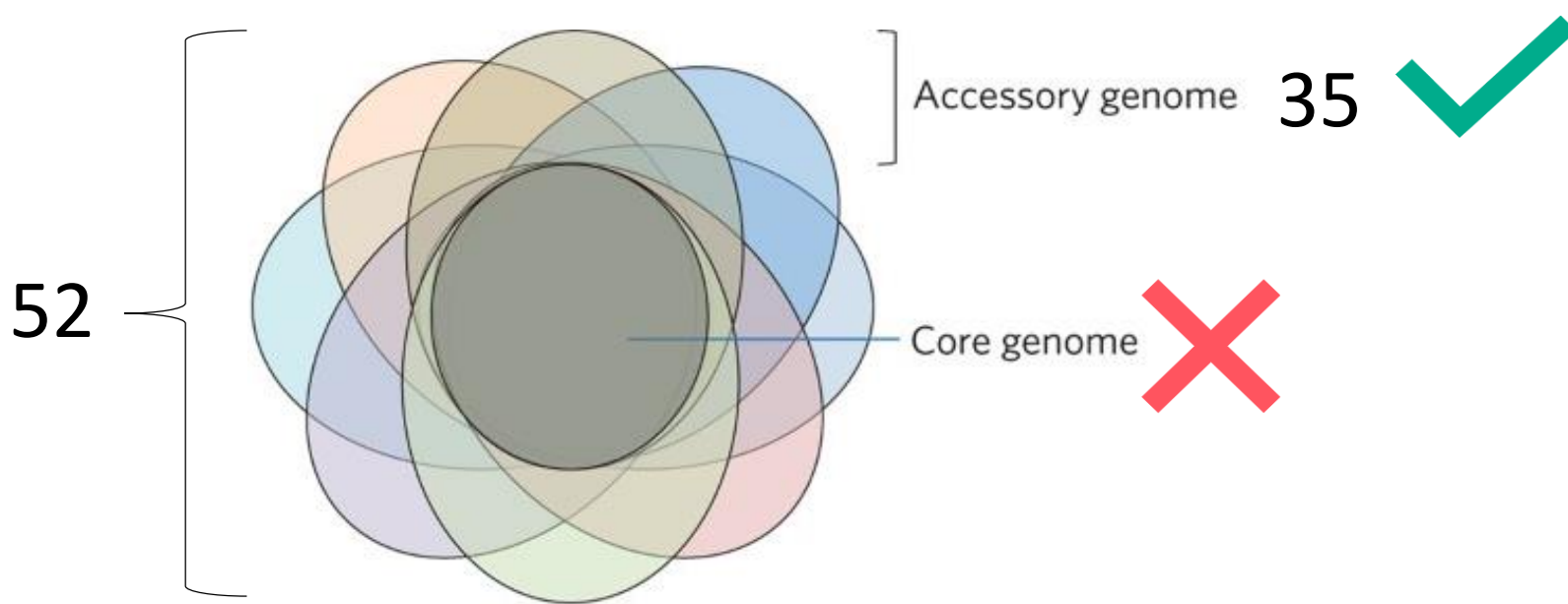


Figure 1. Core and accessory genome repartition of the 52 metals resistance genes identified for the 168 *Salmonella* genomes analyzed.

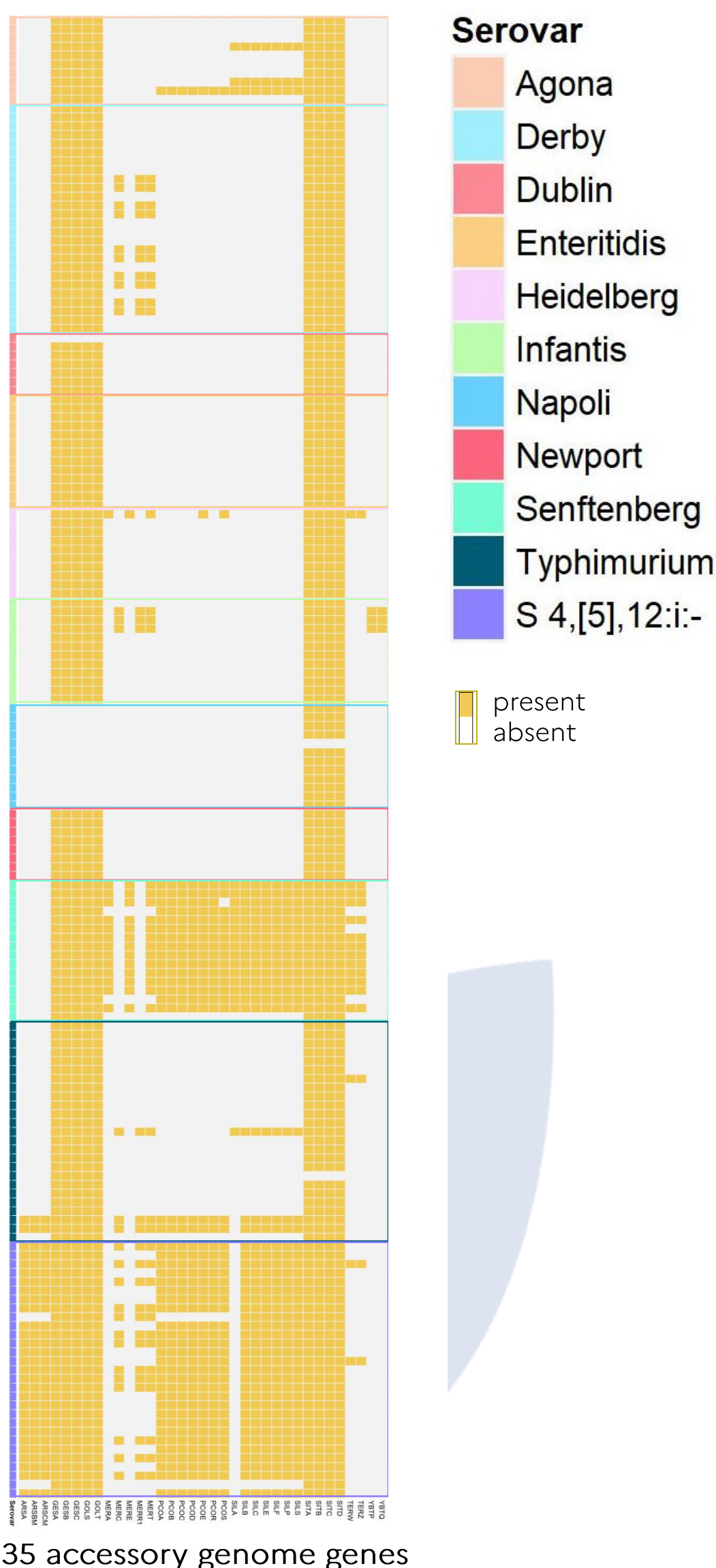
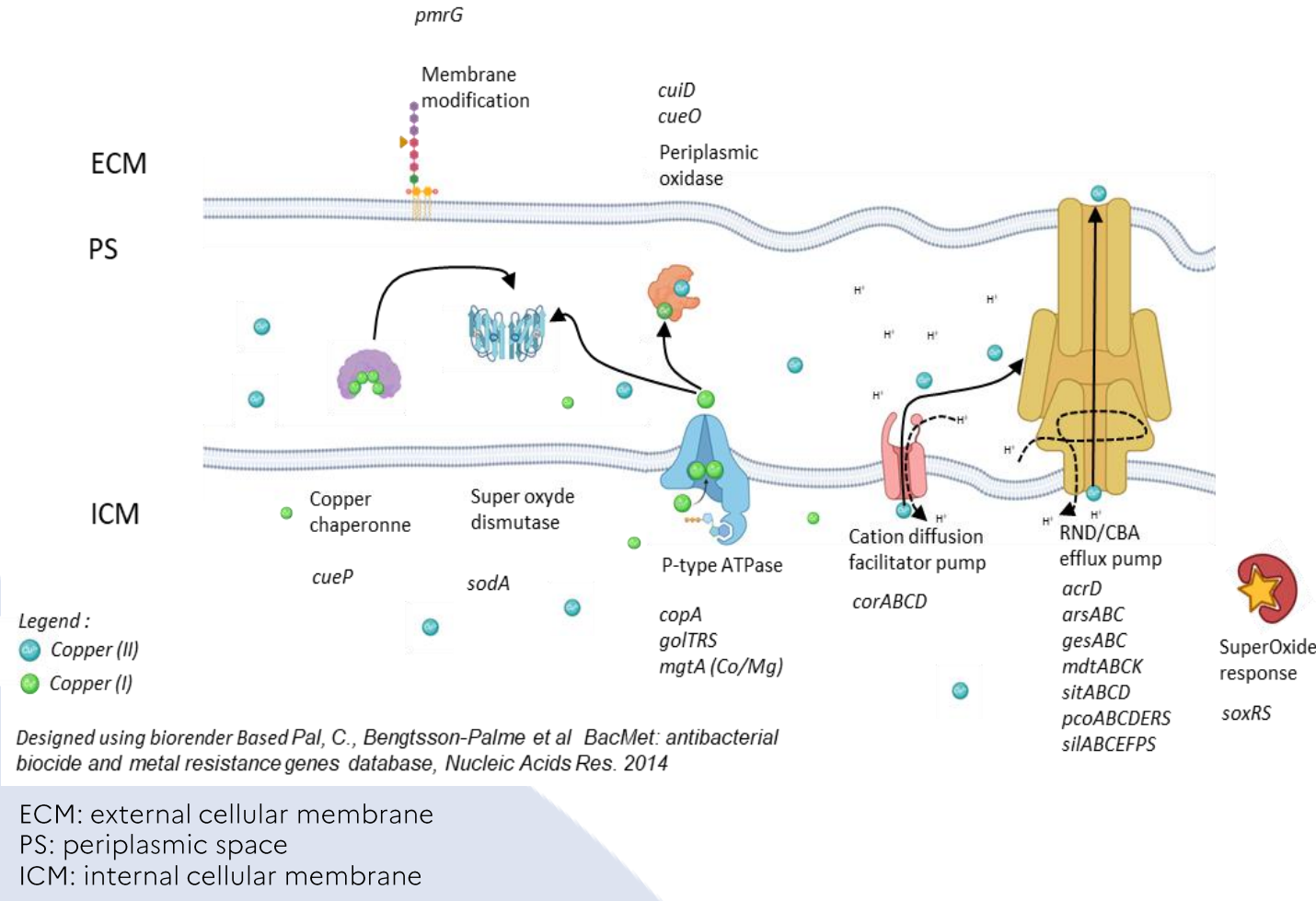


Figure 2. Heat-map of the presence/absence of the 35 accessory genome genes identified among the 168 *Salmonella* genome analyzed.

- 19 genes, implicated in Cu resistance, among the 35 metal resistance genes were selected for transcriptomic analysis:

Gene name	Metals resistance
<i>pmrG</i>	Phosphoglycerate mutase family.
<i>cueP</i>	Implicated in the transfer of copper ions to SodCII
<i>cuID</i>	Multicopper oxidase family (alias <i>cueO</i>)
<i>sosX</i>	Superoxide response regulon regulator
<i>copA</i>	P-Type ATPase cation transporter
<i>golT</i>	P-Type ATPase cation transporter, DNA-binding domain
<i>mgTA</i>	P-Type ATPase cation transporter
<i>corA</i>	Metal ion transporter
<i>acrD</i>	RND superfamily
<i>arsA</i>	Anion-transporting ATPase
<i>gesA</i>	RND superfamily, MFP family
<i>mdtA</i>	Linked to metals resistance
<i>sitA</i>	Fe/Mn transport protein, periplasmic-binding protein
<i>pcoA</i>	Multiple copper oxidase PcoA
<i>pcoB</i>	Copper binding protein PcoB
<i>pcoC</i>	Copper resistance system PcoC
<i>silB</i>	Efflux RND transporter periplasmic adaptor subunit SIIB
<i>silC</i>	Efflux RND transporter outer membrane channel SIIC
<i>rpoD</i>	RNA polymerase sigma 70 factor

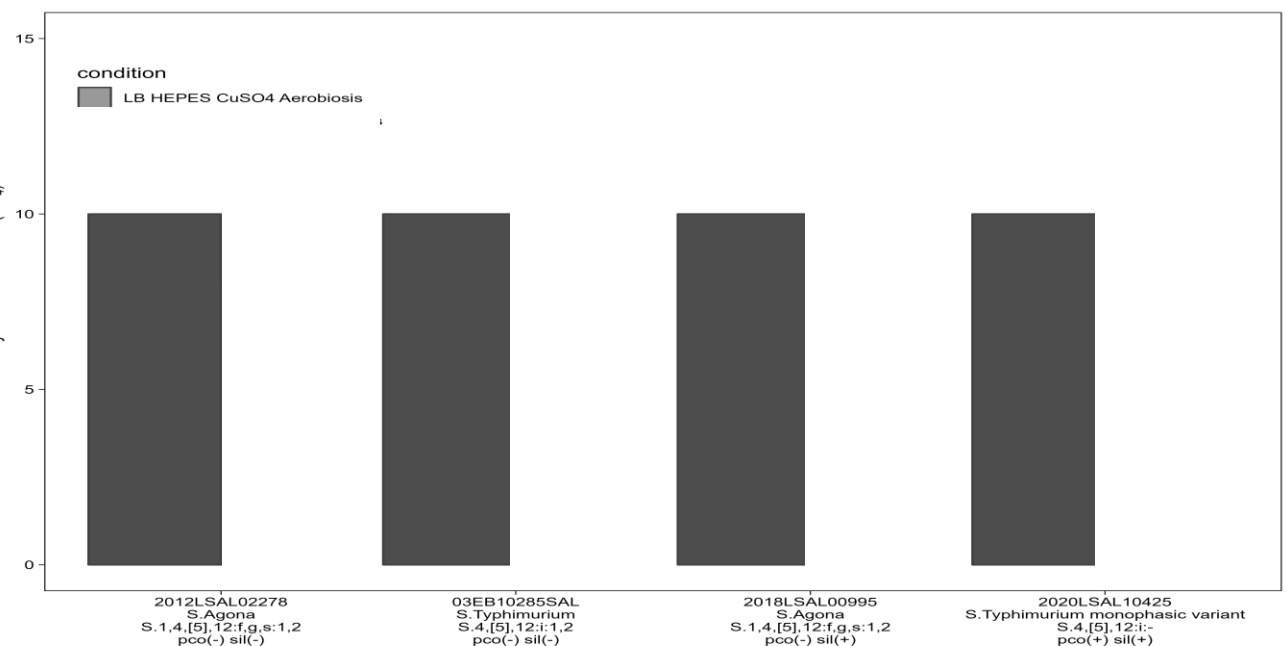


- 4 strains with clusters *pco* and *sil* present/absent were selected for phenotypic analysis:

Strain	Characteristics
03EB10285SAL	<i>S. Typhimurium</i> <i>sil</i> (-) <i>pco</i> (-)
2012LSAL02278	<i>S. Agona</i> <i>sil</i> (-) <i>pco</i> (-)
2018LSAL00995	<i>S. Agona</i> <i>sil</i> (+) <i>pco</i> (-)
2020LSAL10425	<i>S. 4,[5],12:i:-</i> <i>sil</i> (+) <i>pco</i> (+)

2. Phenotypic approach

In aerobic growth condition with copper (CuSO₄) the 4 strains have the same MIC (5mM CuSO₄) despite the different genomic profile (Fig.3)



Figures 3 and 5. Minimal inhibitory concentration of copper sulfate against a panel of strains in aerobiosis and anaerobiosis. In light grey is represented aerobiosis condition; in dark grey the anaerobiosis.

but reduction of the growth kinetics in presence of copper (Fig. 4)

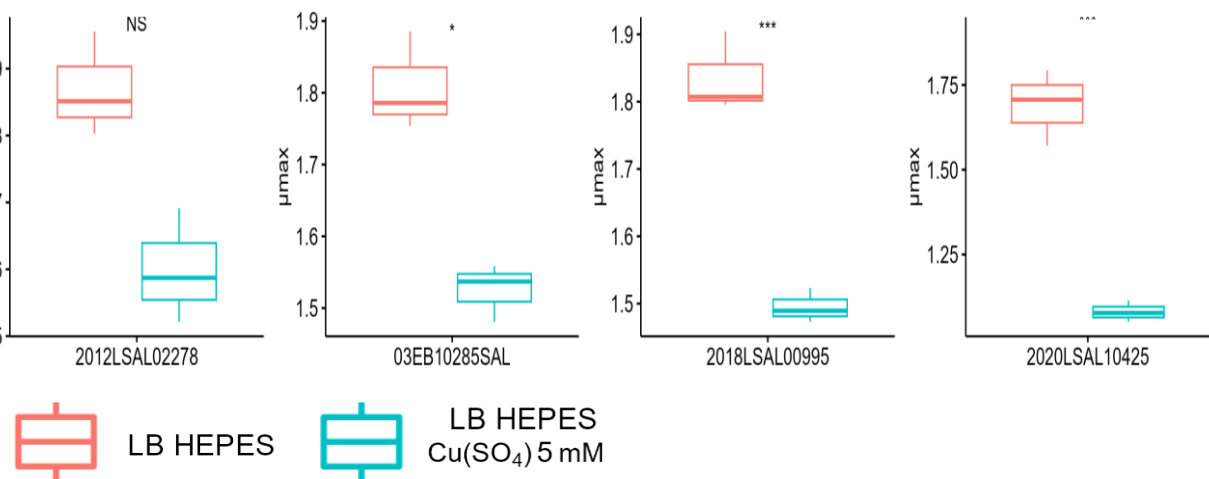
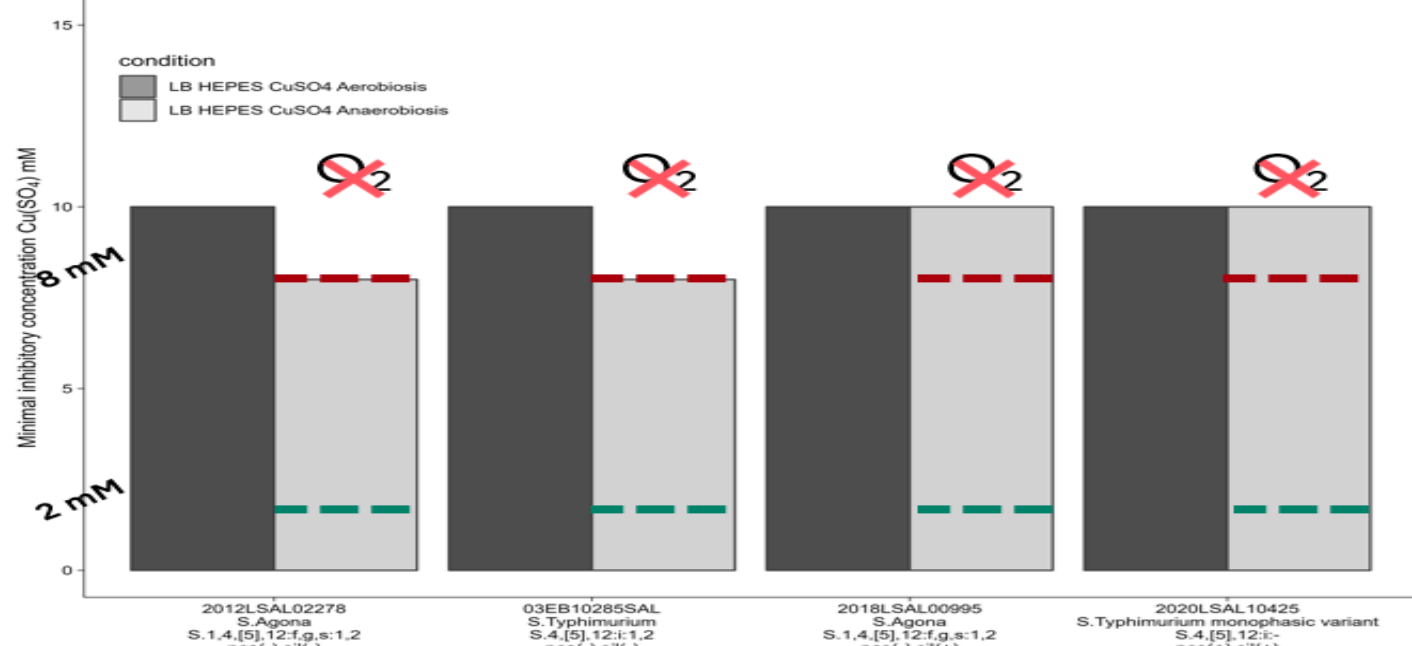


Figure 4. Means of growth rate for strains in basal and copper complemented medium. In red basal condition (LB HEPES); in blue copper supplemented at 5mM (LB HEPES Cu 5mM).

In anaerobic growth condition with copper (CuSO₄) the 2 strains without *pco* and *sil* genes have a lower MIC (4mM CuSO₄) (Fig. 5)



a reduction of the growth and an accumulation of intracellular copper (Fig.6)

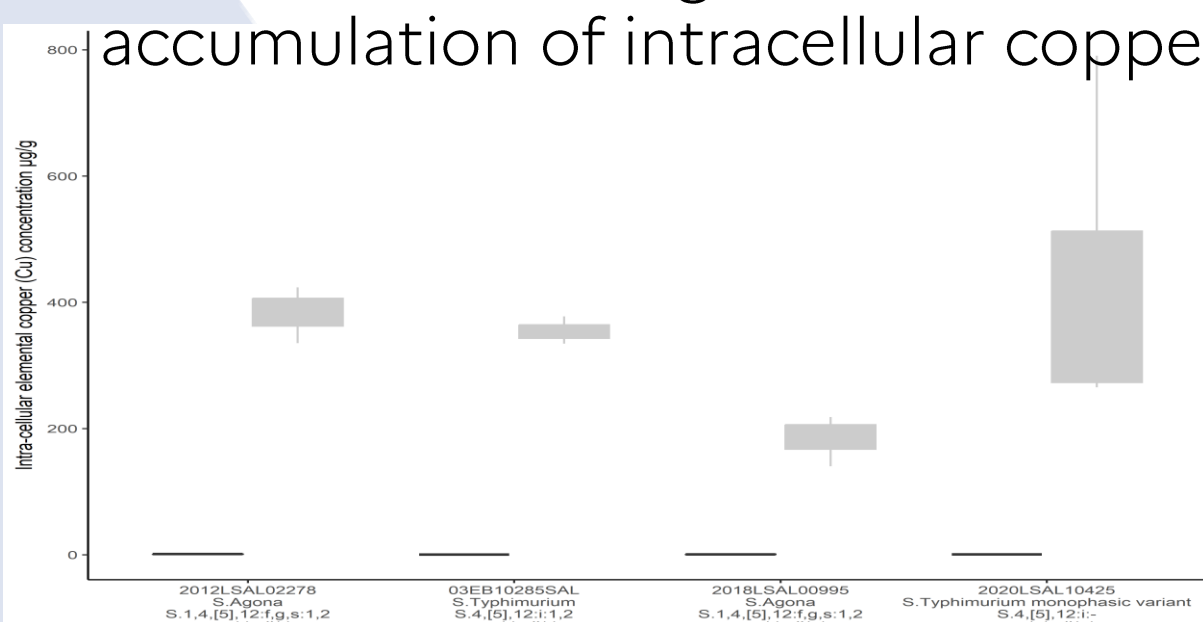


Figure 6. Intracellular copper concentration of the 4 strains was quantified by mass spectrometry.

The presence of *pco* and *sil* clusters did not affect the MIC, growth kinetics and mass spectrometry analyses in aerobiosis. However, in anaerobiosis, strains carrying *pco* and/or *sil* clusters were advantaged with both higher growth resistance rate and greater biomass production suggesting that copper resistance likely plays a role during intracellular life of *Salmonella* in the host.

3. Transcriptomic approach

- Within the 52 genes identified, the 19 involved in copper metabolism and resistance were prevalent within the 168 genomes analyzed. Interestingly, *S. Senftenberg* carry 25 of the 89 SGI-4 genes with both *pco* and *sil* operons and some *S. Agona* strains (n=3/10) encoded *sil* cluster, confirming the mobile nature of these genomic elements.

- The transcriptomic analysis of *pco* and *sil* genes confirmed their involvement in copper resistance, especially *sil* operon (Fig. 7)

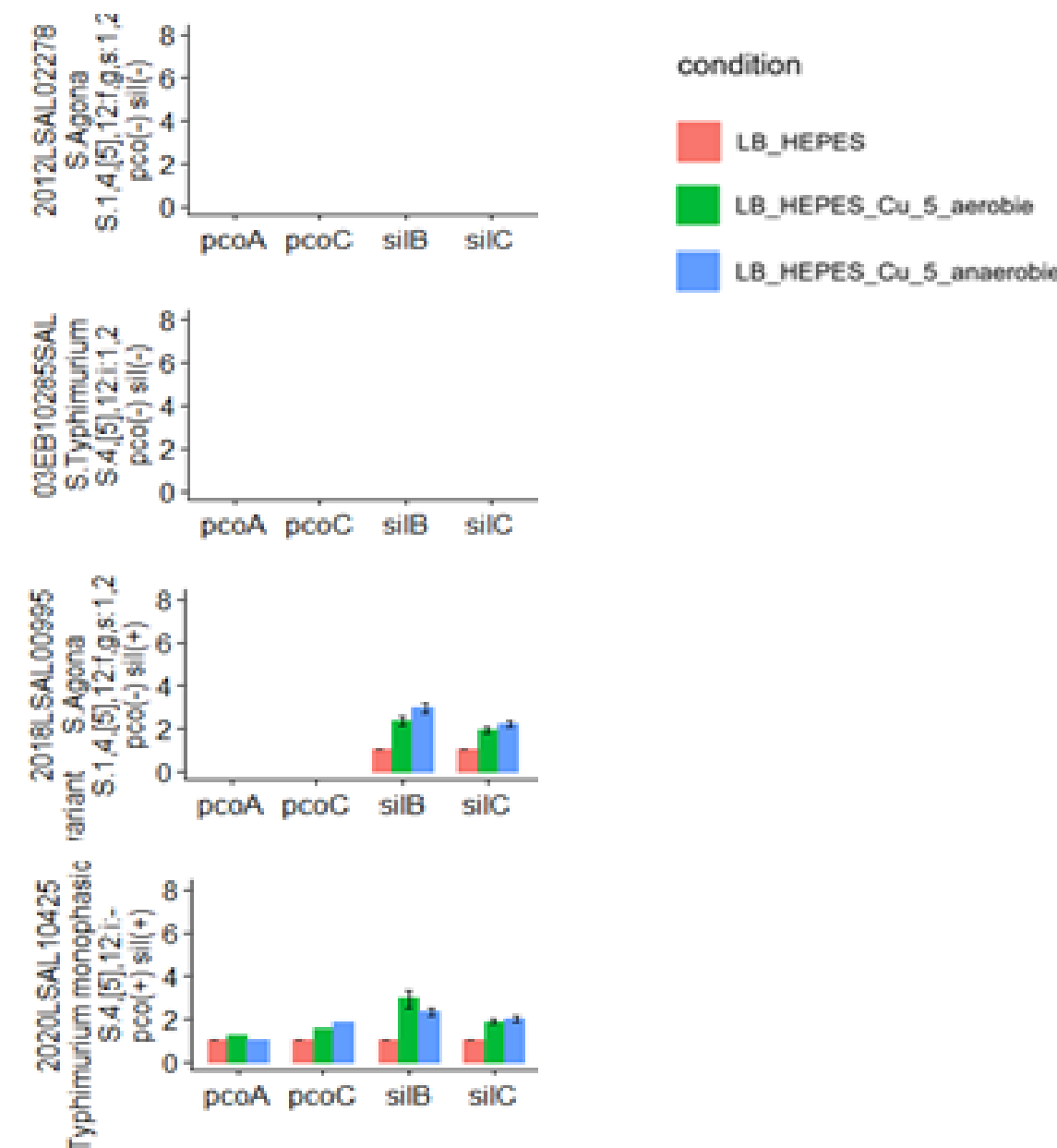


Figure 7. Relative expression of 4 of the 19 genes in copper supplemented LB HEPES under aerobiosis, anaerobiosis regarding basal condition. In red basal condition (LB HEPES in aerobiosis), in green copper supplemented medium in aerobiosis and in blue copper supplemented medium in anaerobiosis. No data means that the gene was absent in the genome.

Finally, our results underlined that the copper resistance appears to be a key factor in the survival of *Salmonella* in the host and suggest that relationship between heavy metal resistance and antibiotics for *Salmonella* would deserves further study.

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References or website-links:

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