Characterization of *Lactobacillus reuteri* isolates to inhibit *Campylobacter* colonization in chicken

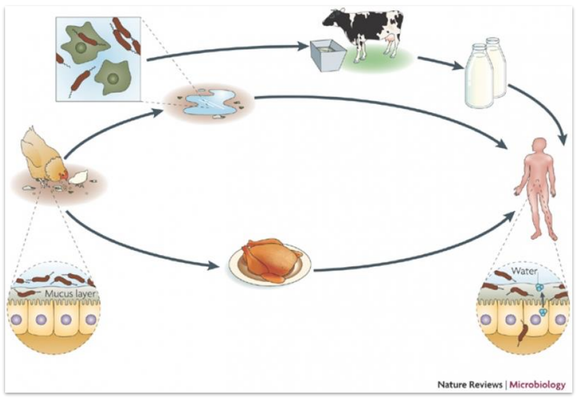
Paul Tetteh Asare[1], Clarissa Schwab[1], Christophe Lacroix[1] , Anna Greppi[1]*

[1]Institute of Food, Nutrition and Health. Laboratory of Food Biotechnology, ETH, Zurich
*anna.greppi@hest.ethz.ch

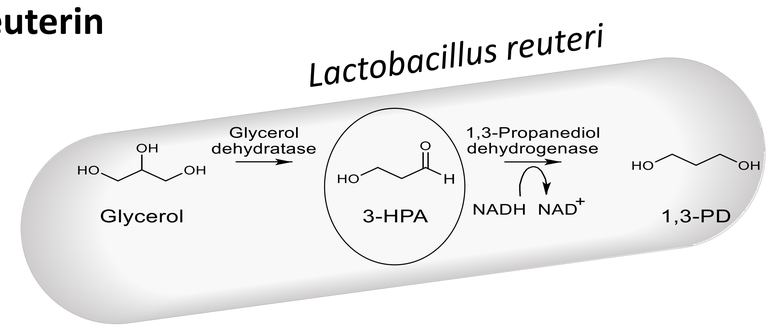
INTRODUCTION & HYPOTHESIS

Novel approach to control *Campylobacter* contaminations in chicken: reuterin

73 % of chickens worldwide test positive for *Campylobacter*



Campylobacter infection cycle



- GI-track *Lactobacillus*
- Forms biofilm in the crop of chicken
- Produces reuterin
- Reuterin is a broad spectrum antimicrobial

Reuterin system: antimicrobial

***In ovo* administration to prevents *Campylobacter* colonizazion**

APPROACH

L. reuteri isolation

- Chickens sampled in 6 different farms in Switzerland and bacteria isolated from crop and faeces
- *L. reuteri* isolates confirmed by 16S rDNA gene sequencing (bak4, bak11w)

Reuterin production characterization

- Reuterin production efficency verified by PCR on *pduC* gene, using degenerated primers (pduC-F and pduC-R) **[Fig. 1]**
- Reuterin-production confirmed *in vitro* by a colorimetric method: reuterin + DI-tryptophan + concentrated HCl **[Fig. 2]** and quantified by IC-PAD **[Tab. 1 & Fig. 3]**

Reuterin vs *Campylobacter*

- Minimum inhibitory concentration (MIC) values of reuterin against *Campylobacter coli* and *Campylobacter jujeni* chicken isolates quantified by micro broth dilution methods **[Fig. 4]**

Strains characterization

- *L. reuteri* isolates grouped by ERIC-PCR (ERIC1R and ERIC2) **[Fig. 5]**
- Sensitivity of isolates to 8 different antibiotics (AB) were tested with AB strips and microbroth dilution
- Presence of AB resistance genes tested by PCR with AB resistance specific primers

RESULTS

200 bacterial strains isolated from chicken crop and faeces, 70 *L. reuteri* → 51 *L. reuteri* isolates produce reuterin → 25 reuterin producing *L. reuteri* strains → 4 *L. reuteri* strains with high reuterin production and desireble AB resistance proile were selected.

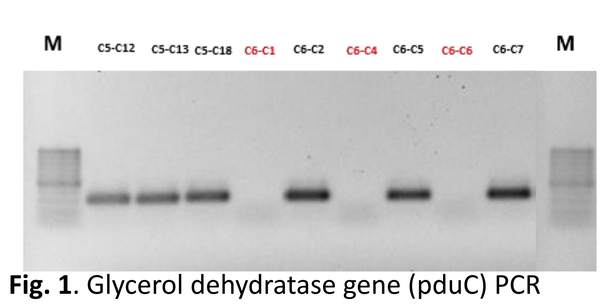


Fig. 1. Glycerol dehydratase gene (pduC) PCR

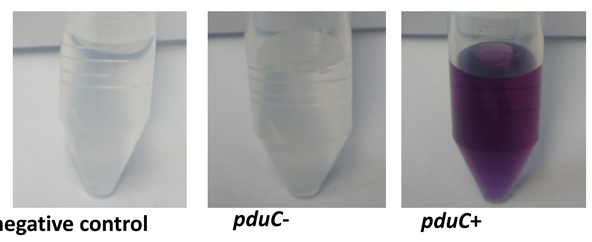


Fig. 2. Colorimetric method to detect reuterin production by *L. reuteri* isolates. *pduC- /pduC+* represents the strain not/habouring the gene responsibe for reuterin production

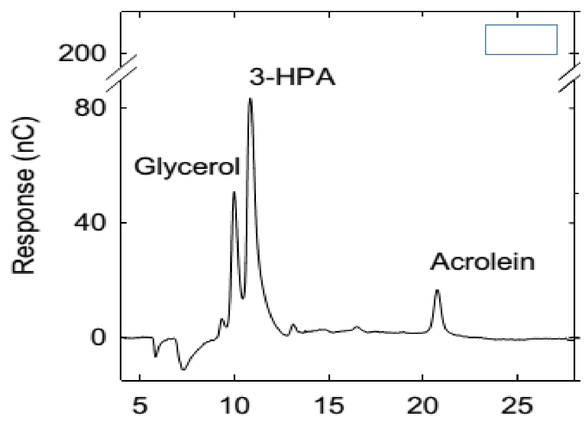


Fig. 3. Chromatograph of reuterin quantification using IC-PAD

(3-HPA)	< D.L.	100 mM	100-200 mM	>200 mM
N° isolates	11	14	21	**5**

Tab. 1. Reuterin (3-HPA) production by *L. reuteri* strains measured by IC-PAD and number of strains per group

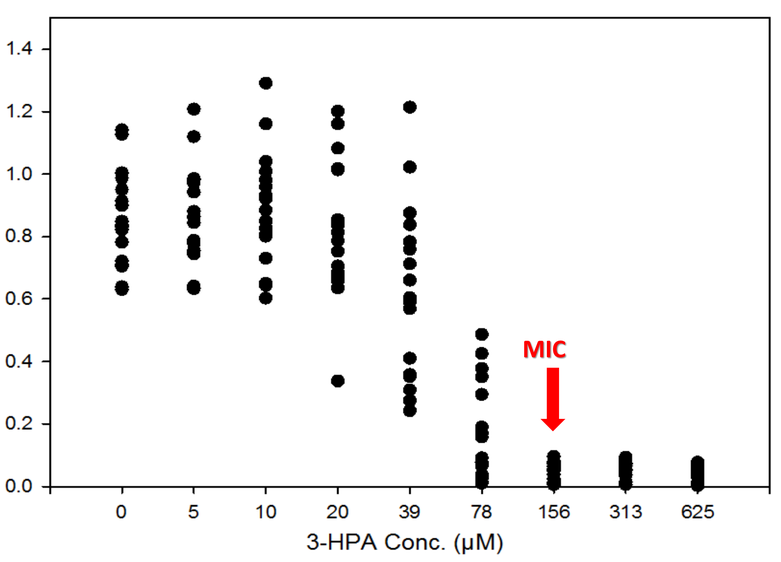


Fig. 4. A scattered plot of MICs in the micro-broth dilution test against 17 C*ampylobacter* species (13 *C. jejeni* and 4 *C. coli*). Each dot represents the growth of one *Campylobacter* strain

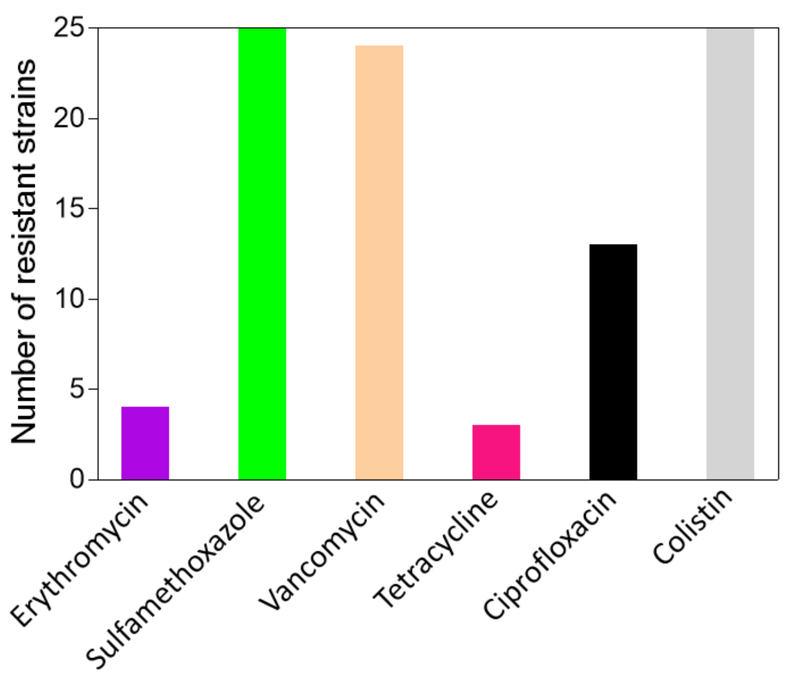


Fig. 6. Antibiotic resistance profile of 25 isolates of *L. reuteri* from Swiss chicken gut against 6 critically important antibiotics. All isolates were resistant to sulfamethoxazole and colistin.

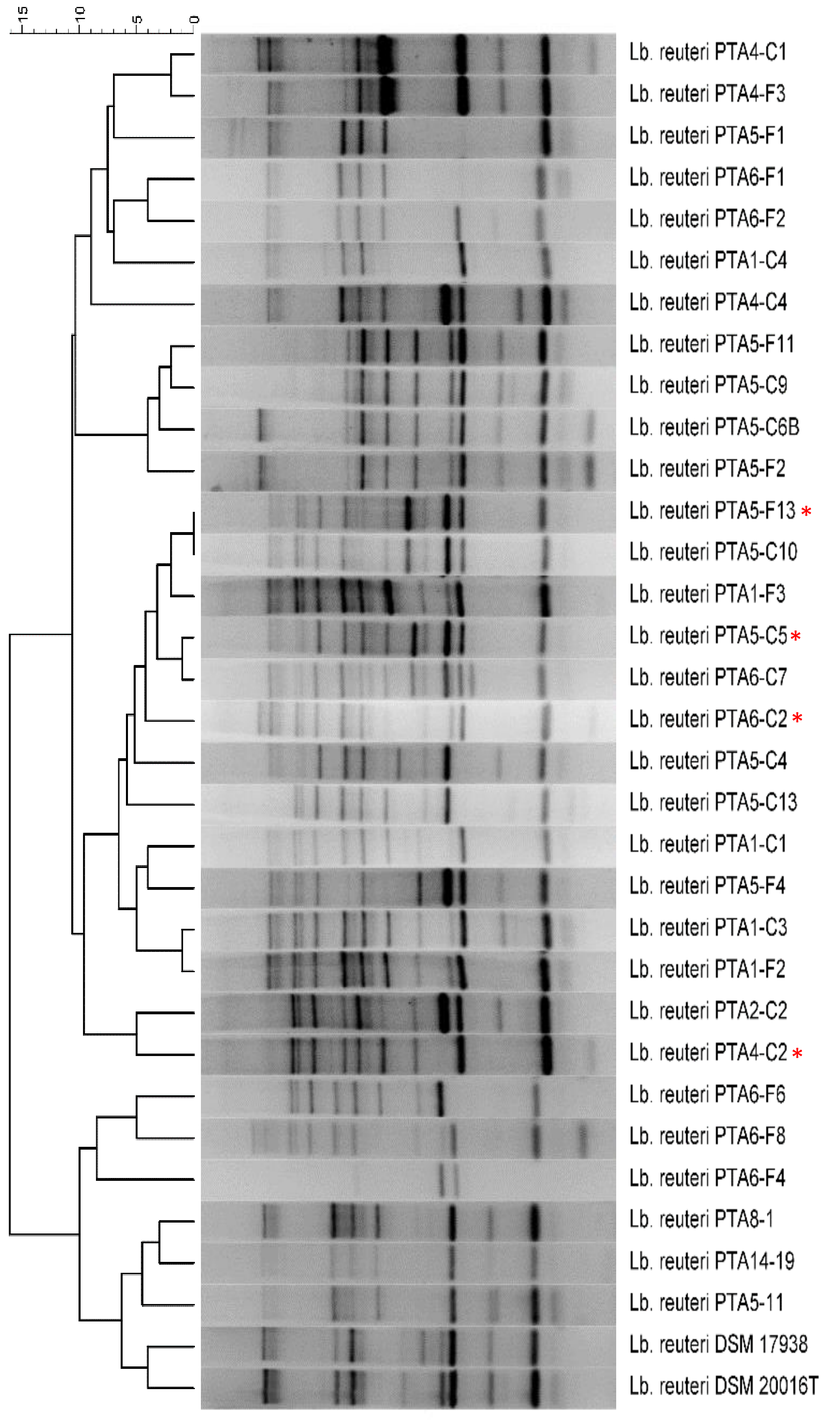


Fig. 5. ERIC-PCR profile of representative *L. reuteri* isolates from chicken crop and faeces demonstrating 25 groups.
* = selected isolate

CONCLUSION

Fifty-one (51) reuterin producing *L. reuteri* were isolated from chicken gut. Based on their ERIC-PCR and resistance profiles against several critically important antibiotics, 4 strains were selected to be used for the next stage of the project. They were sensitiive to **erythromycin, penicillin, tetracycline** and **colistin** and high reuterin production efficiency (150mM from 600 mM glycerol). Whole genome sequence of these strains is currently on-going. *Campylobacter coli* and *jejun*i exhibited high sensitivity to reuterin with MIC between 156 µM 3-HPA. Our results demonstrate the presence of reuteri-producing *L. reuteri* in chicken gut with the potential to produce reuterin that can inhibit the grwoth of *Campylobacter spp*. This property will be tested using the in-vitro gut model (PolyFermS) developed in our laboratory.

Acknowledgments: Tarnutzer Carmen