Isolation of lactic acid bacteria from chickens that demonstrate probiotic properties of autoaggregation and coaggregation with *Salmonella enteritidis*.

S. Savvidou, J.Beal, P.Brooks, University of Plymouth,* School of Biological Sciences, Drake Circus, Plymouth, PL4 8AA, UK, e-mail: *soumela.savvidou@plymouth.ac.uk*

Introduction

The association of salmonella infections with the consumption of poultry products and the fact that in the live bird the *Salmonella* carriage is mainly asymptomatic have led to a demand to find ways of preventing infection of commercially reared poultry and product contamination. One approach is the use of probiotics. Their capacity for adhesion to mucus, ability to autoaggregate and potential for coaggregation with pathogenic bacteria are possible mechanisms for providing competitive advantage in the intestinal microbiota and forming a barrier that prevents colonization of pathogenic microorganisms.

Methodology

•53 lactic acid bacteria (LAB) of chicken origin were isolated from the contents of the crop, caecum and small intestine, and from the mucosa of the crop, jejunum and ileum of three organically farmed chickens.

•**Autoaggregation**: 24h cultures of LAB were centrifuged, washed three times with sterile distilled water, resuspended in PBS and incubated at room temperature in the presence of 10% (v/v) freshly prepared filter-sterilised own supernatant fluid. Autoaggregation was taken as positive when clearly visible sand-like particles (formed by the aggregated cells) gravitated to the bottom of the tubes, leaving a clear supernatant fluid, within 2h.

•**Coaggregation** with *Salmonella enteritidis*: 500µl of each LAB suspension were mixed with 500µl of *S. enteritidis* suspension and incubated in vials for 4 h at 37°C under agitation. After 4 hours aggregation the suspensions were scored as above and were prepared for observation by scanning electron microscope. Six randomized fields were evaluated in each sample.

•LAB strains were identified using API CHL kit. BioMéreux (UK) .

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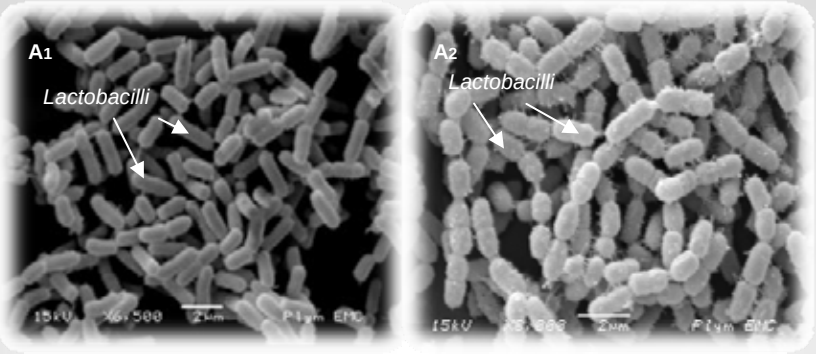

Figure A. Aggregation of two Lactobacillus strains **A1**. Normally aggregating *Lactobacillus sp.* **A2**. Rapidly aggregating *Lactobacillus sp.*

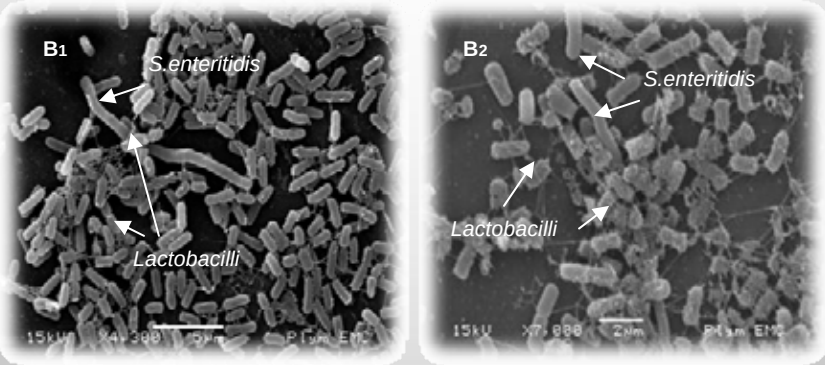

Figure B. Aggregation of two lactobacillus strains with *S.enteritidis* showing strands of LAB aggregating protein adhering to *S. enteritidis*. **B1**. *Lactobacillus sp.* showed partial aggregation **B2**. *Lactobacillus plantarum* showed maximum aggregation .

Results

•20 LAB were non-aggregative.

•11 LAB showed a rapid autoaggregation, within 15minutes.
•12 LAB had a normal reaction and the rest of the strains showed weak autoaggregation activity.

From the 23 LAB that showed normal and rapid aggregative activity:

- 3 LAB showed **no, or almost no, aggregation**
- 9 LAB showed **partial aggregation**
- 6 LAB showed **good aggregation**
- 2 LAB showed **marked aggregation**
- 1 LAB **maximum aggregation**

Conclusions

•Interaction between the origin of the LAB, the intensity of autoaggregation and coaggregation with *S. enteritidis* was not found.

•The *Lactobacillus* strain that has been identified as ***Lactobacillus plantarum*** and has been found to have a rapid autoaggregation activity and a maximum ability to aggregate with *Salmonella enteritidis* will be subjected to further screening for its potential use as a probiotic in chicken nutrition.