

SOY BY-PRODUCTS REUTILISATION: USE OF NATIVE LACTIC ACID BACTERIA WITH PROBIOTIC ATTRIBUTES

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Argentina is becoming a central soy producing country, and waste generated from production and industrialisation is of great environmental concern. Okara (Ok) and tofu serum (TS), having high nutritive and nutraceutical value (high quality proteins, isoflavones, prebiotics), are abundantly discarded by the soy industry as by-products.

Lactic acid bacteria (LAB) are considered safe for human and animal consumption, are traditionally used in the food and feed industries and have a health promoting -probiotic effect for their host. Therefore, we explored the bioconversion of highly contaminating soy by-products by using selected native LAB strains.

Twelve of 356 (3%) LAB isolates from faeces of weaned piglets with good sanitary status from Argentinean farming operations (INTA-p 10, 46, 47, 64, 68, 76, 105, 160, 172, 174, 235, 320) were selected by their probiotic and technological attributes (acidification kinetics and lactic acid production, inhibition and co-aggregation with entero-pathogenic bacteria, porcine bile and low pH resistance), and compared with the commercial LAB strain marketed as probiotic (here named 'strain X') and ATCC LAB reference strains. Bacteria were inoculated into: i) Man Rogosa Sharpe (MRS, LABs reference for positive growth control), ii) TS 100%, iii) TS + Ok 5% w/v, and iv) TS + Ok 5% w/v + whey (1:1; providing 2.5% lactose for LAB nutritional requirements). Sterilized media (200mL; 121°C/15min, soy a-nutritional factors inactivated; pH: 5.5-5.8) were inoculated with pre-activated LAB monocultures ($1.0-5.0 \times 10^9$ CFU), and growth monitored at different time points (t=0, 4h, 24h, 48h, 72h, 7d, 14d, 21d, 28d) by turbidity (od 600nm), viable counts (UFC/mL), pH (ELECTRONICA S.A. pH meter) and metabolic activity (lactic acid production; Reflectoquant kit).

Selected strains were able to grow in all tested media (especially in iii and iv), at comparable levels of MRS, with minor differences among strains, and better than LAB reference strains. These results show the possibility of using selected native LAB strains in soy by-products biotransformation.

Additionally, the sanitary properties of fermented products (media iii and iv) were analysed by challenging them with piglets diarrhoeal isolates (1.0×10^5 UFC): two *Salmonella* (*S. typhimurium* and *S. choleraesuis*) and two β -hemolytic *Escherichia coli*. High inhibition and a bactericidal mode of action were observed even at short incubation times (4h for *Salmonella*), higher than the commercial and ATCC strains, and comparable to the inhibition produced by antibiotics normally used in pig production (Chlortetracycline and Penicillin). Pathogens did not grow after being inhibited even after long incubation periods.

We show encouraging results for using selected native LAB strains (not genetically modified, isolated from autochthonous natural sources) as a cheap, easily transferable, environmentally friendly and with no public health hazard for bio-transforming agro-industrial wastes into high quality food and feed, important for minimizing antibiotics usage in farming operations. At the beginning of the food chain, this technology offers a good means to improve human, animal and environmental health.

Soy by-products re-utilization :

use of *native* Lactic Acid Bacteria with Probiotic attributes.

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CIPAF

CENTRO DE INVESTIGACIÓN Y DESARROLLO
TECNOLÓGICO PARA LA PEQUEÑA AGRICULTURA FAMILIAR



Gustavo Tito – Director IPAF Región Pampeana

"Agricultura Familiar es sinónimo de arraigo"

Lo afirmó en el marco del Tercer Encuentro del Mercosur Ampliado de Máquinas y Herramientas para la Agricultura Familiar que se realizó en el INTA Castelar del que participaron más de 100 Pymes de todo el país.

Dr. Gustavo TITO
Director , **IPAF PAMPEANA**

**" Family Farming is synonymous
of farmers be rooted in Their lands "**



CIPAF

CENTRO DE INVESTIGACIÓN Y DESARROLLO
TECNOLÓGICO PARA LA PEQUEÑA AGRICULTURA FAMILIAR

In Argentina (LAmérica), Family Agriculture generates
65% of the employment in rural areas.



Sur - Sur Interchange
República de Namibia
(22-23.11.2012)



"ProBIO INTA-nativa"

LAB



STRAIN COLLECTION
LABs INTA-nativas

SELECTED STRAINS

native
LABs

native
LABs



BIO-SILAGE

LIVESTOCK

PRODUCTION UNITS

FARM TO FORK



ProBIO INTA-nativa

Use Native Probiotics

- 1- at the beginning of the animal food chain.
- 2- re-conversion of agro-industrial, rural and urban waste/by-products into high quality (functional, probiotized and safe) animal feed.



native LABs
(Lactic Acid Bacteria)



Animal Health and Performance



Environmental Cares
of the Production Unit (*and further...*)



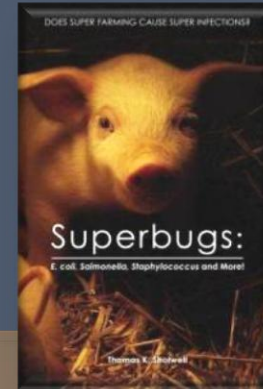
(animal) Agri-Food Safety



AGROPECUARY
INTENSIFICATION

(Antimicrobials)

↓ SANITATION



- ↑ **Antimicrobial resistance**
- ↑ **Virulence**
- ↑ **Food-borne disease**
- ↑ **Environmental pollution**

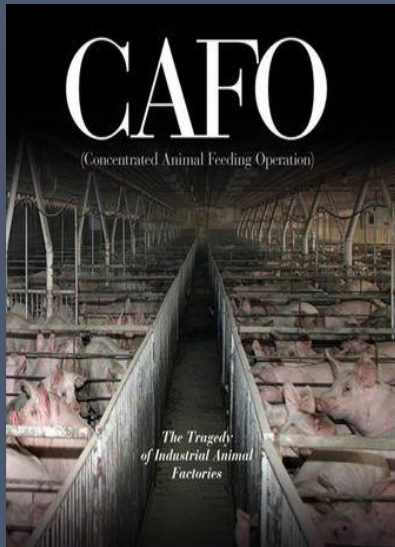
SUPERBUGS: *E. coli*, *Salmonella*,
Staphylococcus (and more is coming...)



80% OF ALL
antibiotics are used on
FACTORY FARM animals



Antibiotic resistance in the natural environment is rising 12.2009



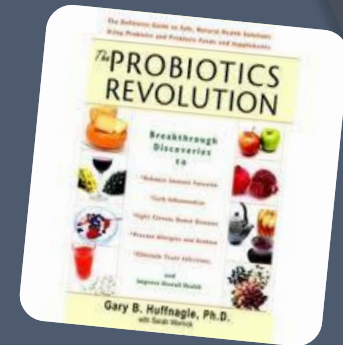
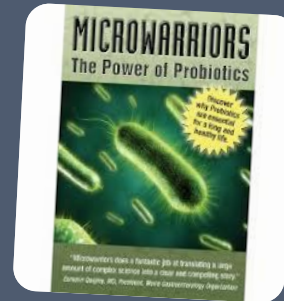
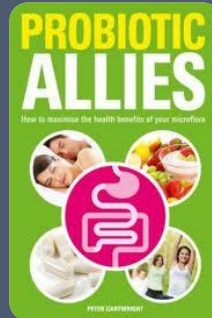
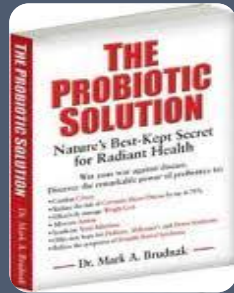
CAFO (Concentrated Animal Feeding Operation) :
The Tragedy of Industrial Animal Factories 07.2011



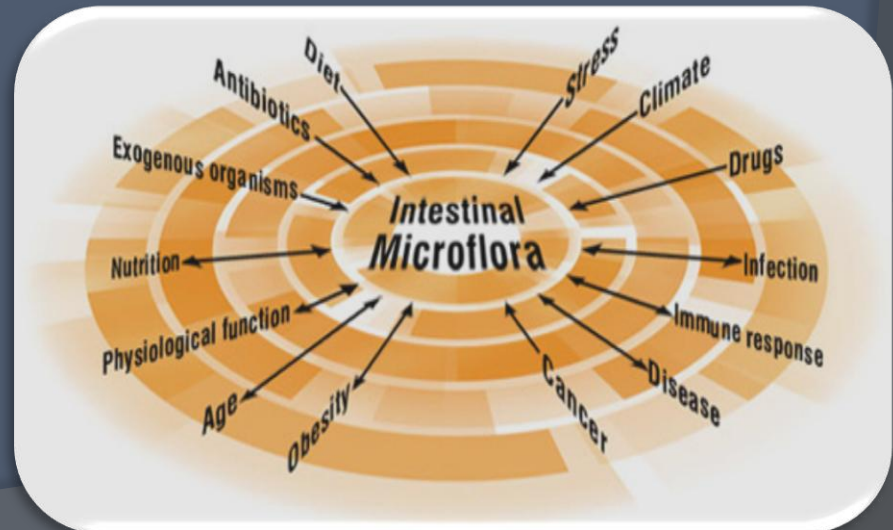
“CONTAGION' CONNECTIONS” : **links among Humans, Animals
and the Environment. A new class of infectious diseases.** 09.2011

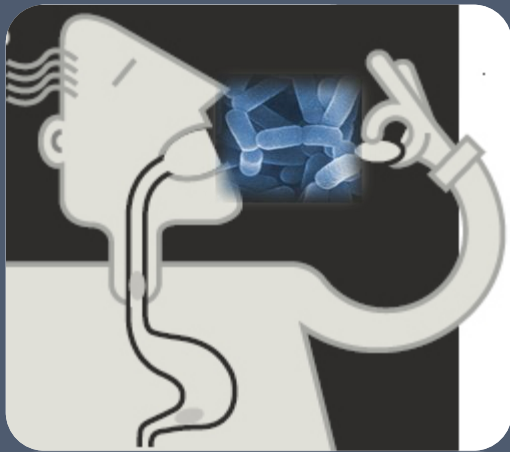
New Superbug Threat from Intensive Pig Farms 09.2012

Encouraging Research of Antimicrobials Alternatives



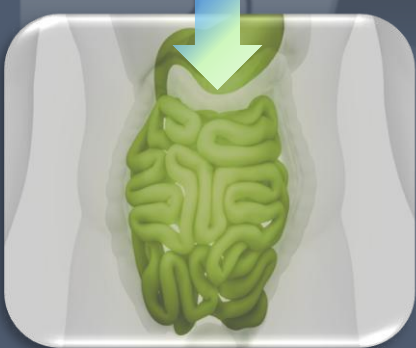
Gastrointestinal Microbiota





“PROBIOTIC CONCEPT”

- microorganisms (**Lactic Acid Bacteria**, **Bifidobacteria**),
- naturally present in the digestive tract (human / animal),
- along lifetime,
- positively acting on the host's health.



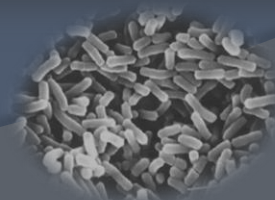
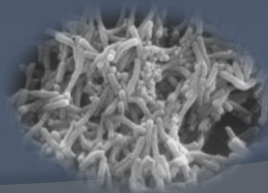
- Strengthen the body's defenses
- Collaborate with beneficial intestinal bacteria
- Fight against bad bugs and their toxins (**NO RESISTANCE !**)
- Help digestion and nutrient absorption
- Prevent chronic diseases (ie. autoimmune, cholesterol, ...)



Microbia claimed to have probiotic attributes

Table 1.1 Microorganisms considered as probiotics. (By Baffoni and Biavati 2008, modified)

Lactobacillus	Bifidobacterium	Enterococcus	Streptococcus	Lactococcus
<i>L. acidophilus</i>	<i>B. adolescentis</i>	<i>E. faecalis</i>	<i>S. thermophilus</i>	<i>L. lactis</i> subsp. <i>cremoris</i>
<i>L. brevis</i>	<i>B. animalis</i>	<i>E. faecium</i>		<i>L. lactis</i> subsp. <i>lactis</i>
<i>L. casei</i>	<i>B. bifidum</i>			
<i>L. curvatus</i>	<i>B. breve</i>			
<i>L. fermentum</i>	<i>B. infantis</i>			
<i>L. gasseri</i>	<i>B. longum</i>			
<i>L. johnsonii</i>	<i>B. thermophilum</i>			
<i>L. reuteri</i>				
<i>L. rhamnosus</i>				
<i>L. salivarius</i>				
Propionibacterium		Yeast		Others
<i>P. freudenreichii</i>		<i>Kluyveromyces lactis</i>		<i>Leuconostoc mesenteroides</i>
<i>P. freudenreichii</i> subsp. <i>shermanii</i>		<i>Saccharomyces boulardii</i>		<i>Pediococcus acidilactici</i>
<i>P. jensenii</i>		<i>Saccharomyces cerevisiae</i>		



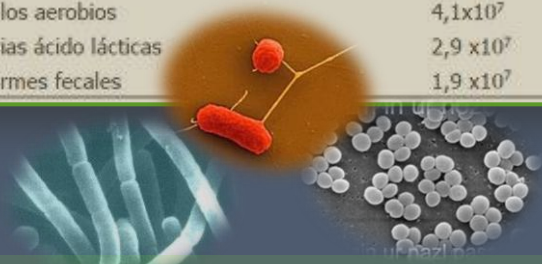
MILK → CHEESE → **WHEY**

by-product



nutrient rich

Parámetro	Promedio
Proteína soluble	8,7-9,4 g/L
Azúcares totales	44,4-47,3 g/L
Acidez	20,2-21,6 Dornic
pH	5,6-5,7
Mesófilos aerobios	4,1x10 ⁷
Bacterias ácido lácticas	2,9 x10 ⁷
Coniformes fecales	1,9 x10 ⁷



HIGH MICROBIAL LOAD
(**pathogens, zoonotic**)



Argentina bigger-high quality exporter
from LAmérica :

- **Infant food** (de-mineralized; \$\$\$)
- **Protein supplement** (\$\$\$)



THOUSANDS LITERS released daily
on the fields and bodies of water ...



FERTILIZING
(**WHEY + FECES**)



HIGHLY CONTAMINANT !

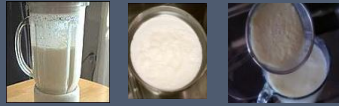
SANITARY PROBLEMS !

SOY BEAN → TOFU → **TOFU SERUM**

by-products



→ SOY MILK → **OKARA**



BOIL (a-trypsin de-activated)

100 g of wet **OKARA** *

Water	81.6 g
Protein	3.2 g
Carbohydrate	12.5 g
Fiber	4.1 g
Calcium	80.0 mg
Iron	1.3 mg
Thiamin	0.02 mg
Riboflavin	0.02 mg
Niacin	0.1 mg

* **TOFU SERUM** : similar composition

Uses of **OKARA** :

- Animal Feed
- (Food _ Japan, China, ...)
- (Fertilizer)

Same barriers for using

OKARA, TOFU SERUM and **WHEY** :

- RAPID DEGRADATION
- HIGH COSTS FOR DRYING and FREEZING (\$\$\$)

OKARA , TOFU SERUM and **WHEY** FACING THE SAME PROBLEMS :

MUST BE PROCESSED QUICKLY, EFFICIENTLY (and ECONOMICALLY).

Fermented pig liquid feed: nutritional, safety and regulatory aspects

C. Plumed-Ferrer and A. von Wright

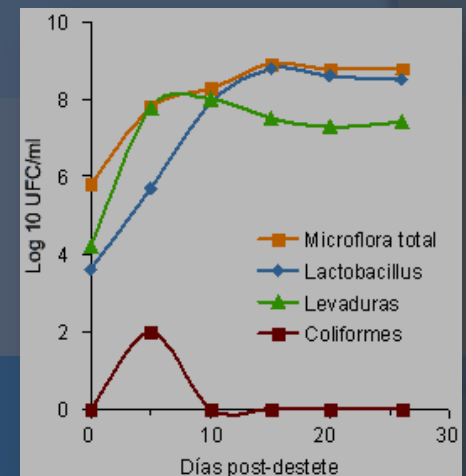
Journal of Applied Microbiology **106** (2009) 351–368

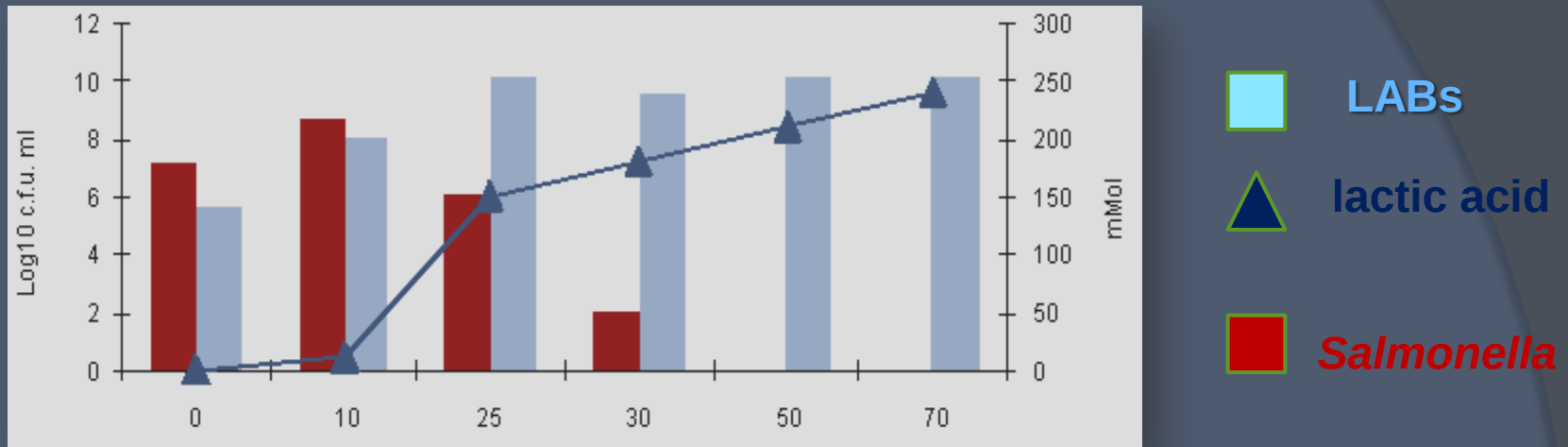
Last decade, **FERMENTED LIQUID FEED** by using **PROBIOTIC BACTERIA** has been much investigated as an **alternative for the antibiotics as growth promoters in animal production**, a practice been banned in the EU since 2006 .



“ PROBIOTIC ” FERMENTED LIQUID FEED :

- ▲ feed utilization, digestibility and growth performance (pigs) ;
- ▼ pathogenic bacteria in feces (*Salmonella*, *E. coli*, *Mycotox-fungus*, ...) ;
- ▲ intestinal cells health .





Pig production units are frequently contaminated with *Salmonella*.

Inoculation of **LABs** into the food (FLF) and/or directly applied to the animal reduce the presence of *Salmonella* and the production unit wastewaters.

Indigestible fraction of okara from soybean: composition, physicochemical properties and in vitro fermentability by pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

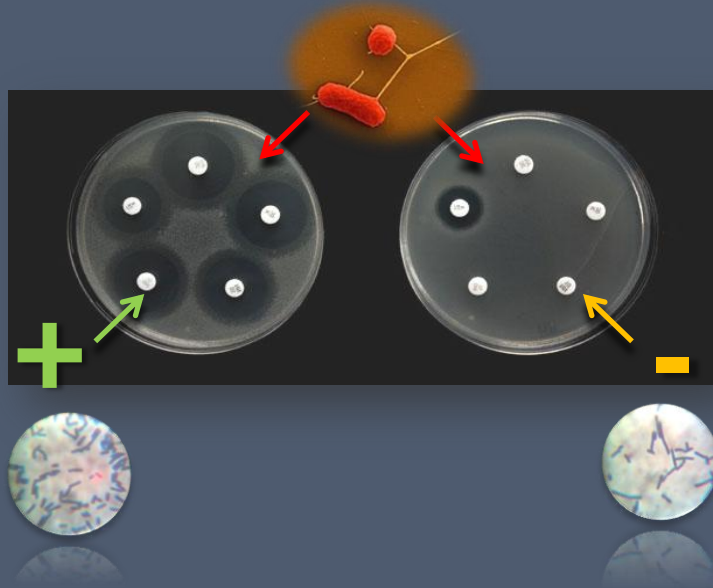
Irene Espinosa-Martos · Pilar Rupérez

Eur Food Res Technol (2009) 228:847

	<i>L. acidophilus</i>	
Fermentation time (h)	24	48
Fermentation parameters		
Total SCFA	1.08 ± 0.32*	0.76 ± 0.24*
Acetic:propionic:butyric ^b	57:41:0	82:15:0
Fermented substrate (%)	5.7 ± 1.6 [†]	6.1 ± 1.1 [†]
Lactic acid	0.7 ± 0.1*	0.6 ± 0.1*
Δ pH	-1.06 ± 0.01 [†]	-0.73 ± 0.07*

Selection of probio INTA-native LABs

✓ Sanitary properties: Inhibition against Super Bugs



Salmonella typhimurium

Salmonella choleraesuis

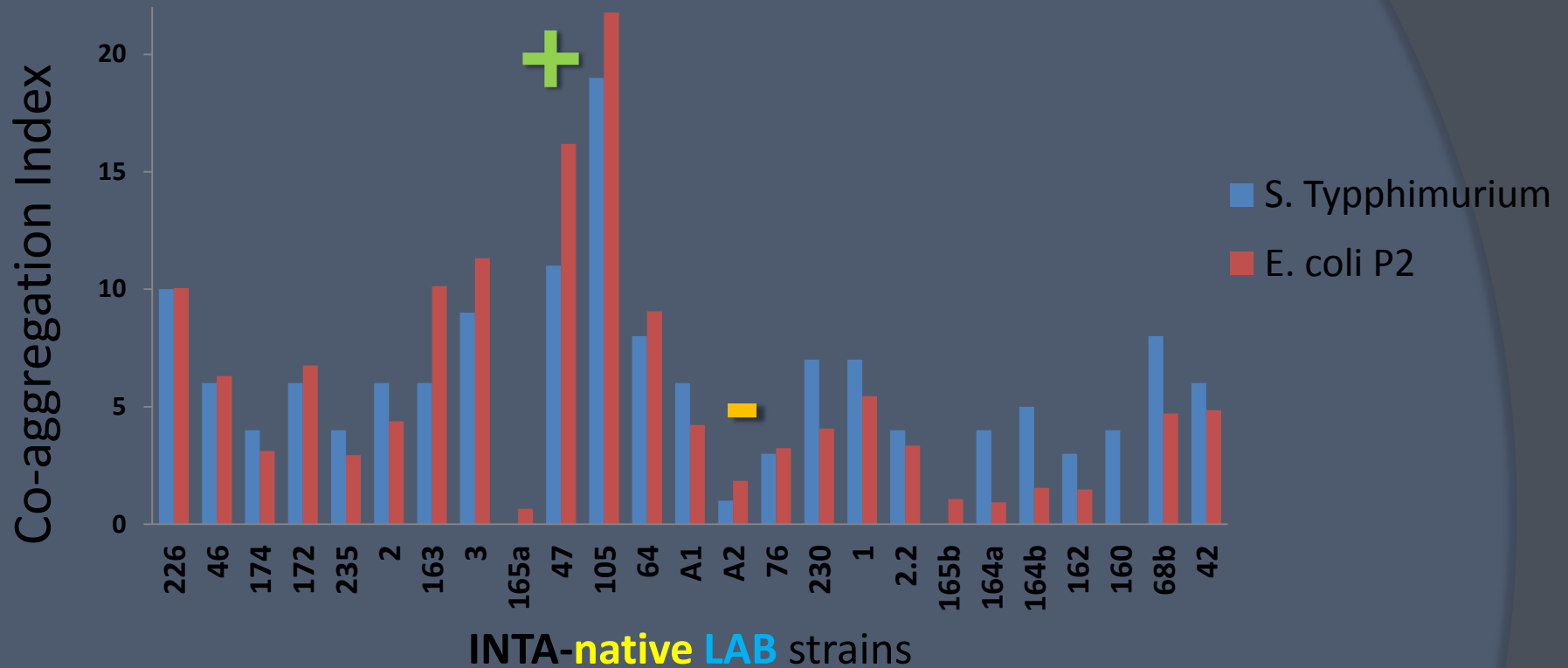
β -hemolytic *Escherichia coli* (2 strains)

- Field isolates (no laboratory strains)
- Pig deadly infected (active pathogens)
- Multi-Antibiotic Resistance

✓ Auto-aggregation



✓ Co-aggregation with Super Bugs



✓ Cell Surface Hydrophobicity

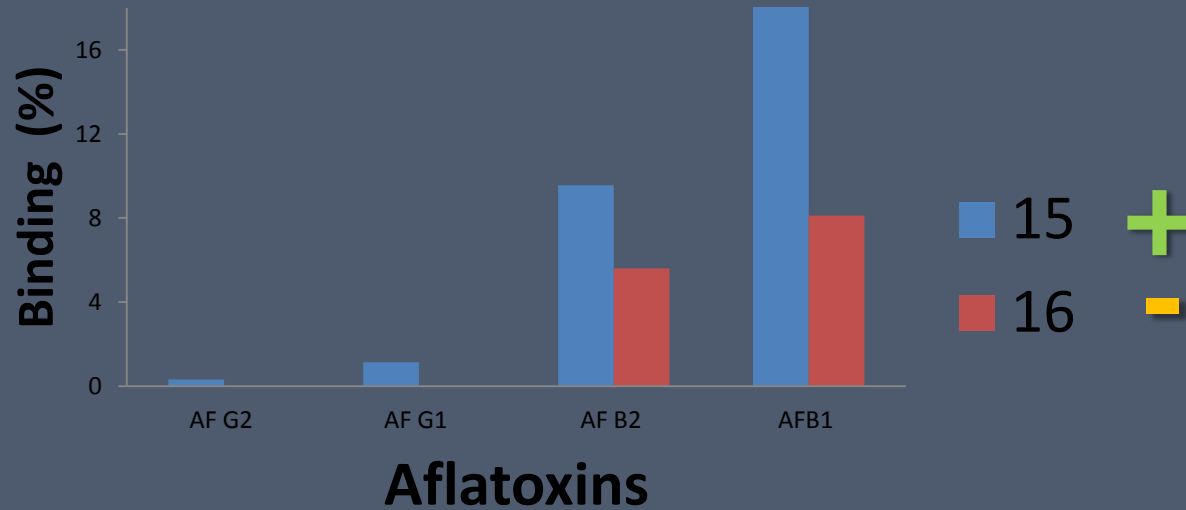


+



-

✓ Mycotoxin binding (Aflatoxins)



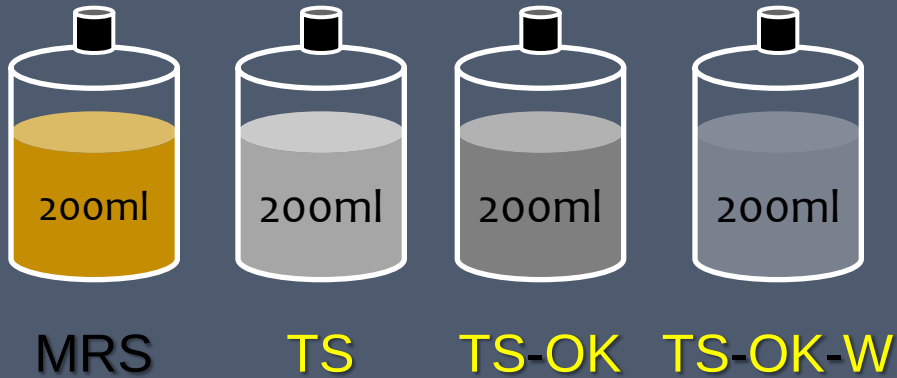
✓ Porcine bile resistance



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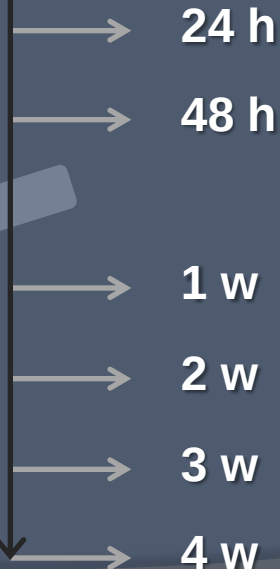
EXPERIMENTAL

10^9 UFC



- **INTA-native LAB** (13 selected strains)
- **LAB ATCC reference strains** (2):
 - Lactobacillus plantarum*
 - Lactobacillus fermentum*
- **LAB commercial strain** (probiotic)

- 30 C
- agitation



- **LAB** counts
- pH
- **Inhibition against "Super Bugs"** (4 strains)
- Lactic Acid determination

RESULTS

(published soon)

- **LAB ATCC reference strains** (*Lactobacillus plantarum* and *L. fermentum*) and the **LAB commercial probiotic strain** showed similar growth (yield rate and acidification speed) in the 3 investigated “by product” combinations, similar found in the MRS **LAB** reference media. (**LAB** : nutritionally fastidious bacteria).
- **INTA-native LABs** showed similar growth to the **LAB ATCC reference strains** and the **commercial probiotic** in the studied media (not significant differences found).
- **Interestingly:** selected **LABs INTA-native** strains (**INTAp** 16, 235, 300, 313, 312, 314 and 315) showed superior performance, growth and survival rate (reduction of 2.5 orders of magnitude in 4 weeks / 30 C).

- **TS** and **OK** containing media shown to have a protective activity on bacterial viability in the acidic-fermented state ($\text{pH} < 4.00$): bacterial counts at 1, 2 and 3 weeks were 2,5 order higher than in MRS.

- Selected **INTA-native LAB** strains (P4, 235, 297, 312, 300, 313, 314 and 315) showed almost a complete inhibition at 24 h fermentation against the all 4 pathogens tested (*Salmonella typhimurium* and *choleraesuis* , and 2 strains of β -hemolytic *Escherichia coli*), and even higher than the **commercial probiotic**.

Therefore: **this would ensure the sanitary status of the fermented by-products for animal nutrition (antimicrobials avoidance).**

- **Interestingly:** Inhibition had a bacteriostatic fashion in most strains in most strains, **but antibiotic-like bactericidal** selected strains will be studied further (**scaling up:** 20-200 L fermentations).



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