ADVANCES ON THE USE OF PROBIOTICS IN SHRIMP CULTURE

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TIMELINE PROBIOTICS

1900

Human application

1970

Modern concept

1980

Liquid products - Brewing

1st Aquaculture use (Kozaka 1986)

1990

Isolation of strains from farms

Vibrio antagonism (Moriarty 1998)

2000

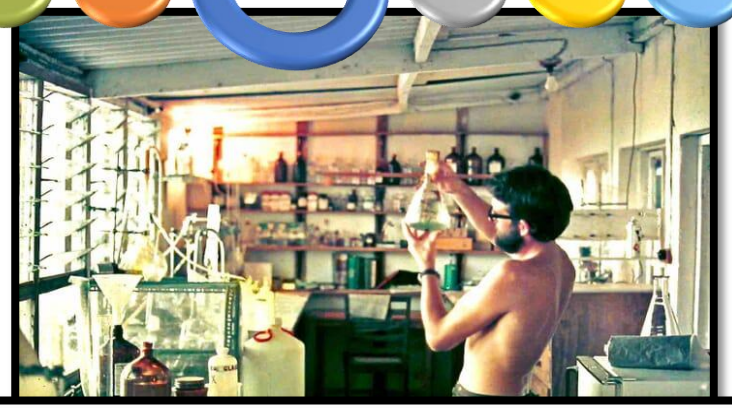
Applied molecular biology

Verschuere (2000)

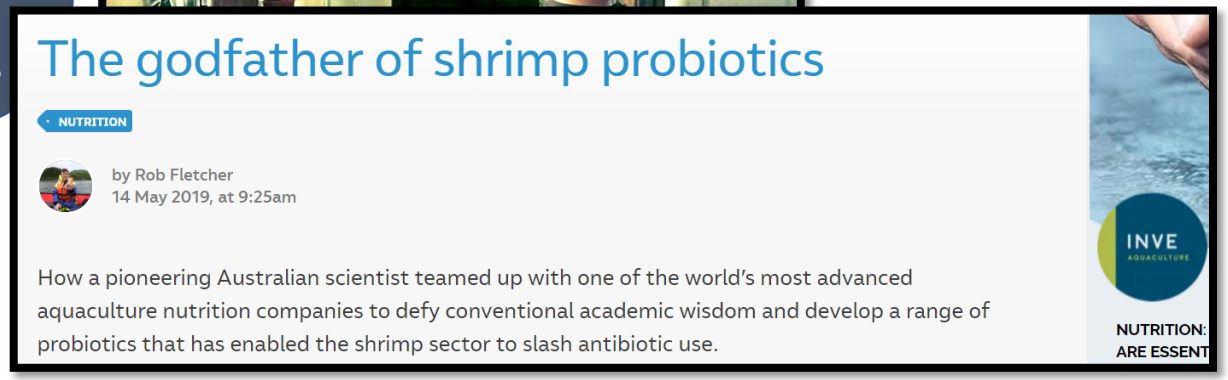
Widespread use

2019...

Next generation Probiotics?



Against the use of antibiotics!



The godfather of shrimp probiotics

NUTRITION

by Rob Fletcher
14 May 2019, at 9:25am

How a pioneering Australian scientist teamed up with one of the world's most advanced aquaculture nutrition companies to defy conventional academic wisdom and develop a range of probiotics that has enabled the shrimp sector to slash antibiotic use.

INVE AQUACULTURE

NUTRITION:
ARE ESSENT

RESEARCH ON PROBIOTICS

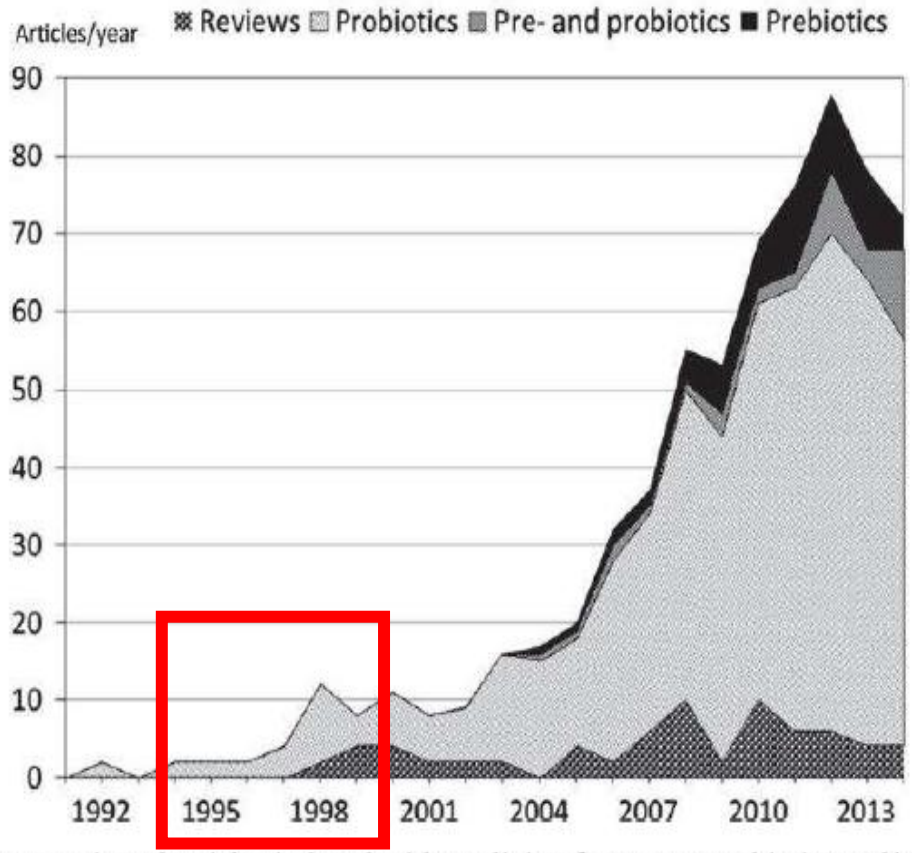
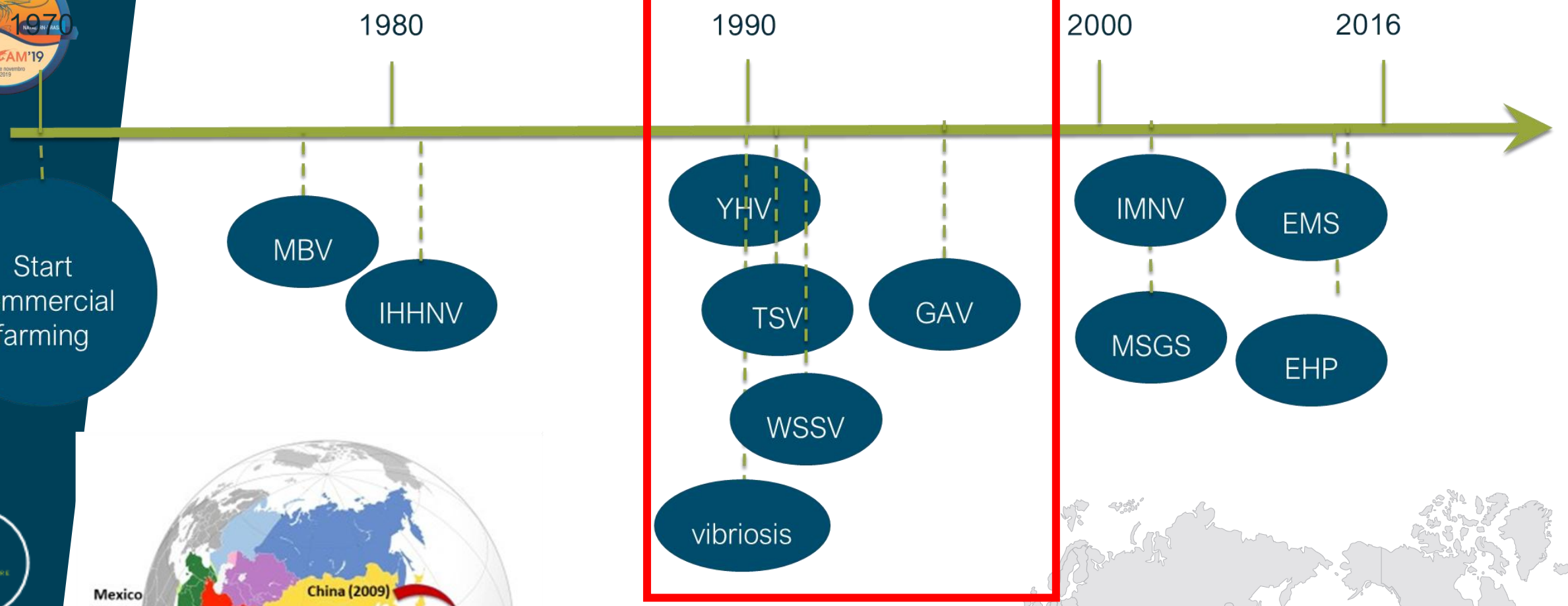
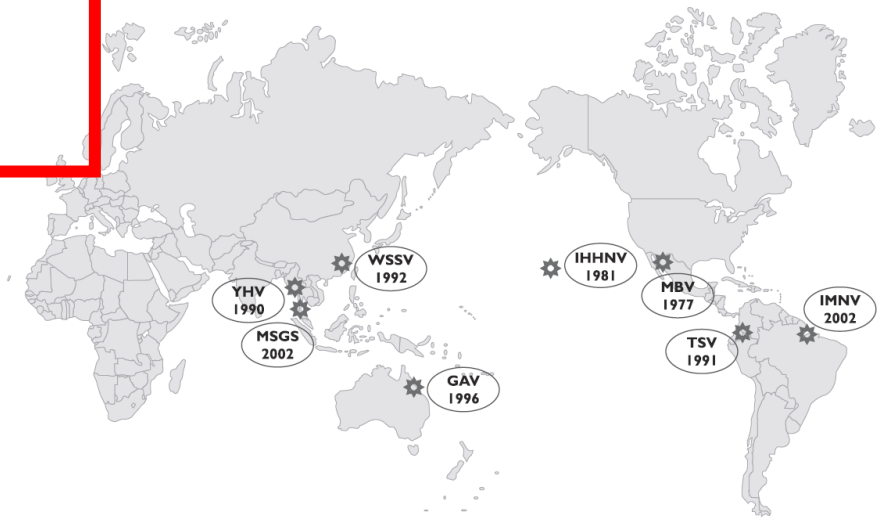


FIGURE 21.1 Annual production of peer-reviewed articles indexed with explicit reference to probiotics and/or prebiotics, and application to finfish or live feed organisms (rotifers and *Artemia*; search based on titles, keywords and abstracts). The total numbers of items published each year were broken down into specific reviews and experimental reports about either prebiotics, or probiotics, or both (which did not necessarily correspond to a synbiotic approach). The counts were stopped by the end of June 2014, and simply doubled for the last year (thus underestimated). The search was not exhaustive, but sufficient to illustrate the trend of fast increase in the recent years.

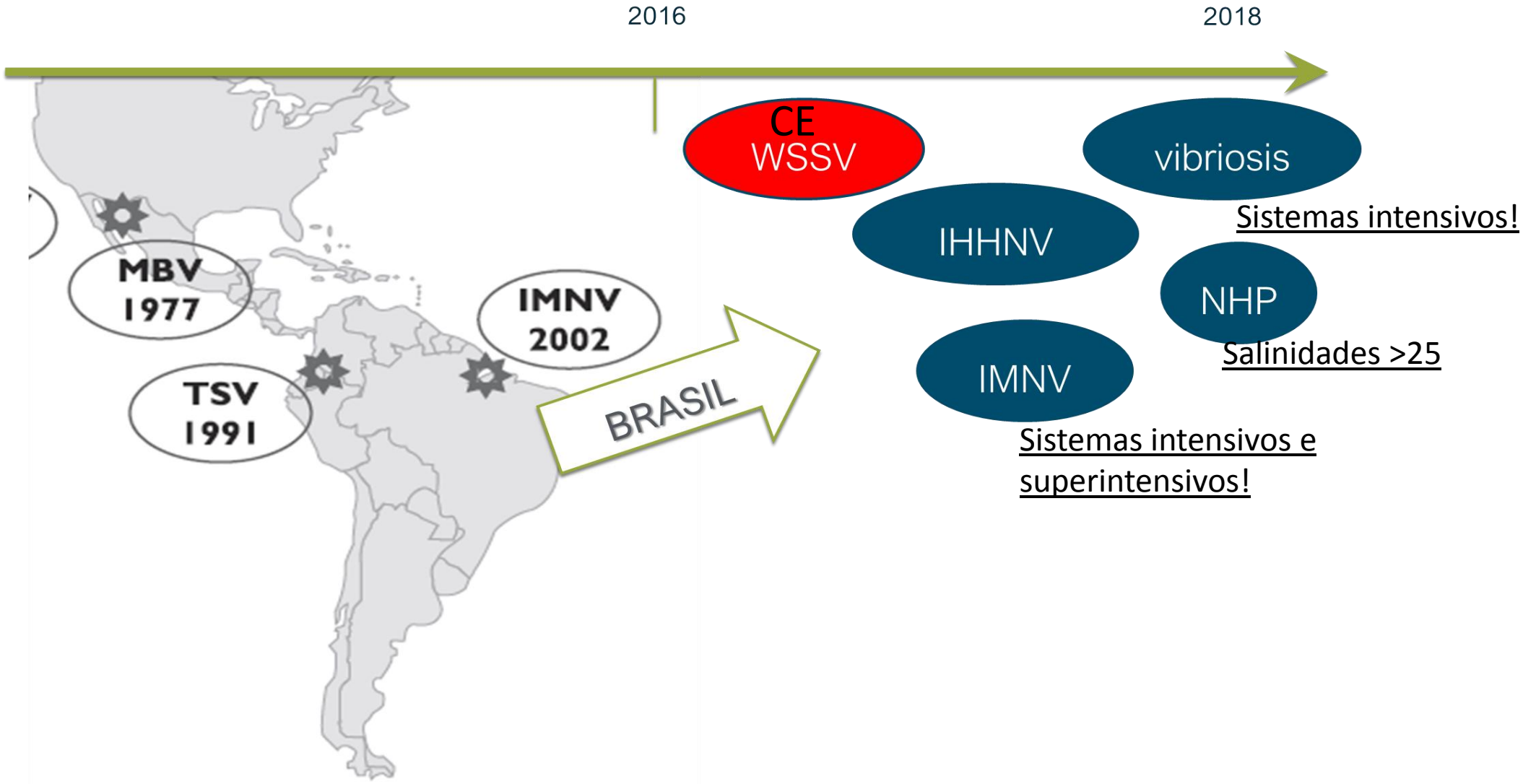
Gatesoupe 2016

PARALLEL WITH DISEASES





PARALLEL WITH DISEASES



DISEASES X FOOD SAFETY X USE OF ANTIBIOTICS

A North American panel of retail and foodservice experts agreed that antibiotic-free was already important to their customers, and was likely to remain a key concern.

"Consumers absolutely care about this," said Walmart's Trevyr Lester. "It may be hard to understand the details, but if you look at the marketplace there's true signals that antibiotic-free, all-natural these are rapidly-growing categories in our stores, and they are demands that are going to be difficult to meet without innovations in healthcare."

https://www.undercurrentnews.com/

DISEASES X FOOD SAFETY X IMPORT BANS

ECUADOR

Ecuador's economy shell-shocked by China shrimp ban

In September, Ecuador's economy was rattled when China banned its shrimp, the country's second most profitable product after oil

Share

Génesis Lozano October 4, 2019

Sales of shrimp from Ecuador to China were worth over US$1 billion between January and August this year but were suddenly halted in September for sanitary reasons, casting uncertainty over the trade.

EQUADOR

As três semanas que abalaram a lucrativa indústria do camarão equatoriano

Por questões sanitárias, China suspendeu o principal produto de exportação não petroleiro do país

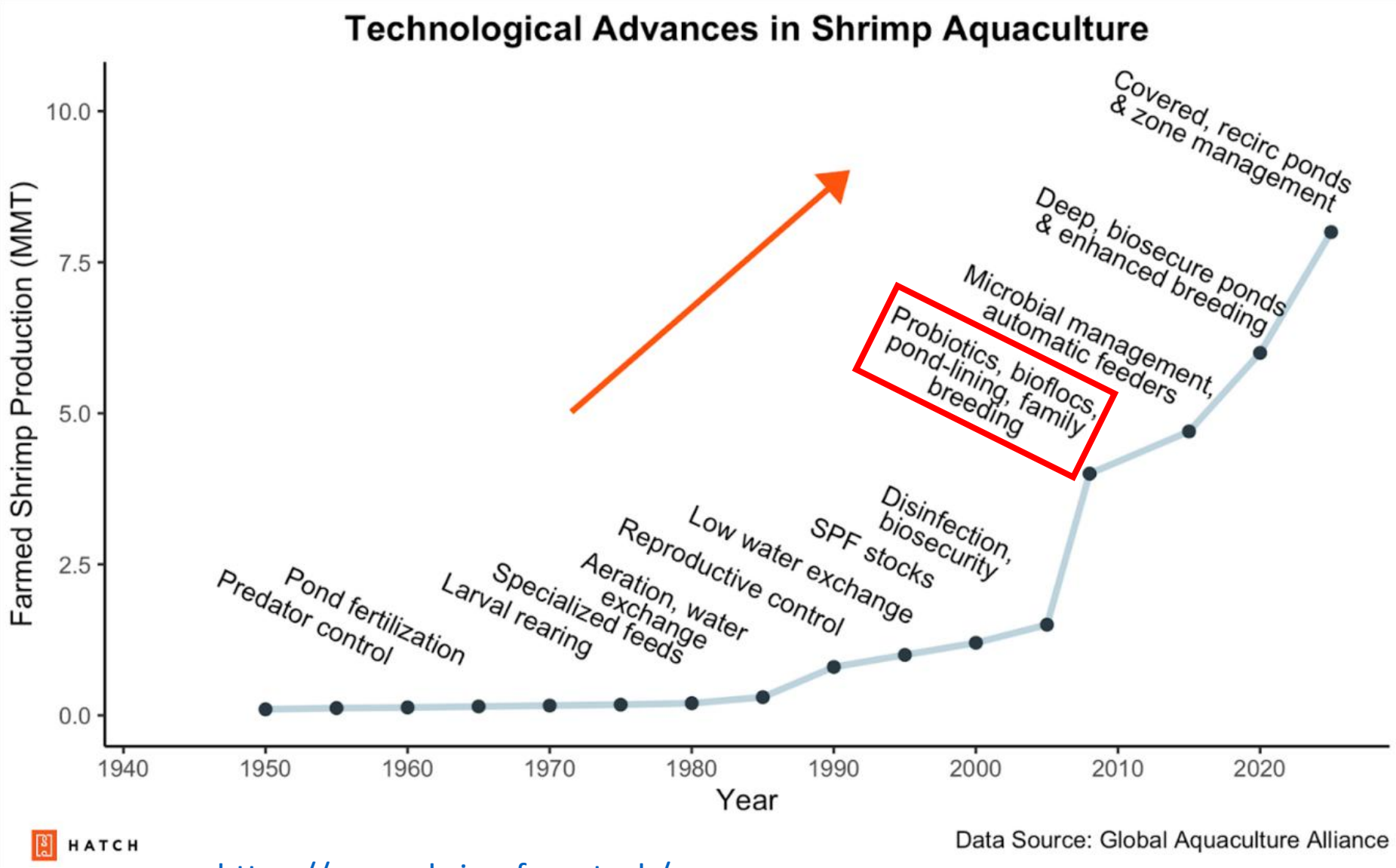
Compartilhar

Génesis Lozano outubro 4, 2019

O objetivo é descobrir novas informações sobre o bloqueio imposto pela China a carregamentos de camarão equatoriano, supostamente contaminados com doenças conhecidas como cabeça amarela e mancha branca.

https://dialogochino.net/30661-ecuadors-economy-shell-shocked-by-china-shrimp-ban/

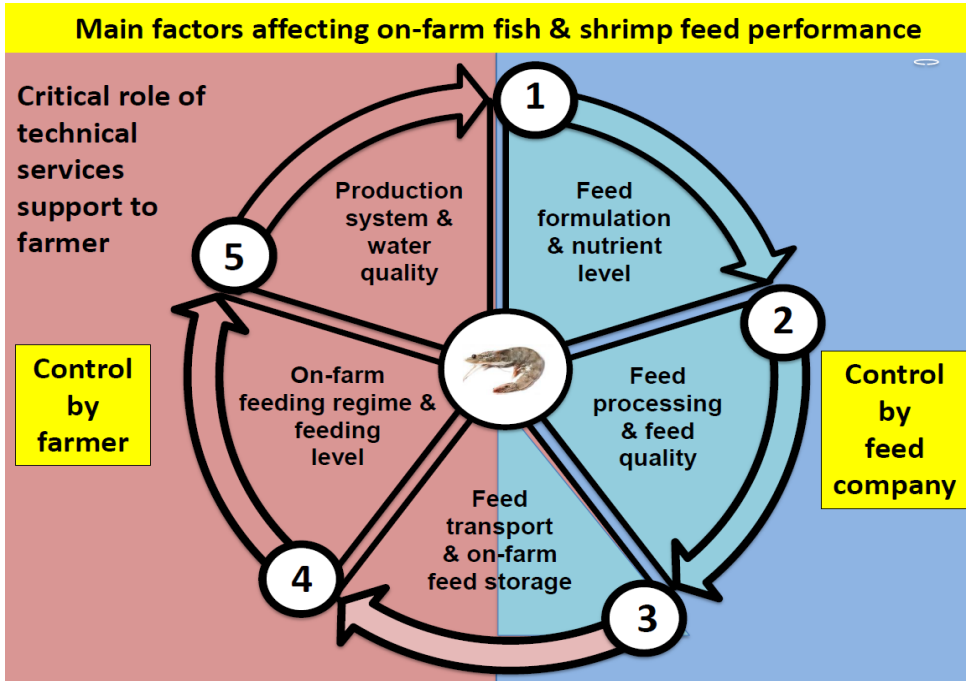
TECHNOLOGICAL ADVANCES AT THE FARMS



https://www.shrimpfarm.tech/

ISSUES WITH PRODUCTION COST

How to handle production and feed costs?



Tacon, Aquafeed Workshop Mexico, 2018

Improve production efficiency:

Lower risk of disease

Stronger PLs

Cost benefit of products/protocols

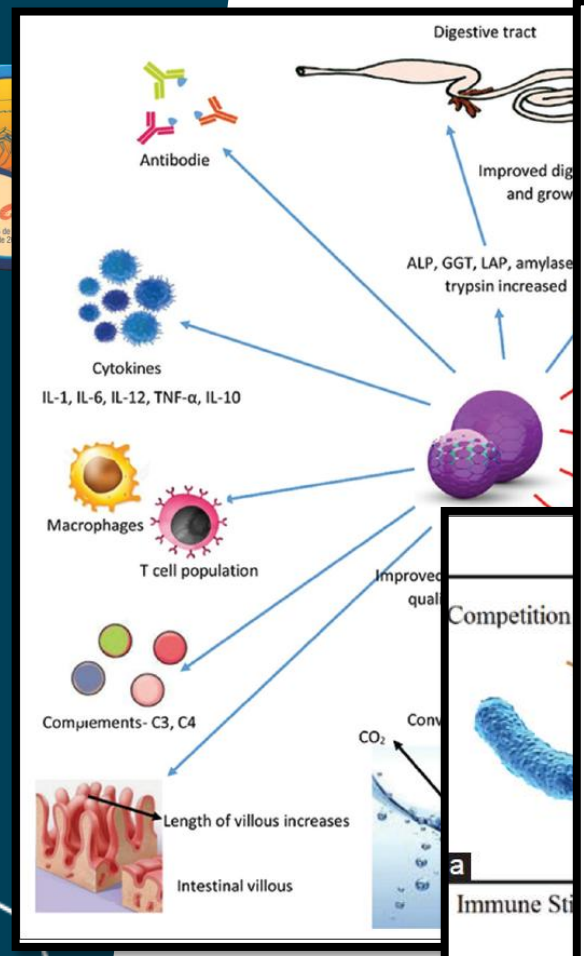


TABLE 2 | Overview of the effects of probiotics against pathogenic bacteria in shellfish.

Probiotic	Pathogen or disease	Shellfish species	Beneficial effects	Reference
GRAM-POSITIVE BACTERIA				
Lactobacillus acidophilus Streptococcus cremoris Lactobacillus bulgaricus	*Vibrio alginolyticus*	Indian white shrimp (*Penaeus indicus*)	Higher survival rate	Ajitha et al., 2004
Lactobacillus plantarum	*Vibrio alginolyticus*	White shrimp (*Litopenaeus vannamei*)	Increased clearance efficiency to *V. alginolyticus* and the survival rate	Chiu et al., 2007
	Vibrio harveyi	White shrimp (*Litopenaeus vannamei*)	Enhanced disease resistance	Vieira et al., 2010
Lactobacillus acidophilus	*Vibrio alginolyticus*	Shrimp (*Penaeus monodon*)	Higher survival ratio	Sivakumar et al., 2012
Enterococcus faecium Lactococcus garvieae	*Vibrio harveyi* and *Vibrio parahaemolyticus*	Shrimp (*Penaeus monodon*)	Enhanced disease resistance	Swain et al., 2009
Pediococcus acidilactici	*Vibrio nigripulchritudo*	Blue shrimp (*Litopenaeus stylirostris*)	Higher survival rate	Castex et al., 2010
Lactobacillus pentosus Enterococcus faecium	*Vibrio parahaemolyticus*	Shrimp (*Litopenaeus vannamei*)	Enhanced the survival rate	Sha et al., 2016
Bacillus subtilis	*Vibrio harveyi*	Shrimp (*Penaeus monodon*)	Enhanced the survival rate	Vaseeharan and Ramasamy, 2003
	Vibrio harveyi	Shrimp (*Litopenaeus vannamei*)	Enhanced the survival rate	Balcázar et al., 2007; Zokaeifar et al., 2012; Liu et al., 2014
Bacillus sp.	*Vibrio harveyi*	Shrimp (*Penaeus monodon*)	Enhanced the survival rate	Rengpipat et al., 1998
Streptococcus phocae	*Vibrio harveyi*	Shrimp (*Penaeus monodon*)	Enhanced the survival rate	Swain et al., 2009
Arthrobacter sp.	*Vibrio parahaemolyticus*	Shrimp (*Litopenaeus vannamei*)	Significantly enhanced the immune parameters and significantly decreased mortalities	Li et al., 2008
GRAM-NEGATIVE BACTERIA				
Streptomyces sp.	*Vibrio harveyi*	Black tiger shrimp (*Penaeus monodon*)	Better survival and growth performance	Das et al., 2010
Pseudomonas aeruginosa	*Vibrio harveyi*	Western king prawns (*Penaeus latisulcatus*)	Improved the survival rate	Van Hai et al., 2009
Pseudomonas sp.	*Vibrio harveyi*	Whrimp (*Penaeus monodon*)	Improved the survival rate	Pai et al., 2010
Alteromonas macleodii Neptunomonas sp.	*Vibrio splendidus*	Greenshell mussel(*Perna canaliculus*)	Improved survival and suppress naturally occurring vibrios	Kesarcodi-Watson et al., 2010
	Vibrio coralliilyticus and *V. splendidus*	Scallop (*Pecten maximus*	Improved the survival rate	Kesarcodi-Watson et al., 2012
	Vibrio coralliilyticus and *Vibrio pectenicida*	Flat oyster (*Ostrea edulis*)	Improved the survival rate	Kesarcodi-Watson et al., 2012
Phaeobacter gallaeciensis, Pseudoalteromonas	*Vibrio coralliilyticus* and *V. splendidus*	Scallop (*Pecten maximus*)	Improved the survival rate	Kesarcodi-Watson et al., 2012
YEAST				
Phaffia rhodozyma Saccharomyces cerevisiae	vibriosis	Shrimp (*Litopenaeus vannamei*)	Improve resistance against vibriosis	Scholz et al., 1999

digestive tract

y through production
l 2012)

, biotin, vitamin B12,
(Vine et al 2006)

sis and antibacterial
nune system

ceptors and immune

eceptors

elated genes (Chiu et

Vijayaran and Kannan 2018

MECHANISM OF ACTION...FARM

PROBIOTIC CHOICE: WHAT TO CONSIDER?

There are still some unanswered questions and obstacles on the use of probiotics!!

*Effects on growth performance

*Price – cost benefit

*Reliability

*Constancy (cfu/g)

*Safety

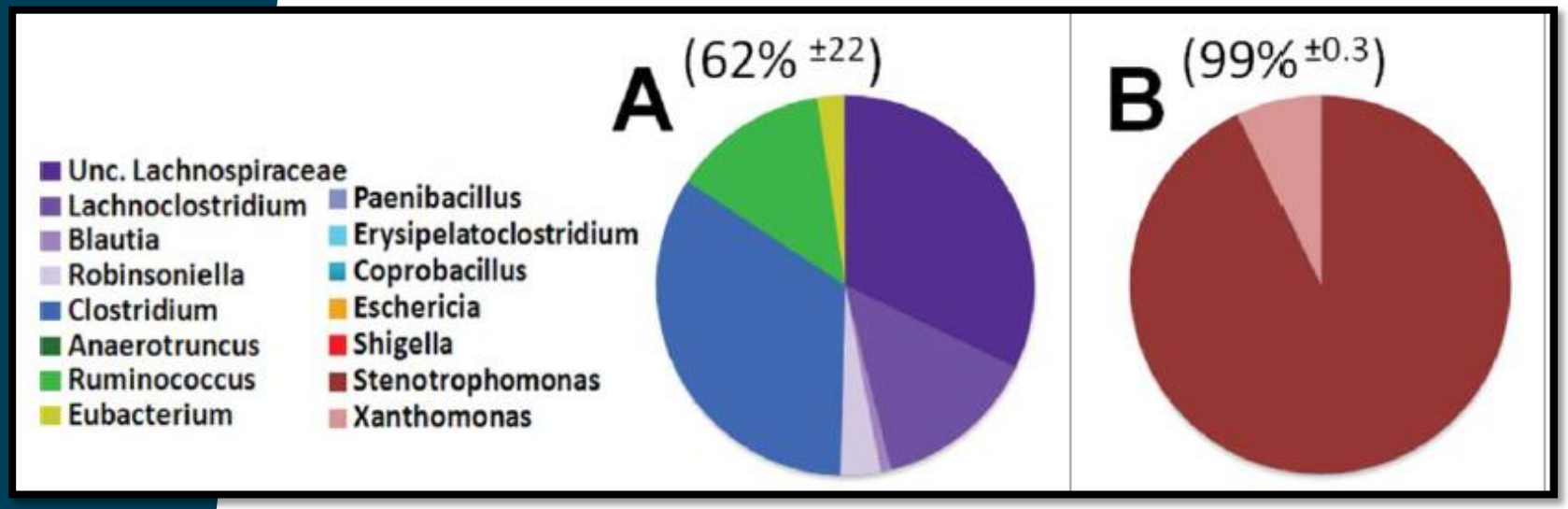
*Quality control

*Production process

*Composition

OVERCOMING OLD OBSTACLES WITH NEW TECHNOLOGY: BRINGING SCIENCE AND FARM KNOWLEDGE TOGETHER TO ALLOW THE BEST CHOICES

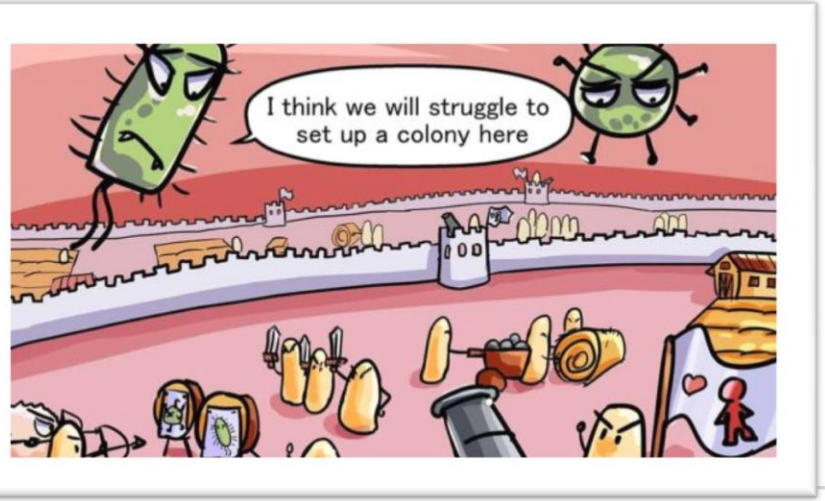
- **Microbial diversity in humans after antibiotics**

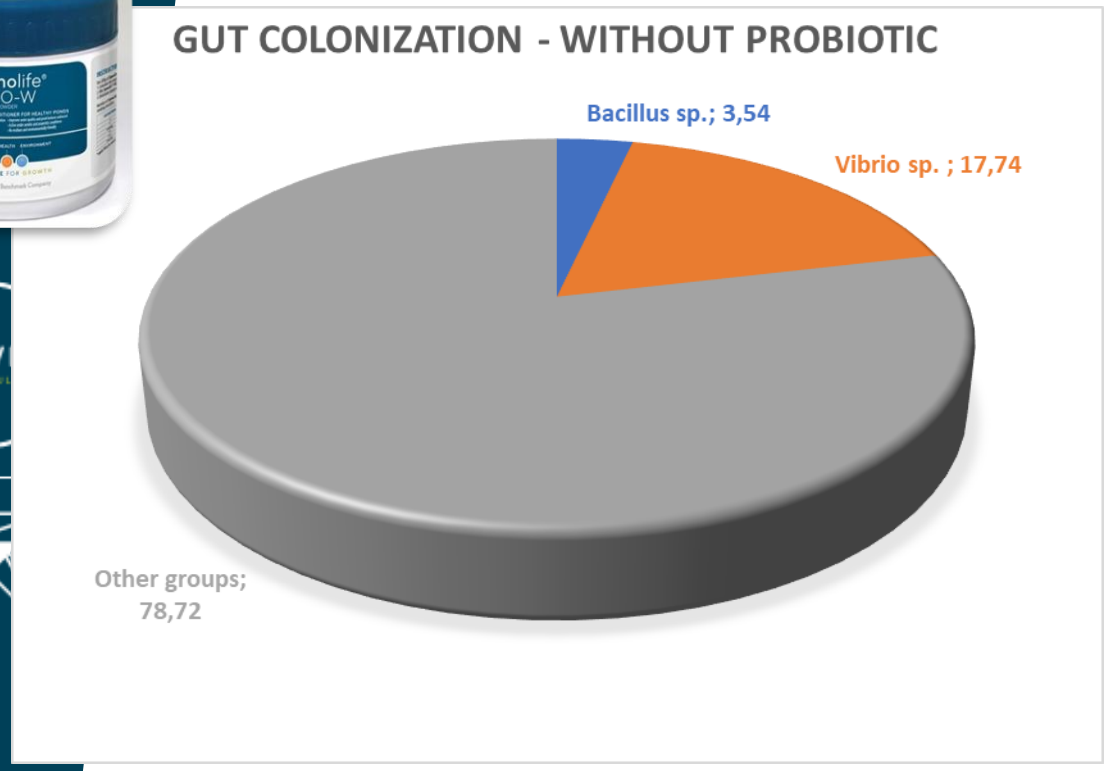


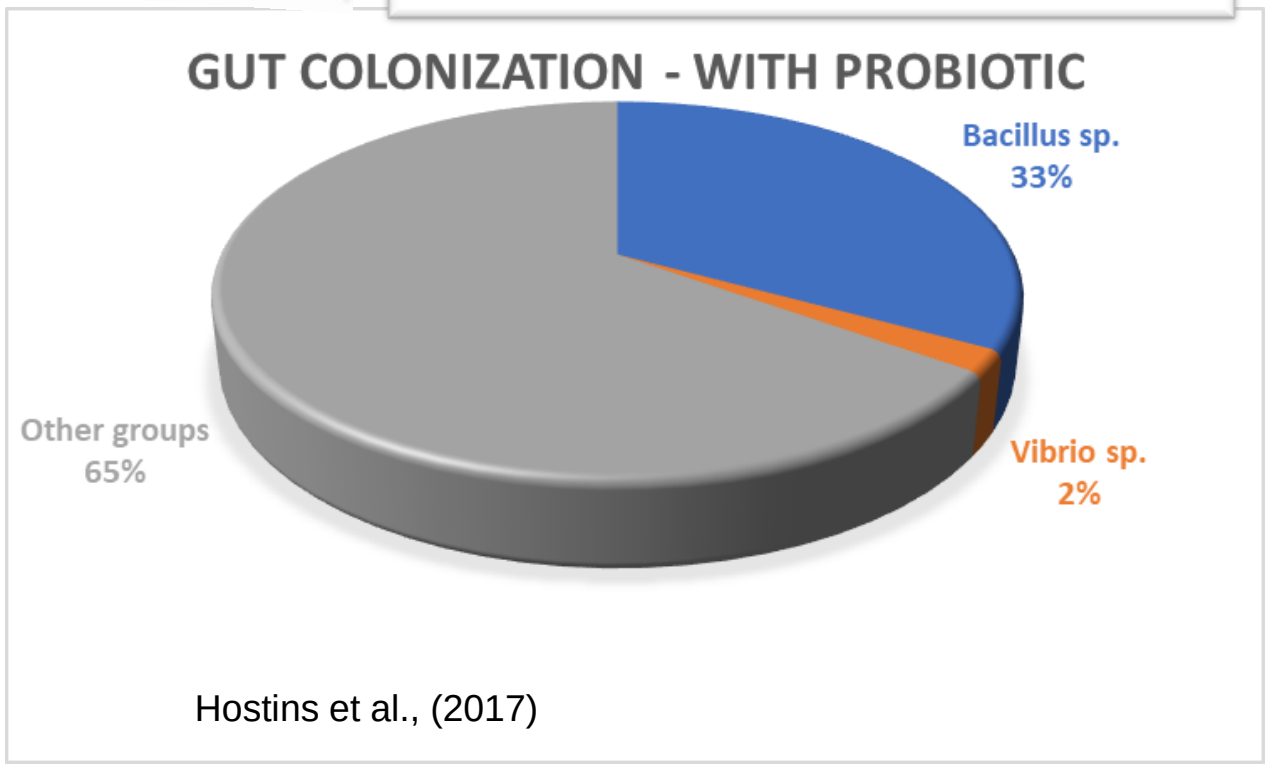
Suez et al., (2019)

Methodological advances in next generation sequencing and molecular biology enable a better understanding of concepts and processes

COLONIZATION DEBATE: CAN ALL PROBIOTICS COLONIZE THE INTESTINAL TRACT?



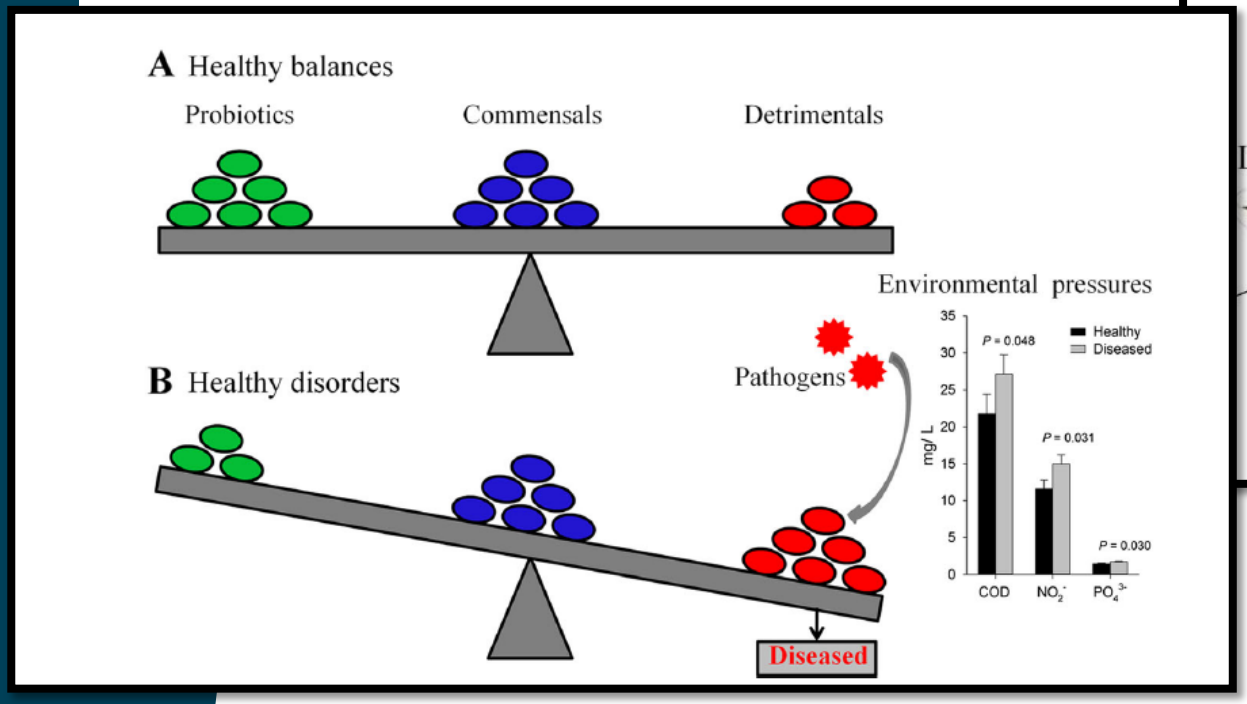


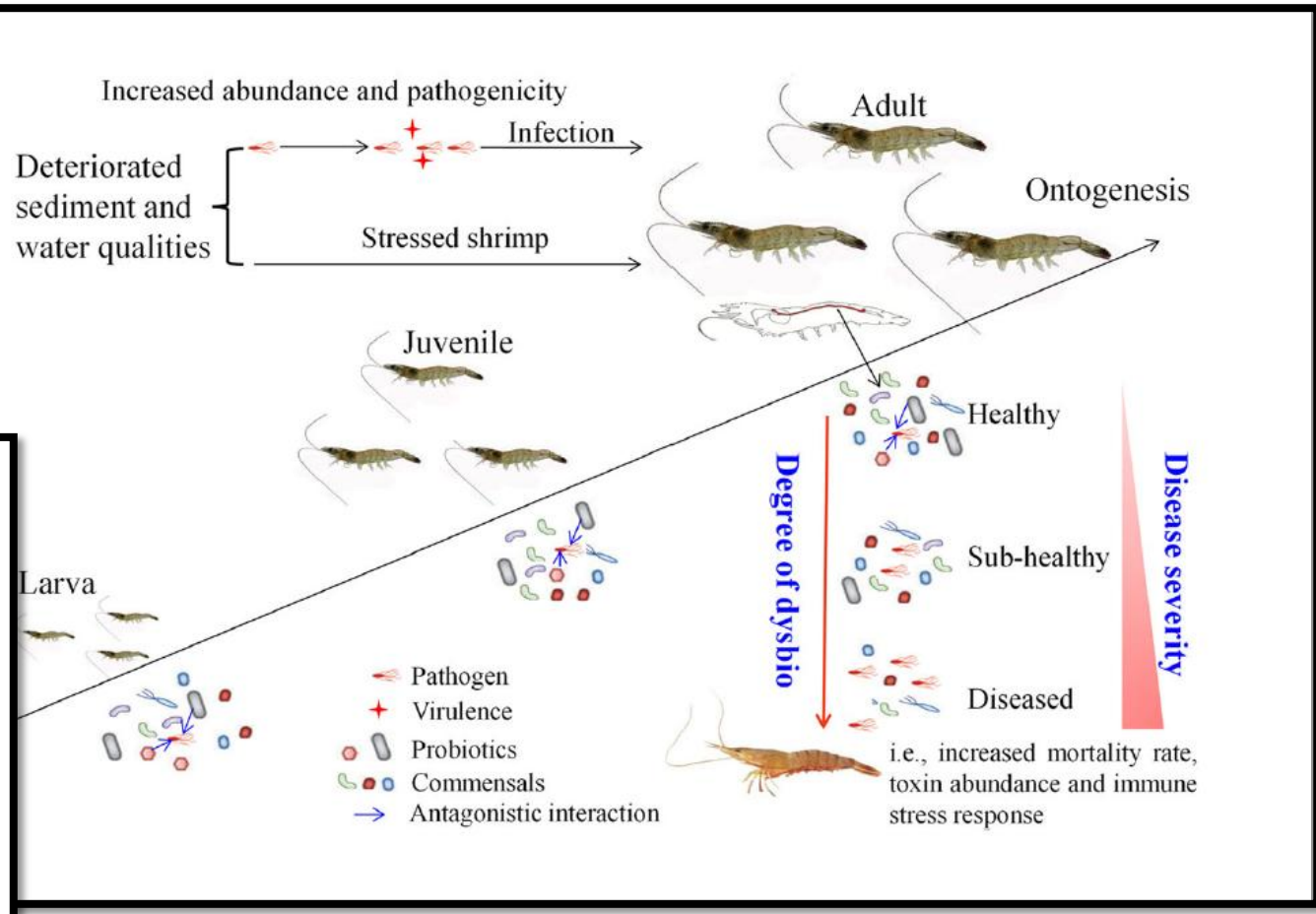


Hostins et al., (2017)

COLONIZATION DEBATE: CAN ALL PROBIOTICS MODULATE GUT MICROBIAL COMMUNITY?

- If the probiotic applied can colonize the tract, it will have a more pronounced role in the modulation of microbiome





Xiong (2018)

OVERCOMING OLD OBSTACLES WITH NEW TECHNOLOGY: BRINGING SCIENCE AND FARM KNOWLEDGE TOGETHER TO ALLOW THE BEST CHOICES

Improved precision, accuracy and repeatability of measures which lead to genuine and not misleading interpretations

*Effects on growth performance

*Price – cost benefit

*Reliability

*Constancy (cfu/g)

*Safety

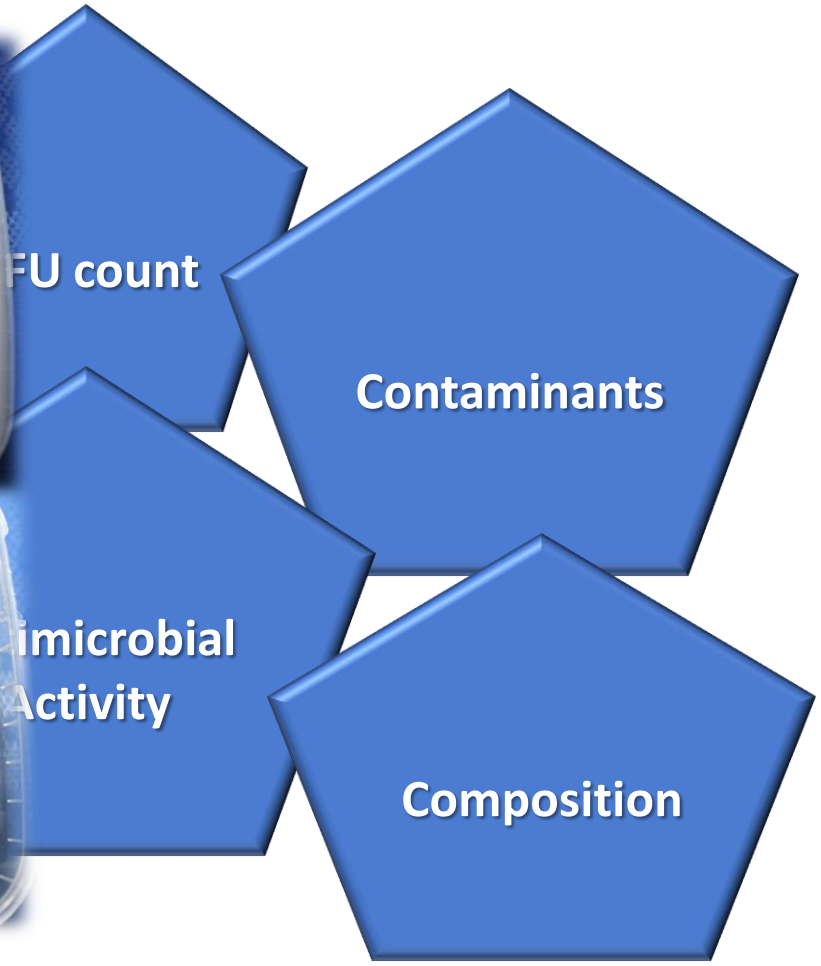
*Quality control

*Production process

*Composition

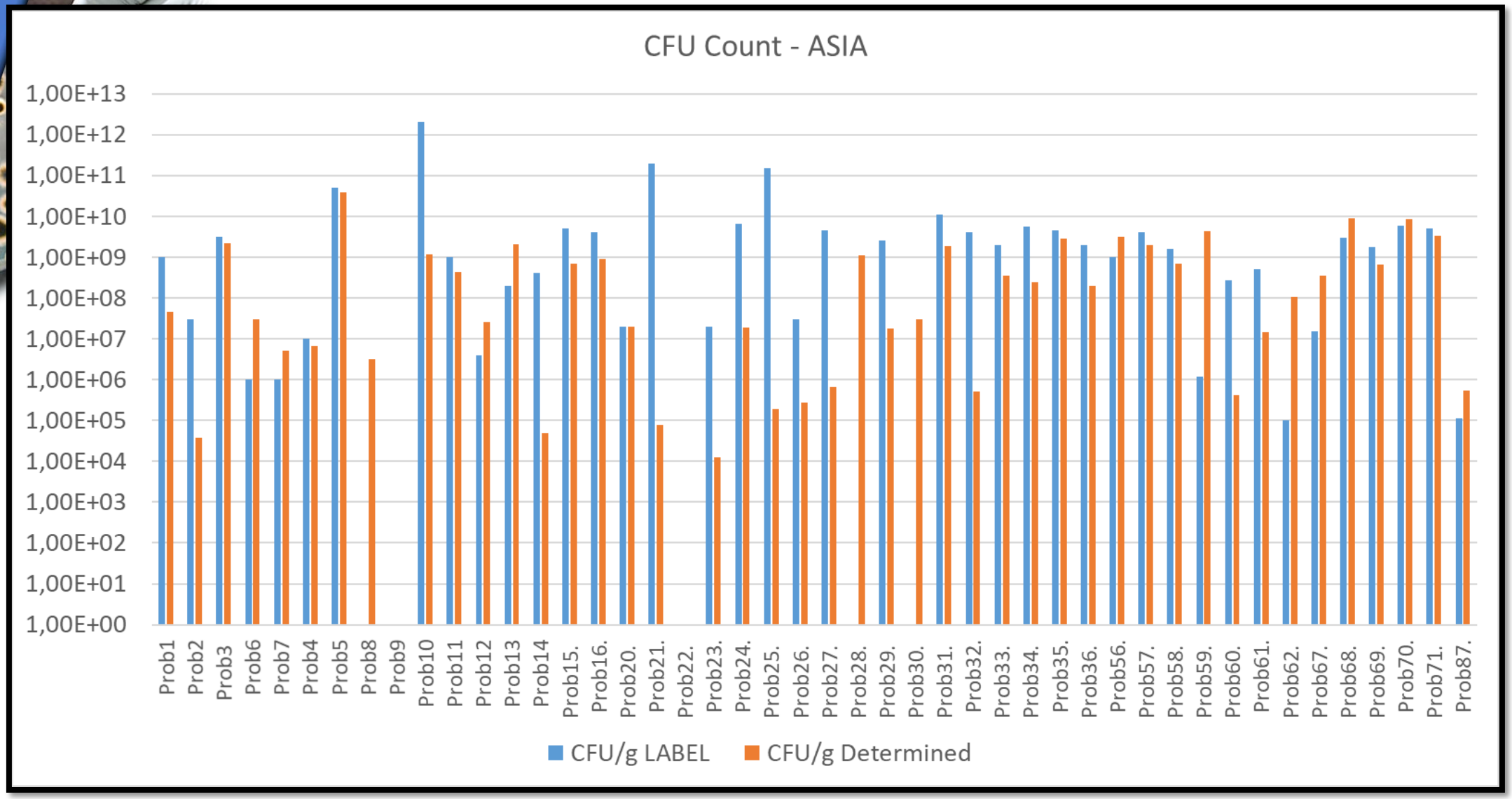
PROBIOTICS SURVEY

- 80 comm
- Asia – Th
  Indonesia
- Americas
  Ecuador,

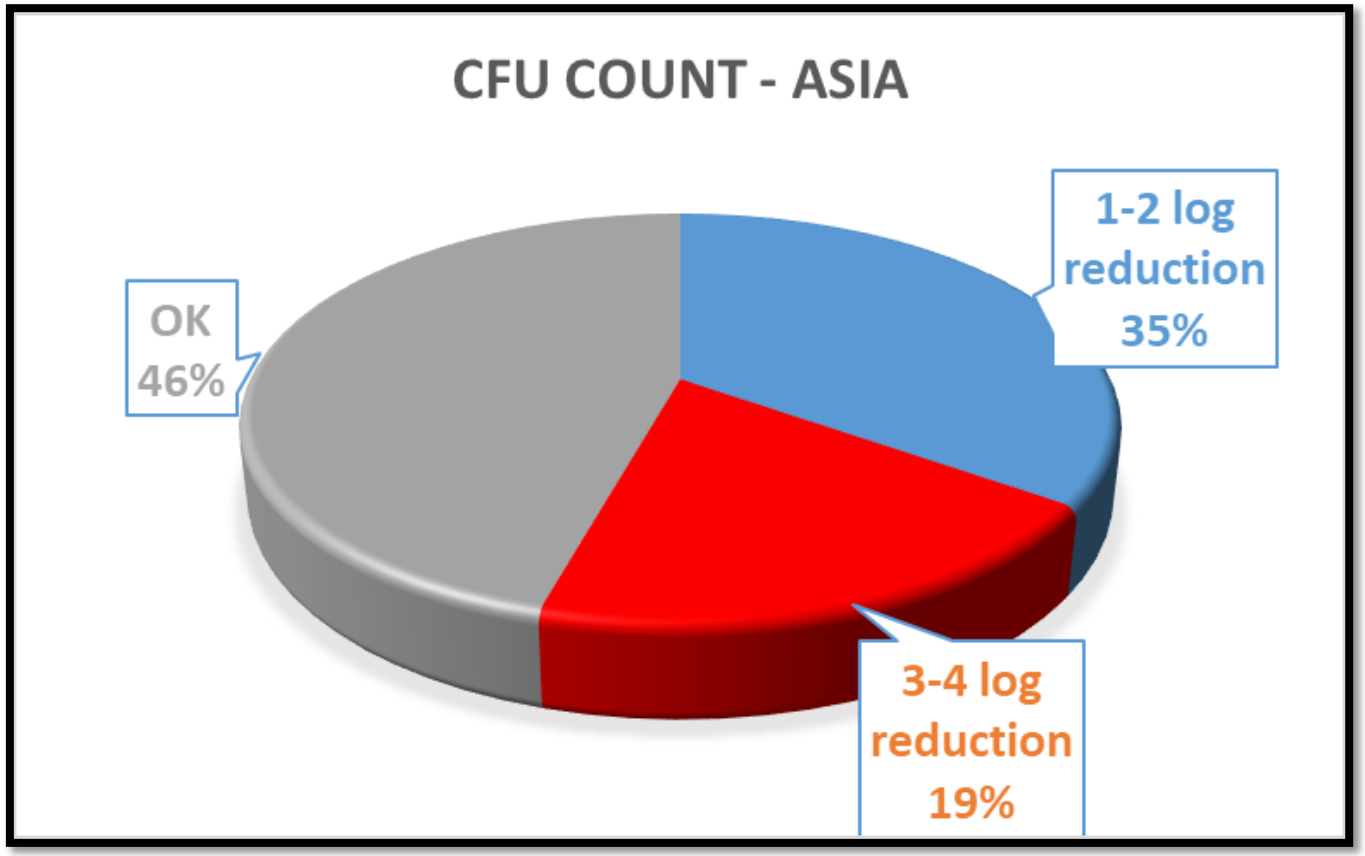


PROBIOTICS SURVEY – DO WE HAVE WHAT WE PAY FOR?

CFU count



PROBIOTICS SURVEY – DO WE HAVE WHAT WE PAY FOR?



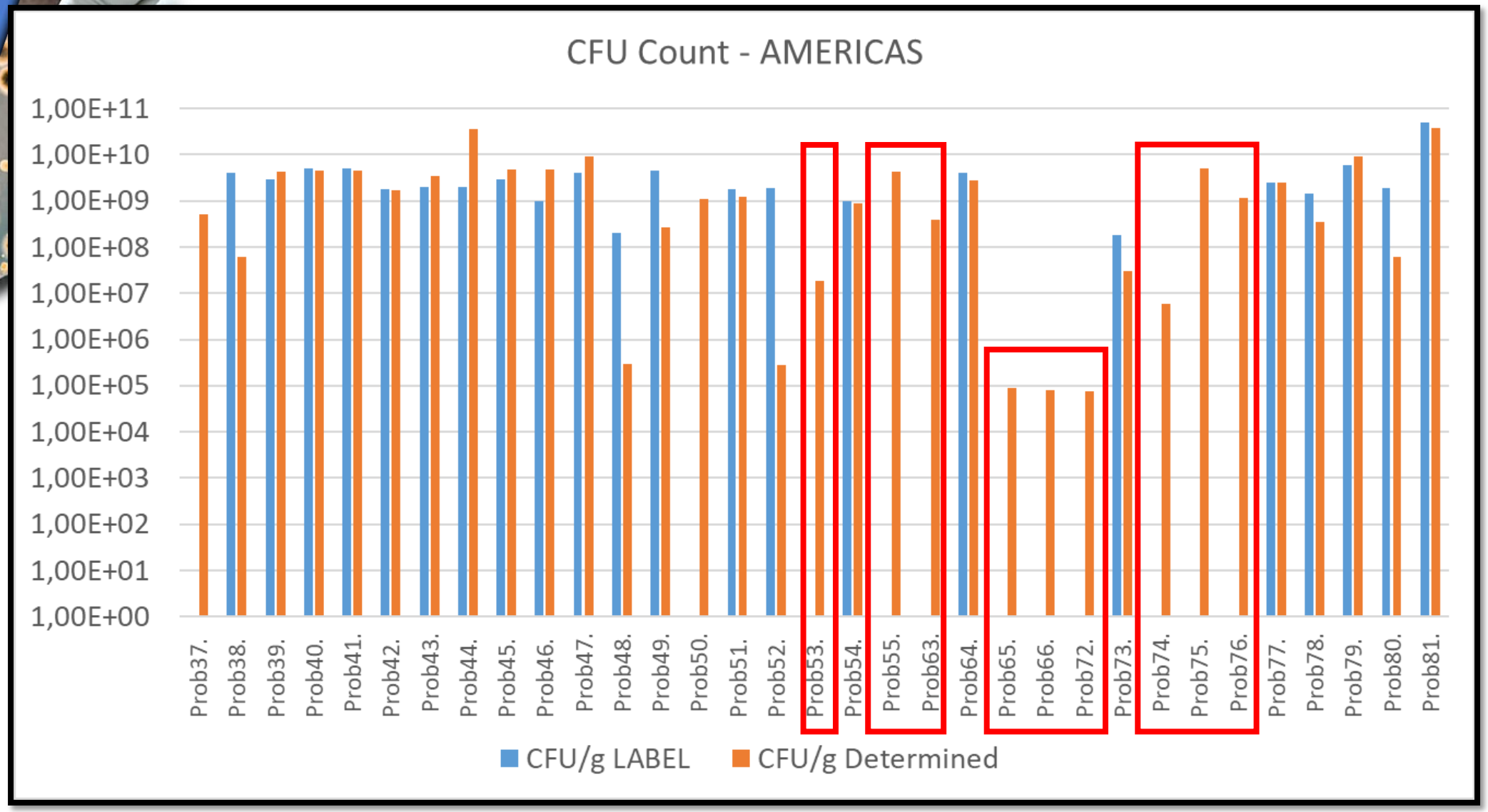
More than 50% of probiotics analyzed have lower number of bacteria than declared on the label!!!

2 products did not declare CFU on the label

Low efficiency – higher costs!!!

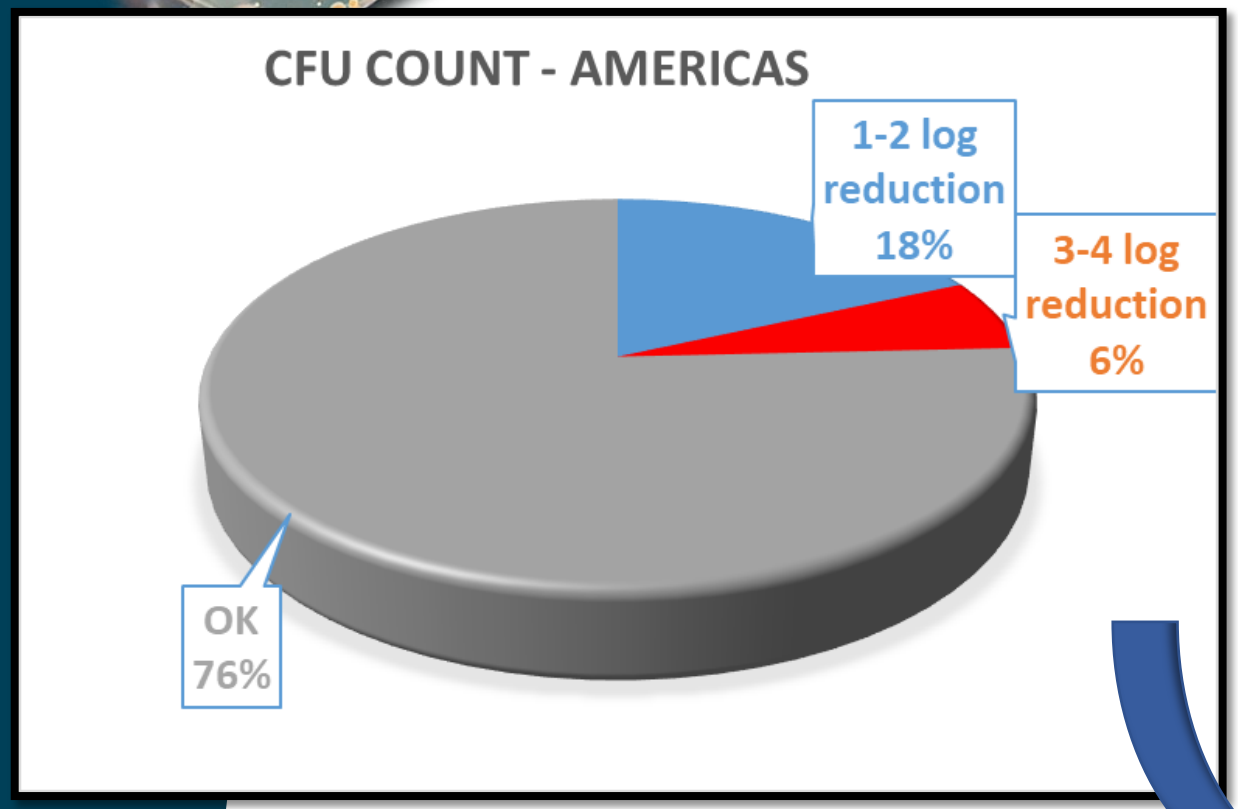
PROBIOTICS SURVEY – DO WE HAVE WHAT WE PAY FOR?

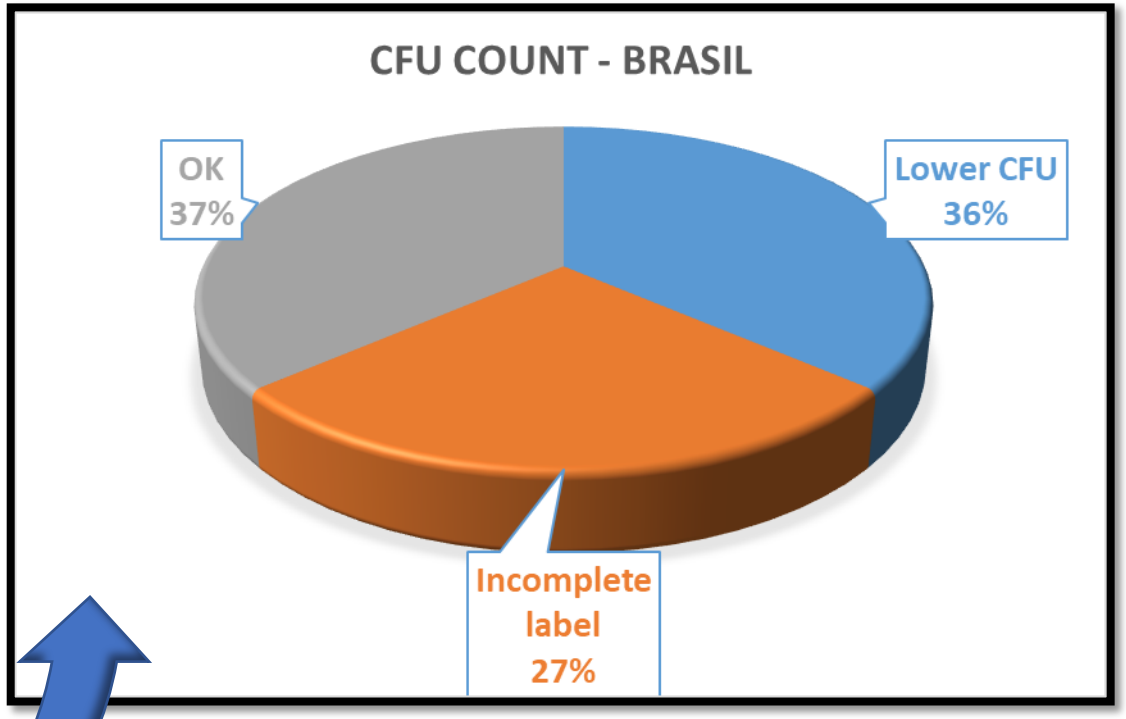
CFU count



PROBIOTICS SURVEY – DO WE HAVE WHAT WE PAY FOR?

- 9 products did not declare CFU on the label – How to evaluate?
- Leads to inconsistency!!!





PROBIOTICS SURVEY – DO WE HAVE WHAT WE PAY FOR?

Example: Probiotic 48: 20U$ dollars/kg – $2*10^8$ cfu/g

Determined: $2,94*10^5$ cfu/g. Reduction in 1000 times bacterial concentration!!

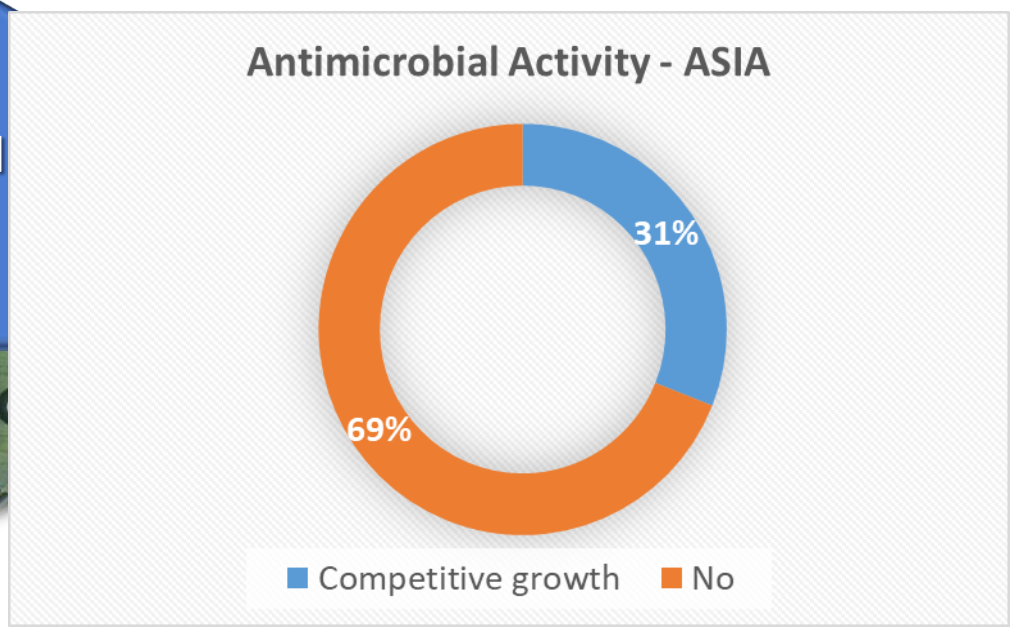
Product becomes 1000 times more expensive!

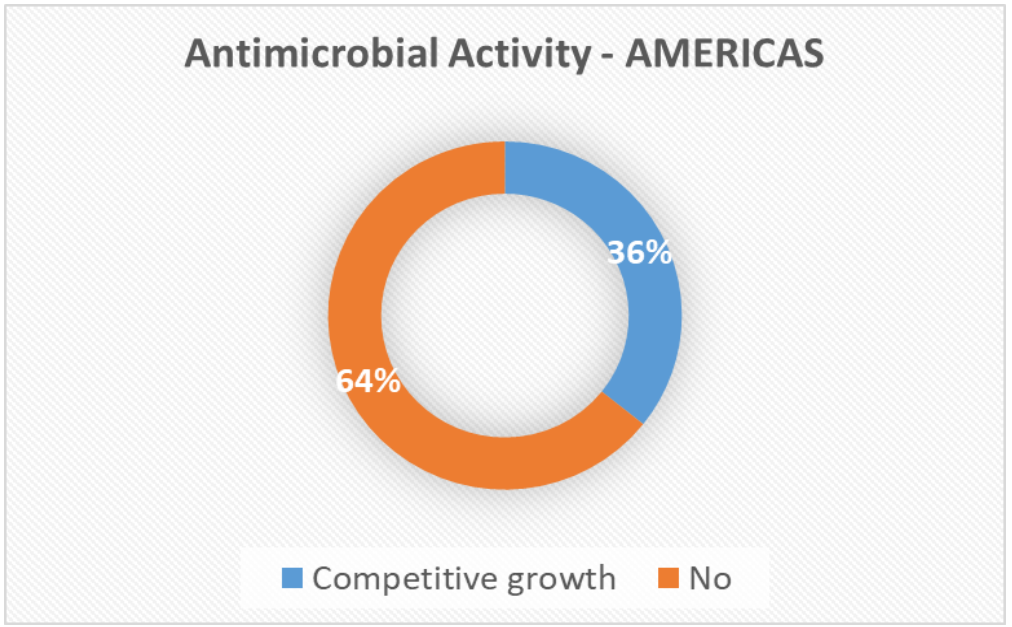
PROBIOTICS SURVEY – FOOD SAFETY

- 3 of the evaluated products showed growth in McConkey agar – gram negative bacilli including coliform organisms and entheric pathogens.
- 2 products from Americas (Ecuador) and 1 from Asia (Indonesia).
- None of the probiotics showed growth in TCBS agar (Vibrio growth).

PROBIOTICS SURVEY – OUTGROWING THE BAD GUYS

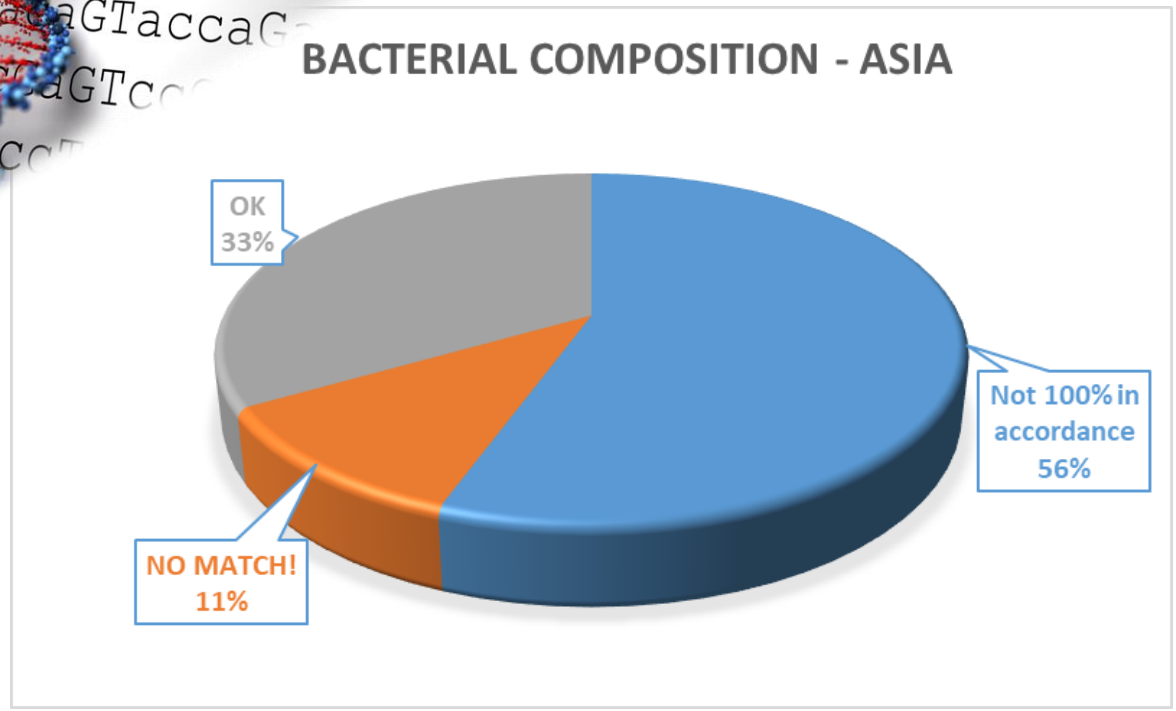
Antimicrobial Activity



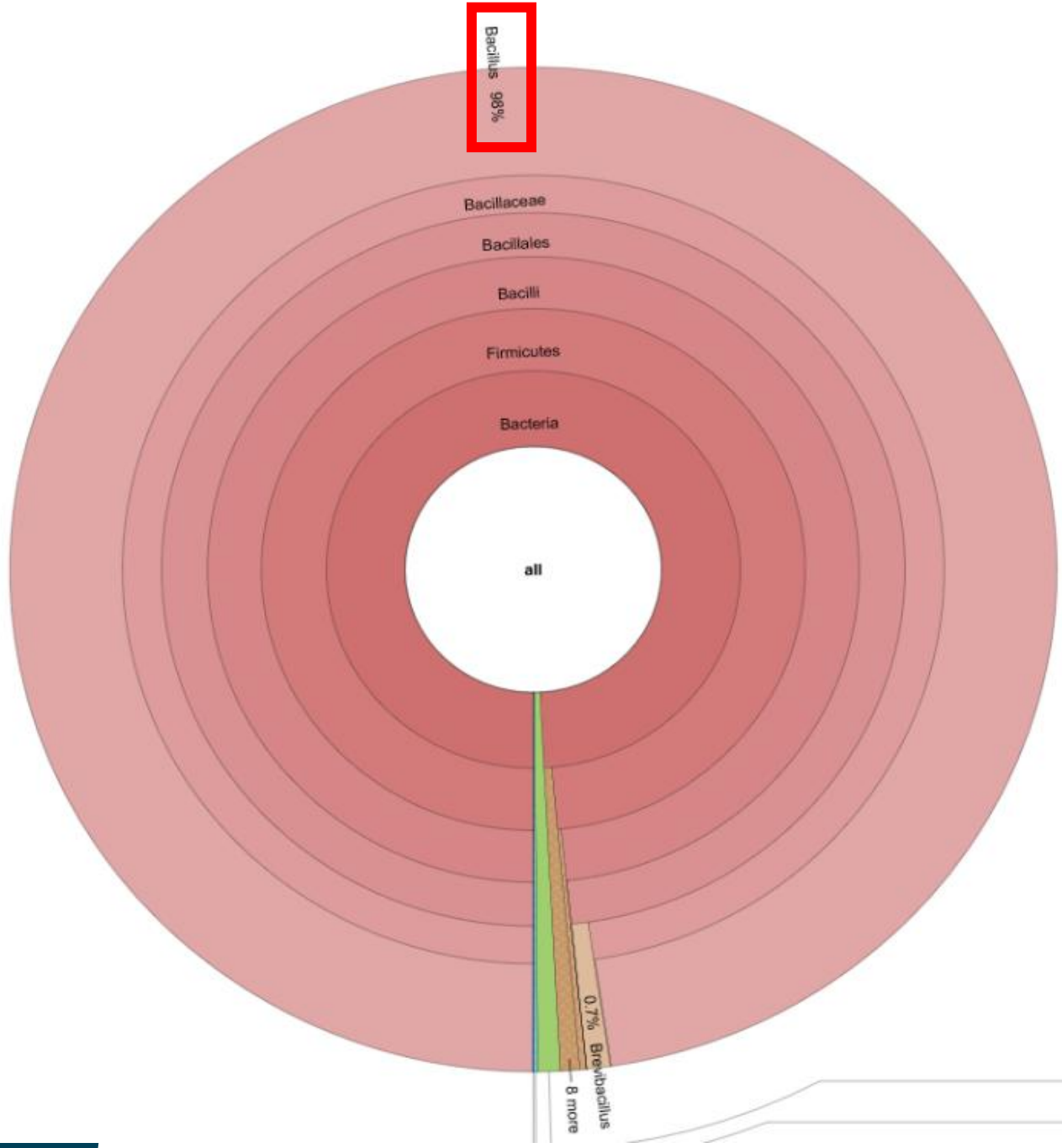


- Only one product showed presence of antimicrobials – chloramphenicol (100ng/kg)
- None of the other probiotics evaluated were positive for antimicrobials – competitive growth with *Vibrio parahaemolyticus*

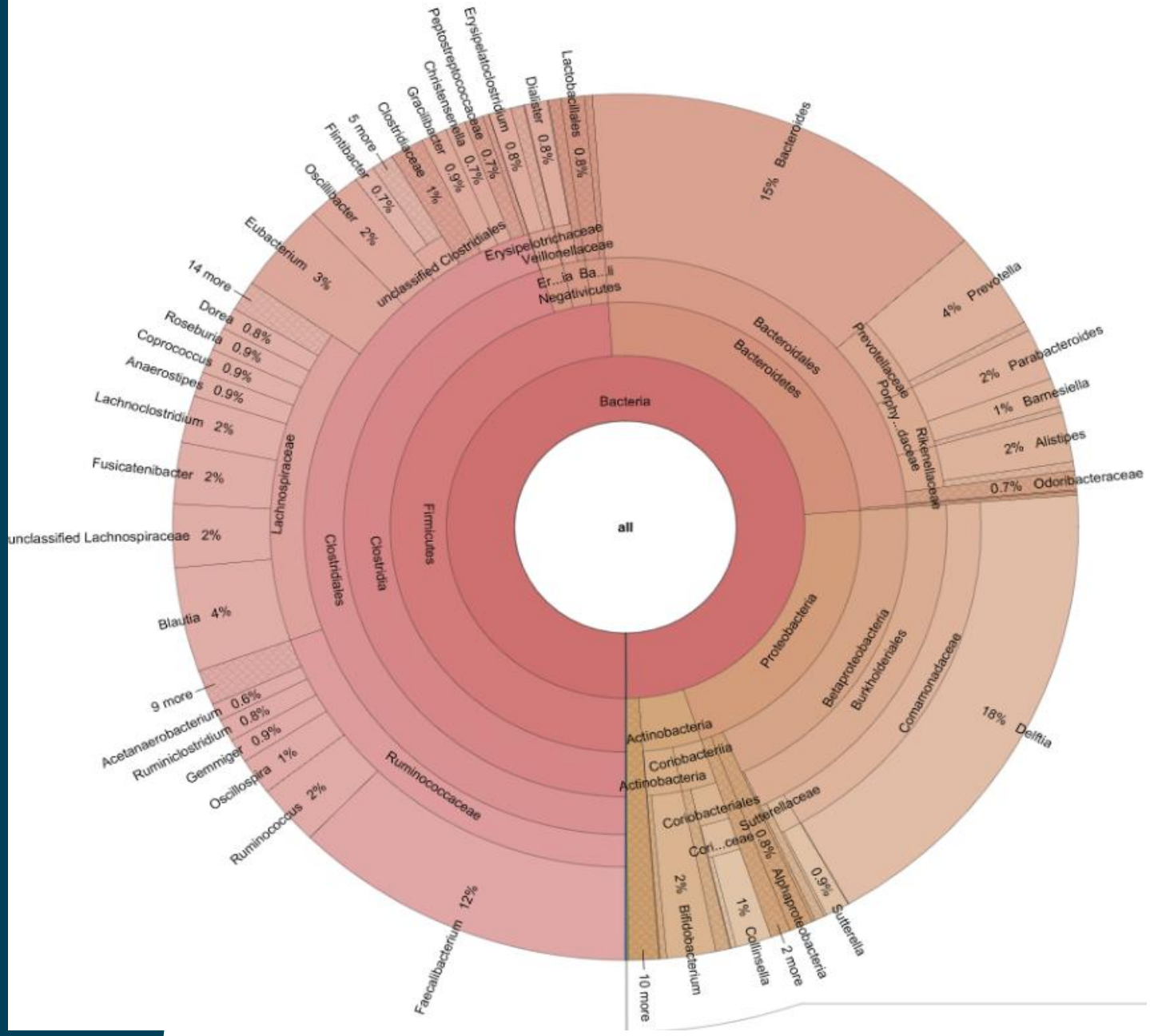
PROBIOTICS SURVEY – ARE THE GOOD GUYS THERE?



- DNA sequencing to confirm bacterial composition declared on the labels
- Extra genera are found in some products or...
- One of the declared species are not found, or in very small percentages.



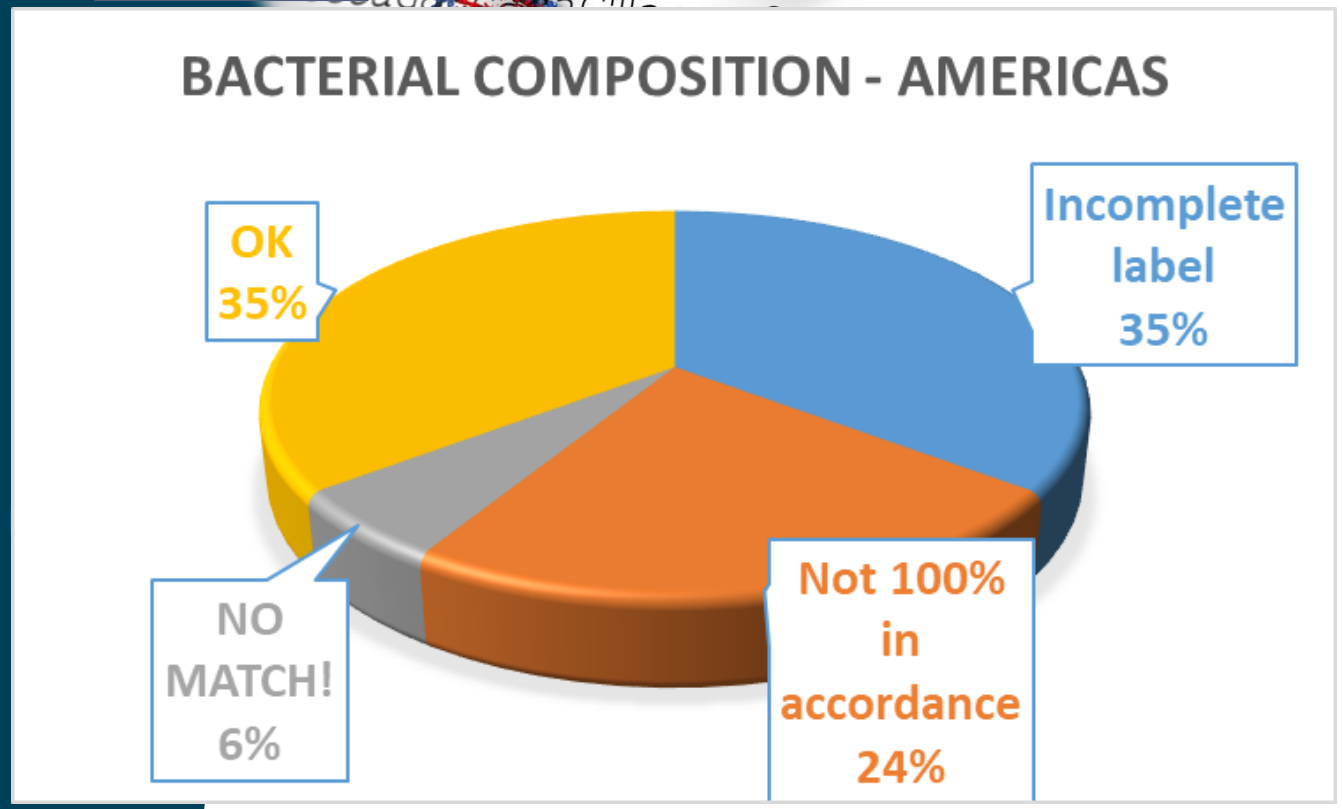
- Product that declares the presence of Bacillus and H2S oxidizing bacteria: Bacillus are found, but **NO H2S** oxidizing bacteria are present!!



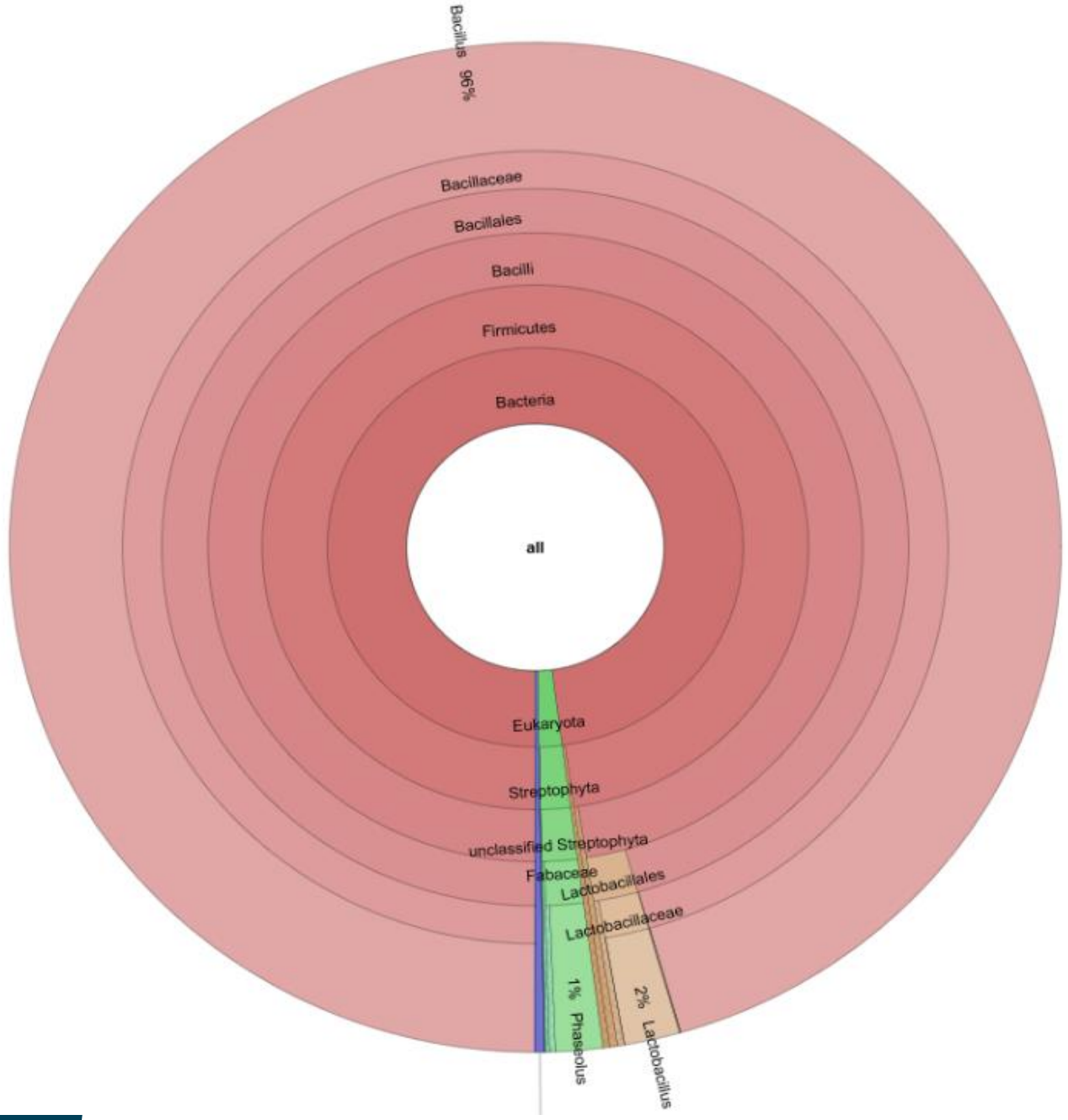
- Product that declares *Bacillus polymyxa, Bacillus licheniformis, Bacillus megaterium:* No Bacillus is found!!

PROBIOTICS SURVEY – ARE THE GOOD GUYS THERE?

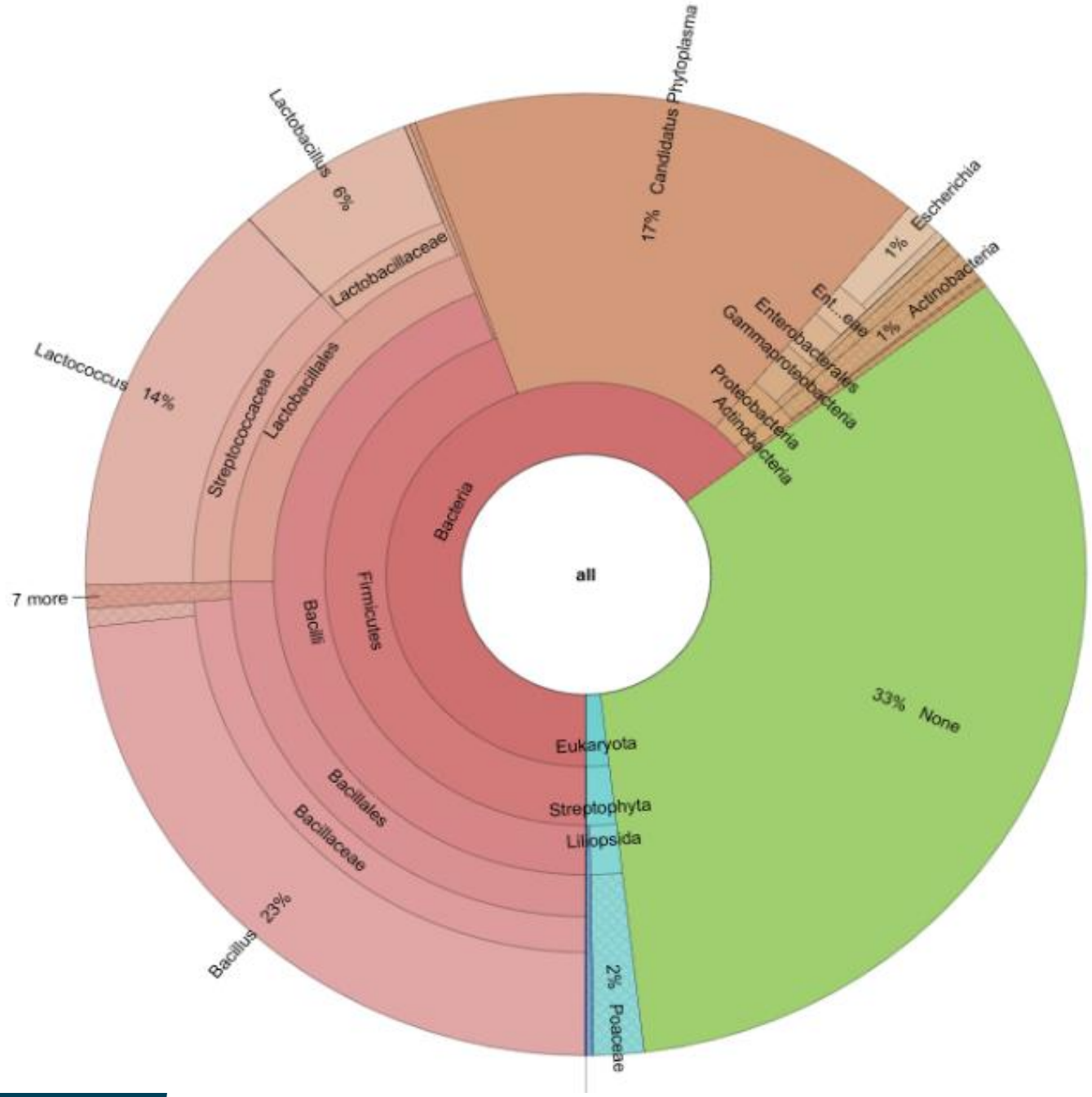
Composition



- 35% of the products did not have any information on composition available on the label – data from sequencing shows Bacillus and/or Lactobacillus.
- Consistency is not reliable!!!



- Product that declares *Saccharomyces cerevisiae, Lactobacillus acidophilus, Enterococcus faecium: 98% Bacillus* is found, almost NO *Lactobacillus or Enterococcus* are found!



- Product that declares *Bacillus sp.*, *Lactobacillus sp.*, *Rhodobacter*, *Nitrobacter*, *Nitrosomonas*, *Saccharomyces sp.* : Only *Bacillus* and *Lactobacillus* found, other species mentioned are not found, or in very low levels

HIGHER PRODUCTION EFFICIENCY: FARM

On-farm approaches to “control costs”…

HIGHER PRODUCTION EFFICIENCY: FARM

On-farm approaches to “control cost”…

From Albert Tacon (USAID workshop, Bangkok 2015)

MICROBIAL RUSSIAN ROULETTE: THE WAY PROBIOTICS ARE PREPARED MATTER!

Survival of pathogenic microorganisms in *kefir*

Sobrevivência de micro-organismos patogênicos em *kefir*

Recebido: 10.12.2010 - Aceito para publicação: 13.03.2012

ABSTRACT

Kefir is a homemade fermented milk produced by adding kefir grains. The domestic handling and the use of raw materials from different standards and sources, and the lack of inspection by qualified professionals, all this classify kefir as a food which might represent potential risks to human health. This study aimed at evaluating the pathogens survival during the kefir fermentation process. Kefir grains were added into portions of UHT skimmed milk which were experimentally contaminated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium and Enteritidis, *Staphylococcus aureus* and *Listeria monocytogenes*. *Salmonella* Typhimurium e Enteritidis sobreviveram por 24 horas no kefir em fermentação. *E. coli* O157:H7, *S. aureus* e *L. monocytogenes* foram recuperados até 72 horas após o início da fermentação. As bactérias patogênicas estudadas, nas concentrações e condições do presente trabalho, sobreviveram por tempo superior àquele normalmente utilizado para a fermentação do kefir preparado artesanalmente, o qual representa perigo potencial para o consumo humano.

Palavras-chaves. *kefir*, leite fermentado, inibição, agentes patogênicos.

CONCLUSIONS

- Advances in technology and molecular biology techniques can bring science and industry together to increase efficiency and predictability of probiotic products
- In parallel with shrimp performance, consistency, reliability and clear information on probiotics should be taken in account to allow better product choices
- Be aware and request clarity on the QC parameters of probiotic suppliers is important to guarantee cost efficiency and safety of the production cycle and end product.

OBRIGADA!