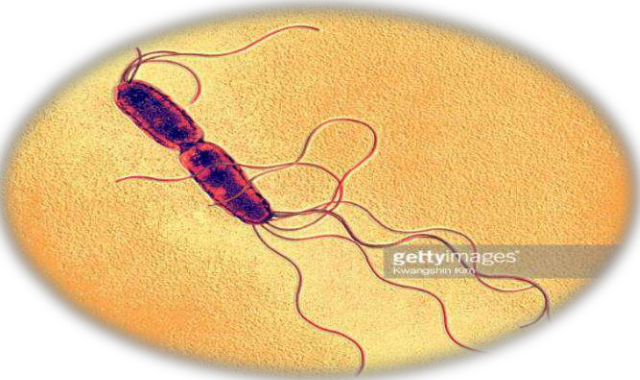


**Effect of *Apis mellifera*, *Melipona beecheii* and *Trigona (Frieseomelitta) nigra* honeys on the expression of virulence genes of *Salmonella enterica* Serovar Typhimurium**



G. Vázquez-Cruz, J. Ramón-Sierra

E. Peraza-López, V. Bustamante-Santillán

Elizabeth Ortiz Vázquez

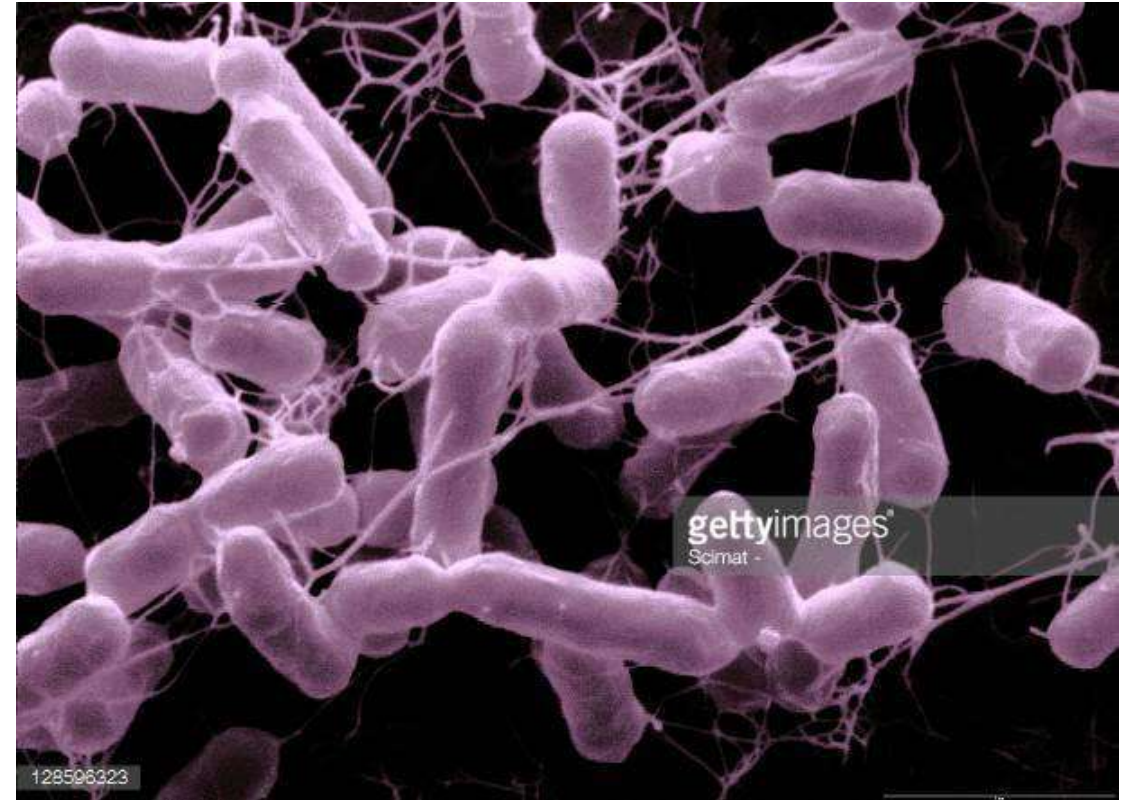


# Diseases Incidence

World Health Organization (WHO): Half of the world population suffer diarrhea.

*Salmonella* is one of main agents of foodborne illness in the world.

US has estimated that 1,412,498 of infections by *Salmonella* no Typhi.



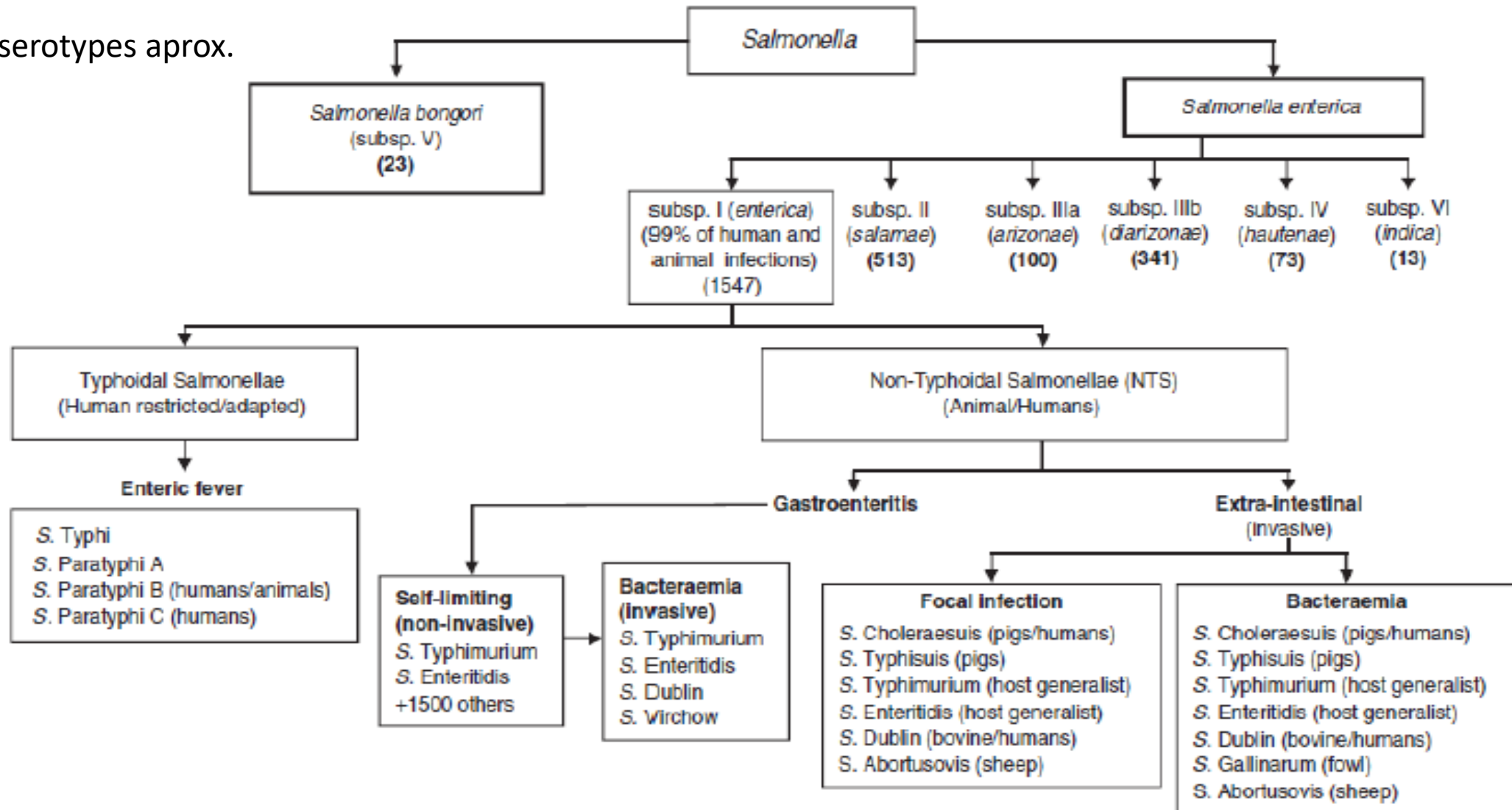
*Salmonella Typhimurium*

(OMS, 2015)



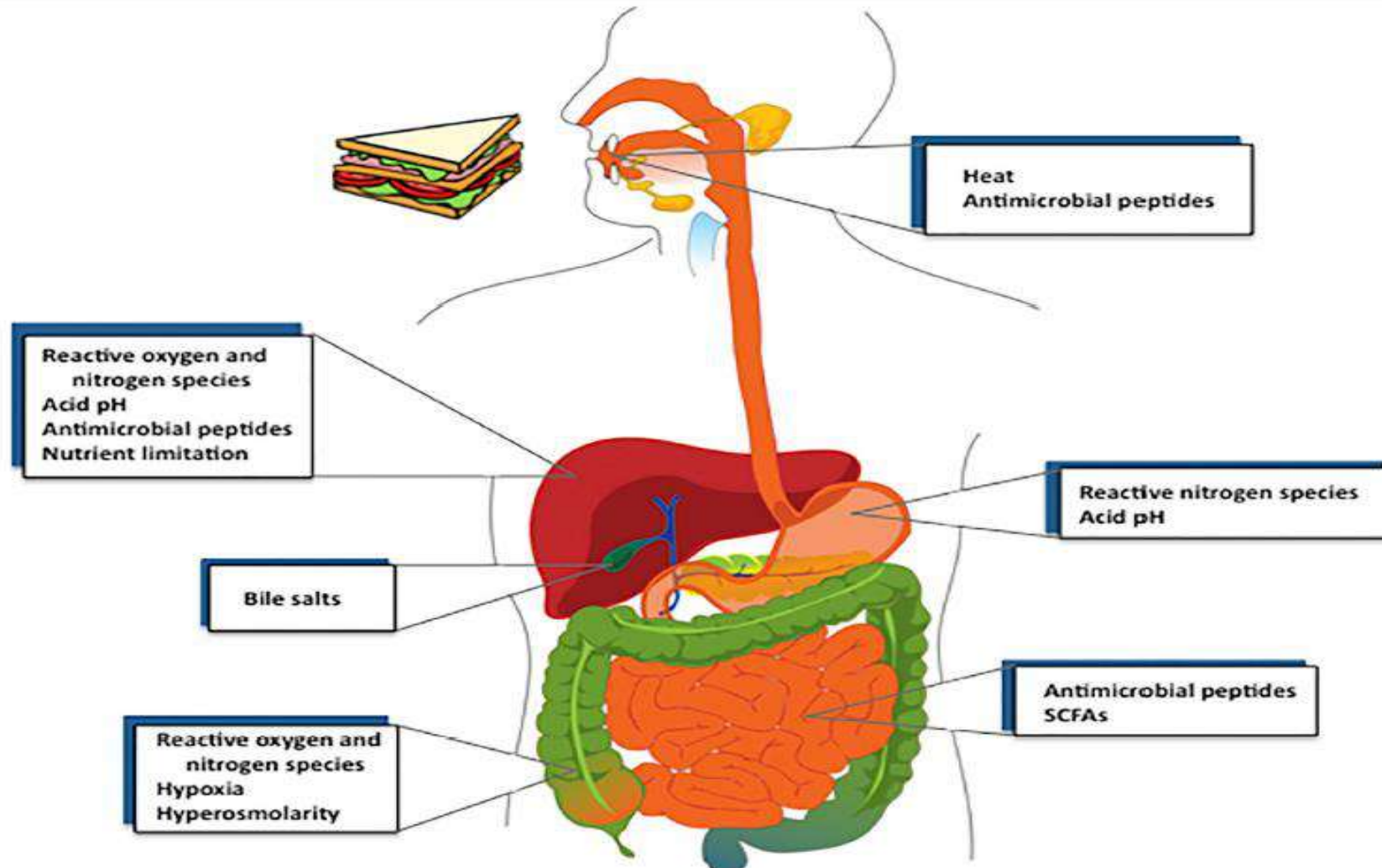
# Salmonella

2500 serotypes aprox.



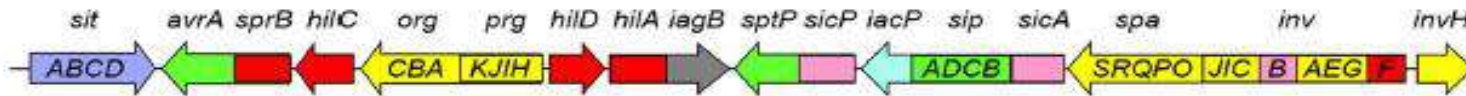
(Sanderson y Nair, 2013)

# Types of *Salmonella* stress in the host

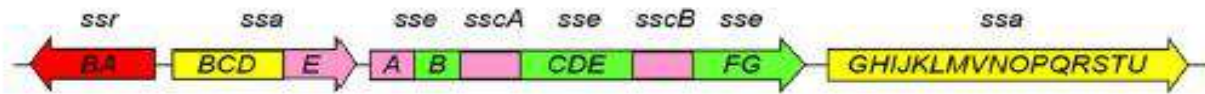


# Pathogenicity islands

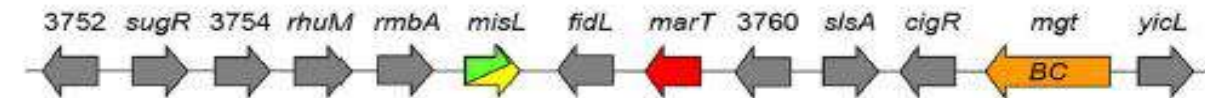
## SPI-1



## SPI-2



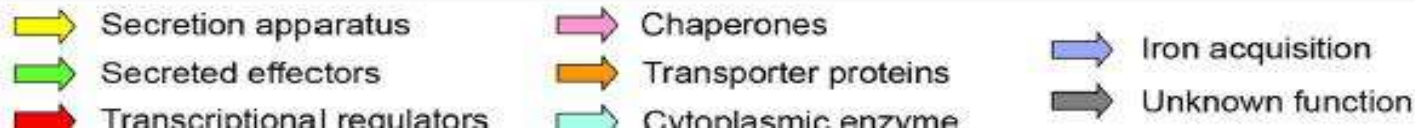
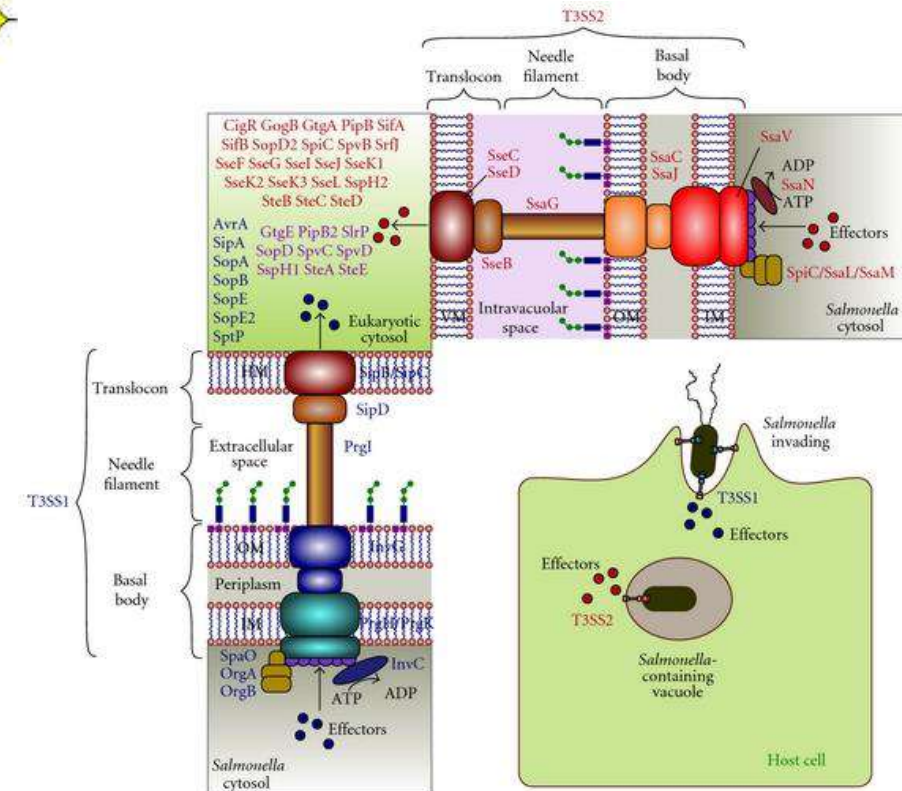
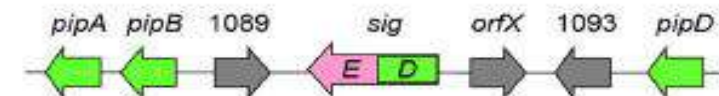
## SPI-3



## SPI-4



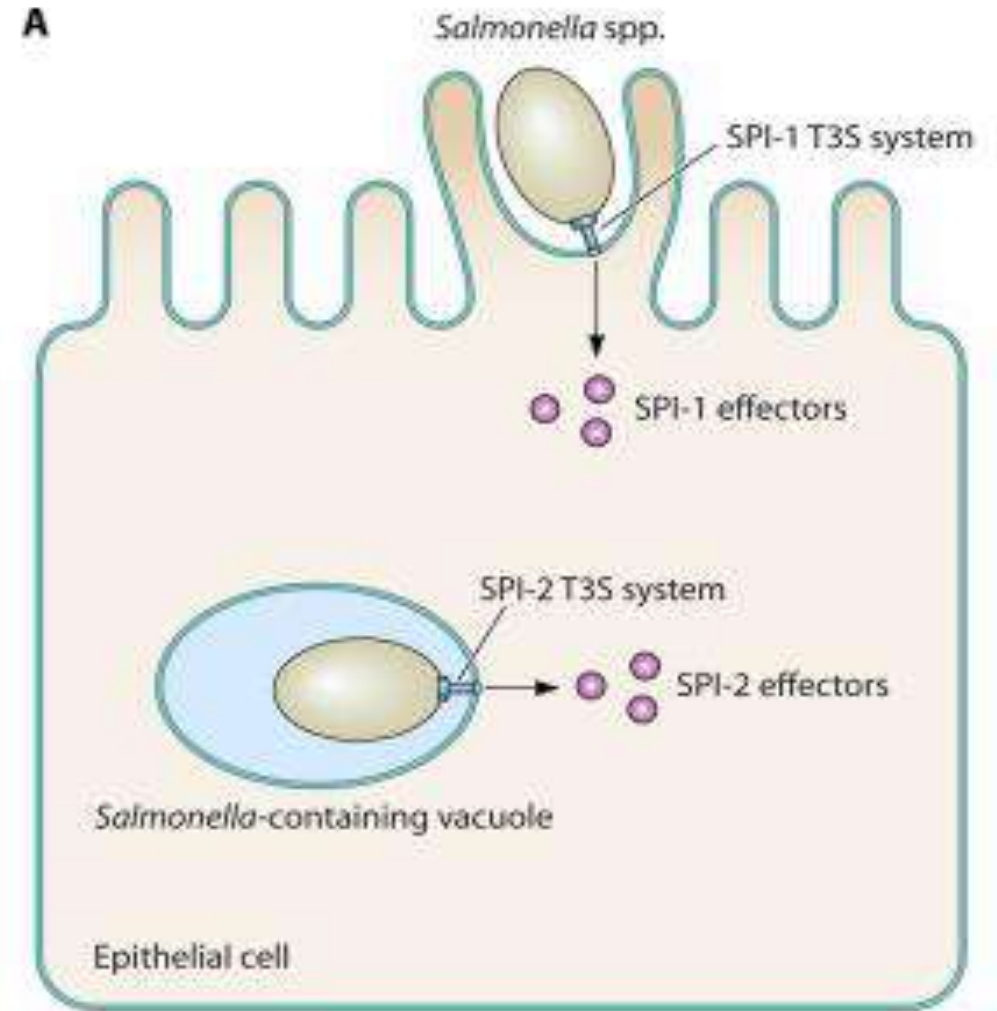
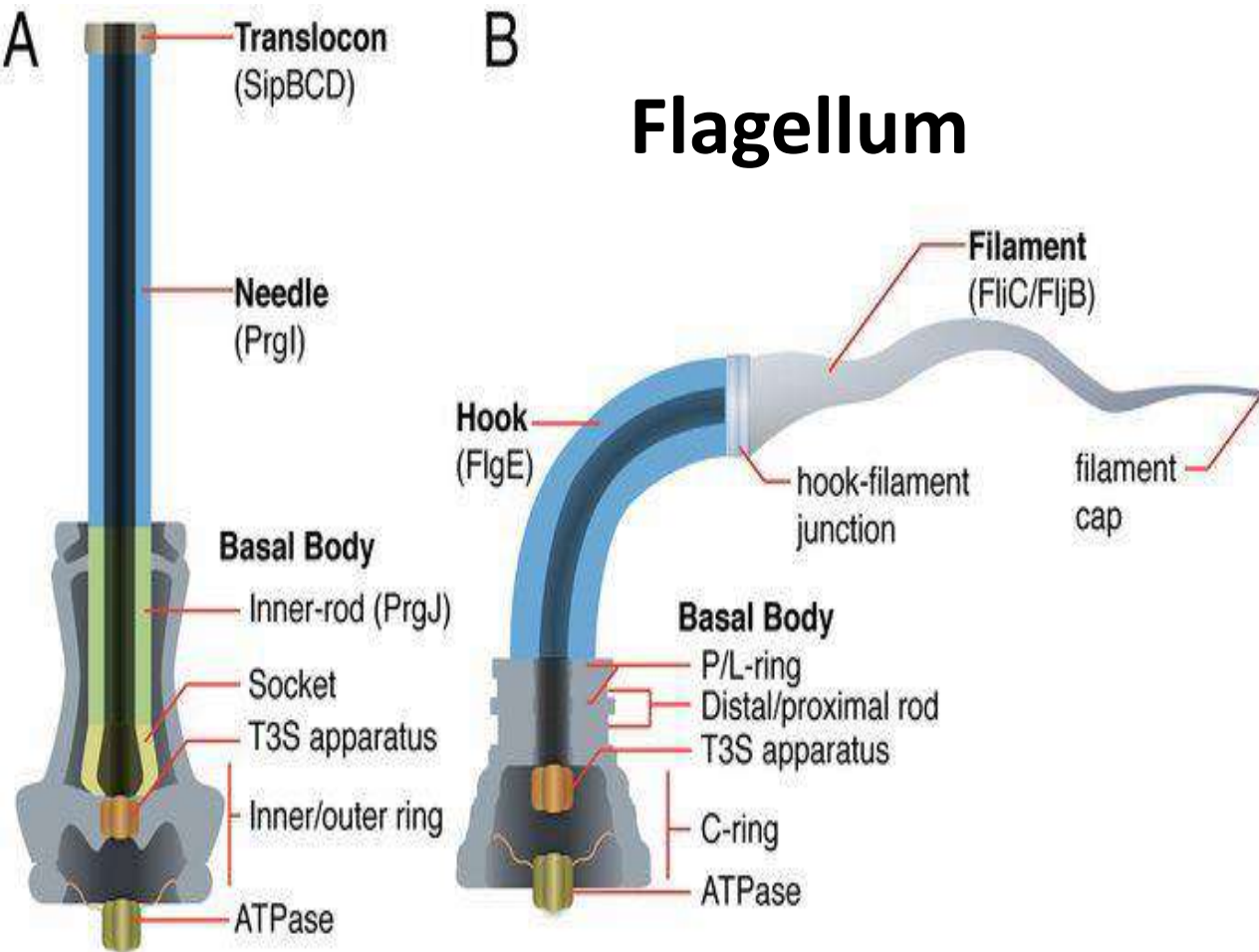
## SPI-5



(Dieyi et al., 2009; Fabrega y Vila, 2013)

# Secretion system type III of *Salmonella*

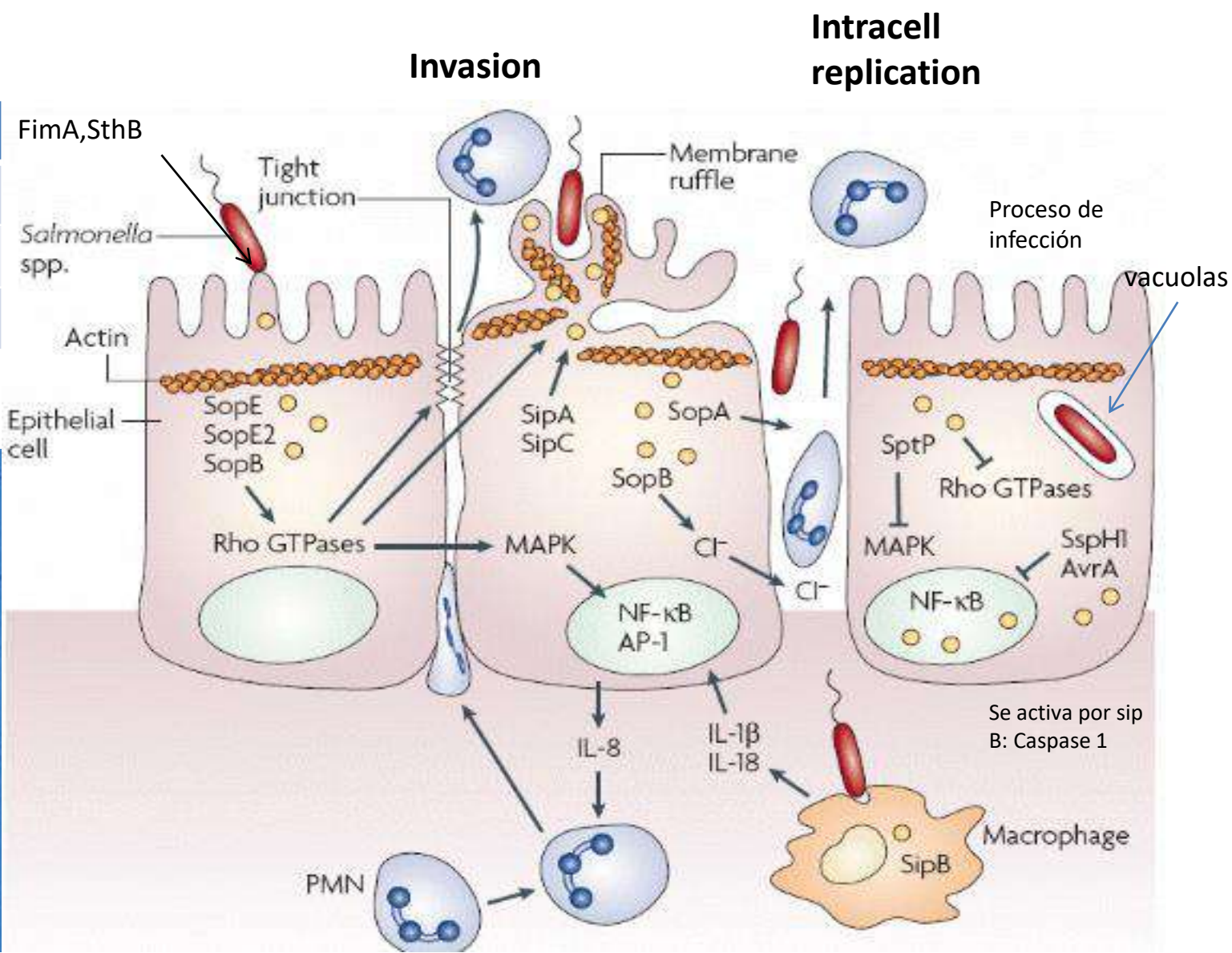
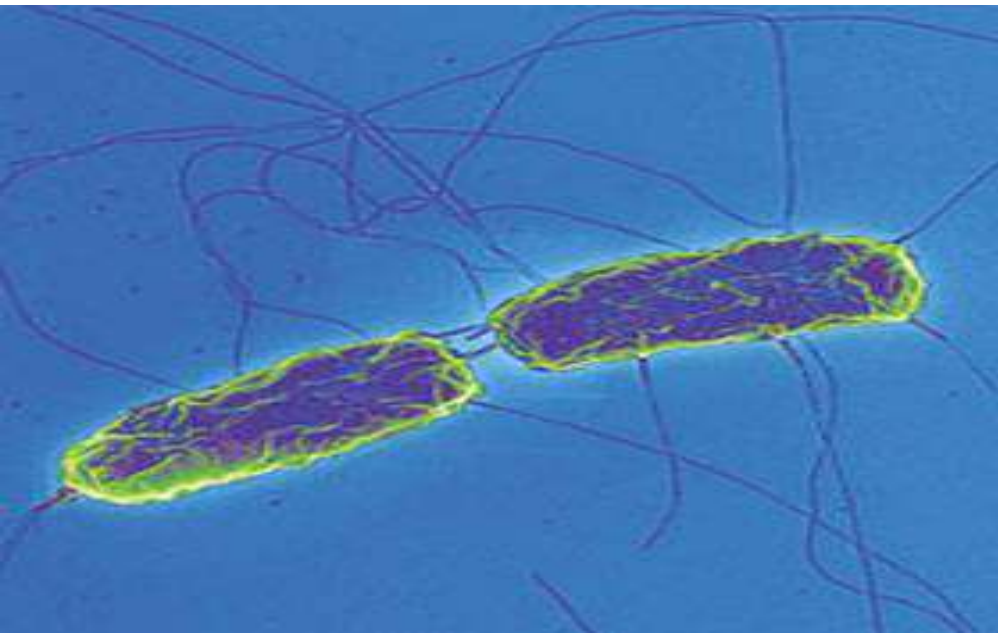
## SST3





# Infection mechanism of *Salmonella* Typhimurium

VIRULENCE GENES	HOUSEKEEPING GENES
InvA	GapA
OrgA	Gnd
FimA	Fbp



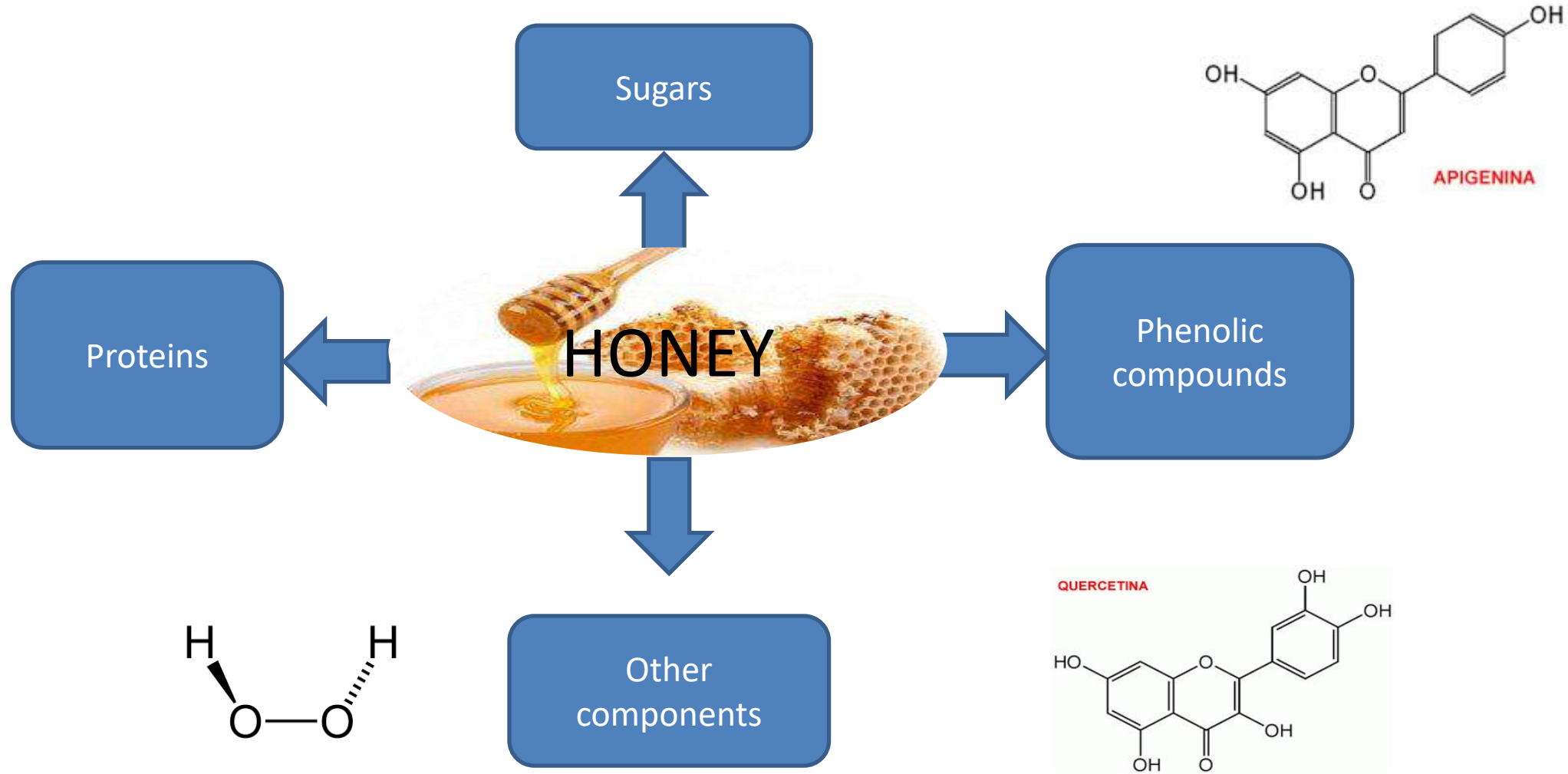
# Antibiotics used against *Salmonella*

Ciprofloxacin (500 mg/12 h), trimethoprim-sulfamethoxazole (160/800 mg/12 h) or amoxicillin-clavulanic acid for 5 days





# Compounds with antimicrobial activity in honey





*Apis mellifera*



*Melipona beecheii*  
(Xunan cab)



*Trigona*  
(*Frieseomelitta*) *nigra*  
Sac Xik

# *Apis mellifera* and stingless bees

Species	<i>Apis mellifera</i>	Stingless bees
Functional sting	yes	no
size	12- 20 mm	1-40 mm
Beehive individuals	30,000 to 40,000	3,000 to 5,000
Honey Production	10 -30 kg/hive	1-2 kg/hive
Selectivity of pecoreo	No	Endemic species
Honey collection	6 times /year	1 time/year

(Gonzalez-Acereto & Quezada-Euan, 2010; Gaytan,2007; Michener,2007)



# Bees distribution

500 stingless bees species, mainly in South America.





# Sample site



Tizimin  
(Trigona)

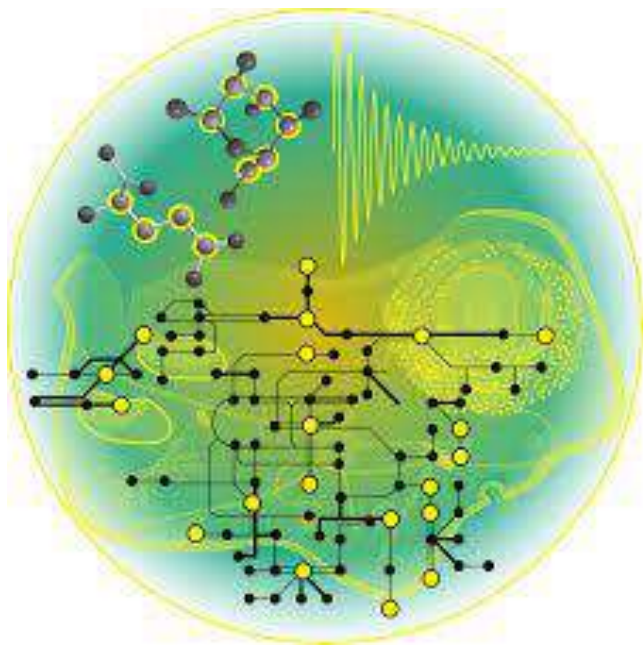


Komchen  
(Apis)



Mani  
(Melipona)





# RESULTS



*mieles de enredaderas y bejucos*  
Obscuras y de sabores fuertes, por su alto contenido de humedad es importante cosecharlas bien maduras para que no fermenten



*mieles de tajonal*  
Abundantes, claras y tiernas, por la composición de sus azúcares tienden a cristalizar rápido



*mieles de ts'its'itche*  
Muy aromáticas, de tonos ámbar y mucho cuerpo, características de la Península



*mieles multiflorales*  
Claras, oscuras, cremosas, delgadas, de sabor intenso, ligeramente saladas, con un toque ácido, ...la variedad es inagotable



*mieles multiflorales de la selva*  
La combinación de néctares de flores de la selva les confiere su intenso sabor y aroma



*mieles de verano*  
Húmedas y fluidas, las últimas de la temporada, no comerciales, se usan para alimentar las colmenas

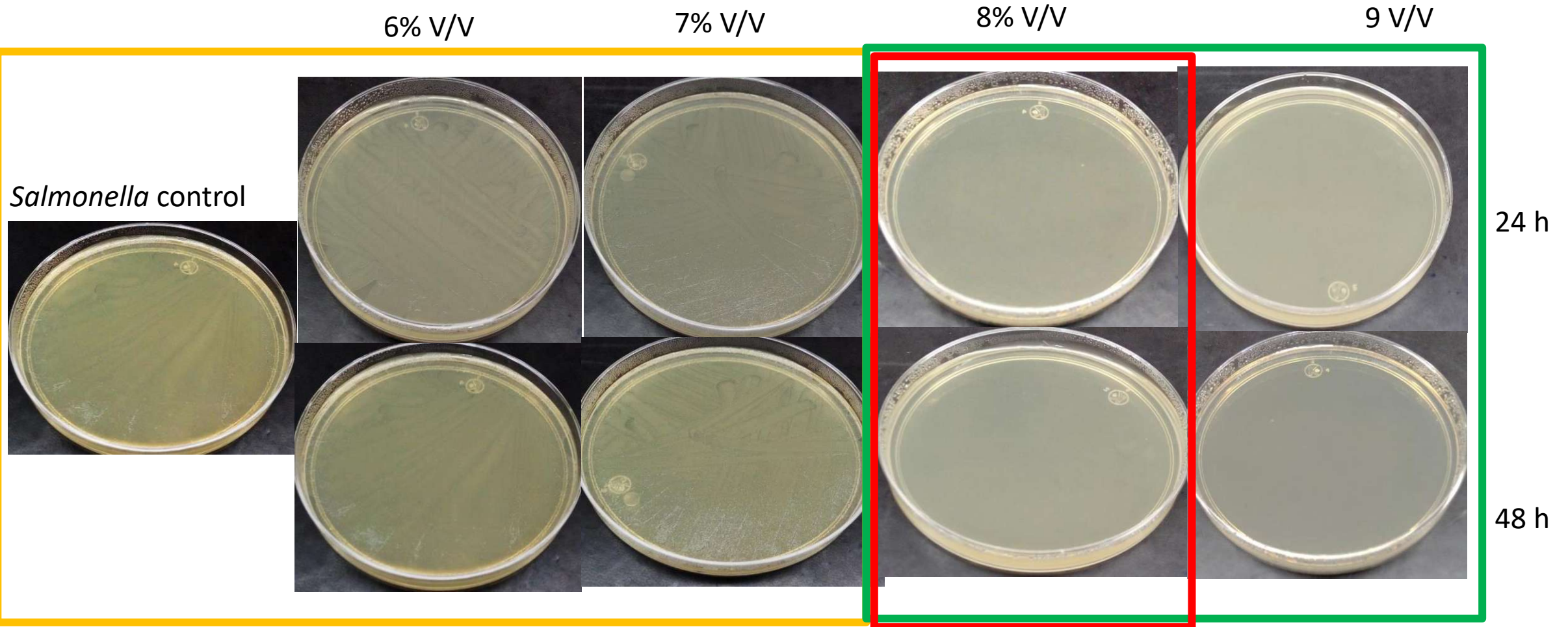


*mieles de abeja melipona*  
Se producen en pequeñas cantidades y se utilizan principalmente con fines medicinales y rituales

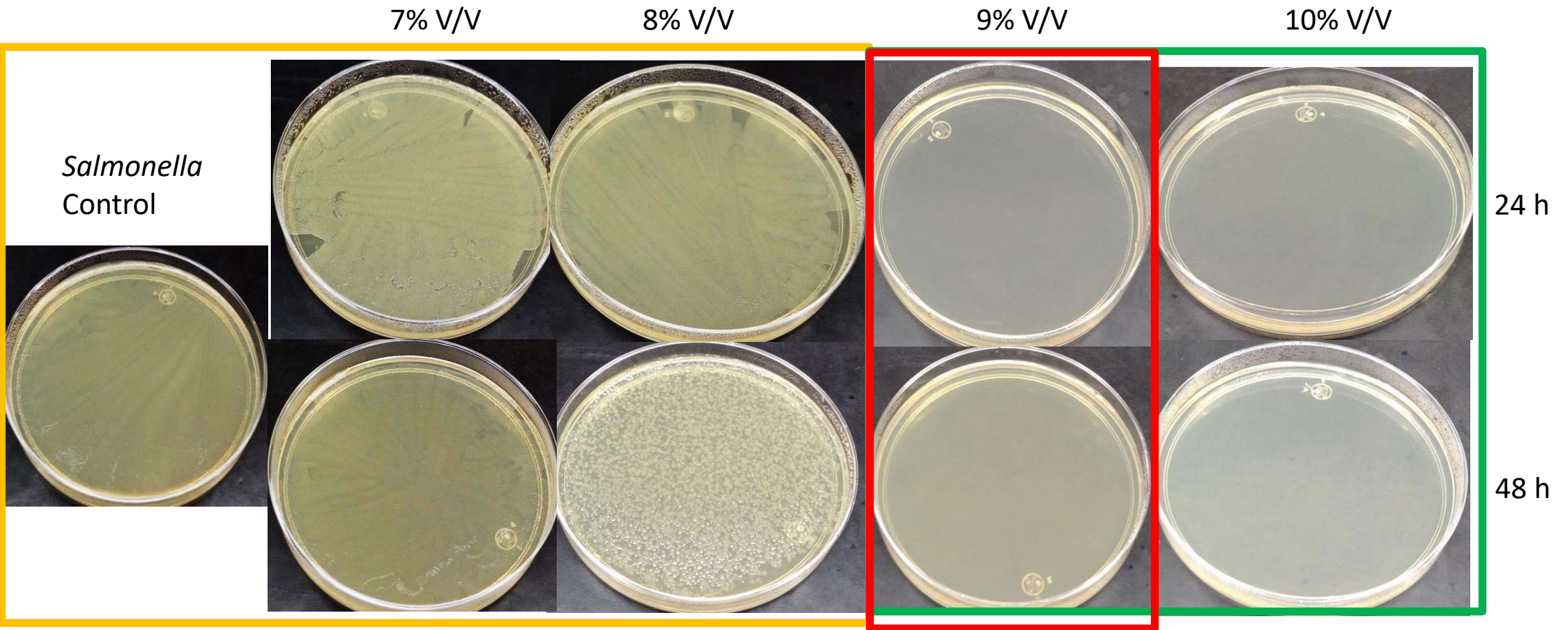
*En su color, aroma y sabor cada miel lleva el sello de su origen*



# Apis honey against *Salmonella*

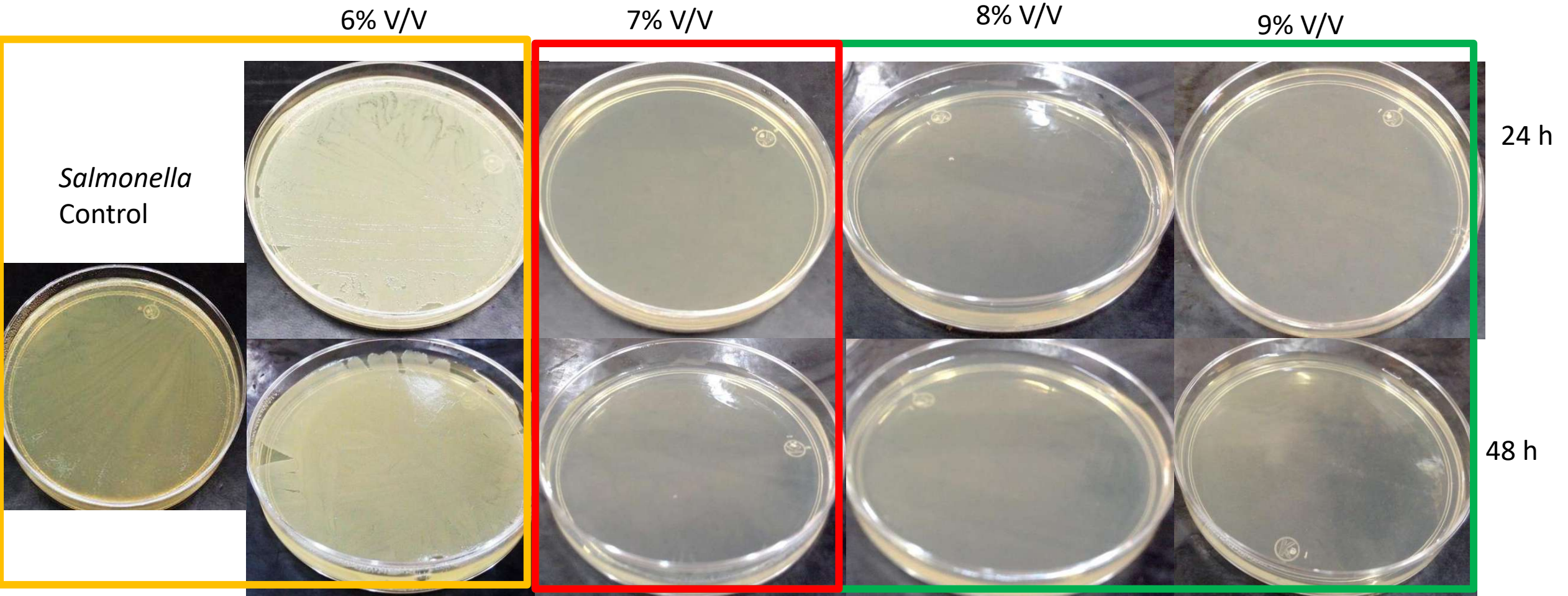


# Melipone honey against *Salmonella*



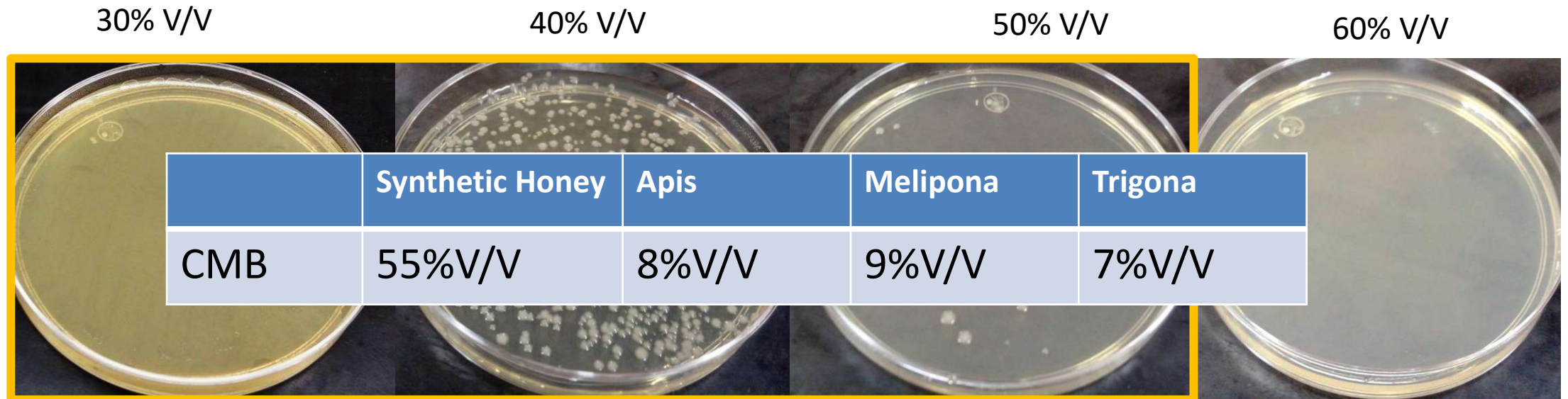


# Trigona honey against *Salmonella*



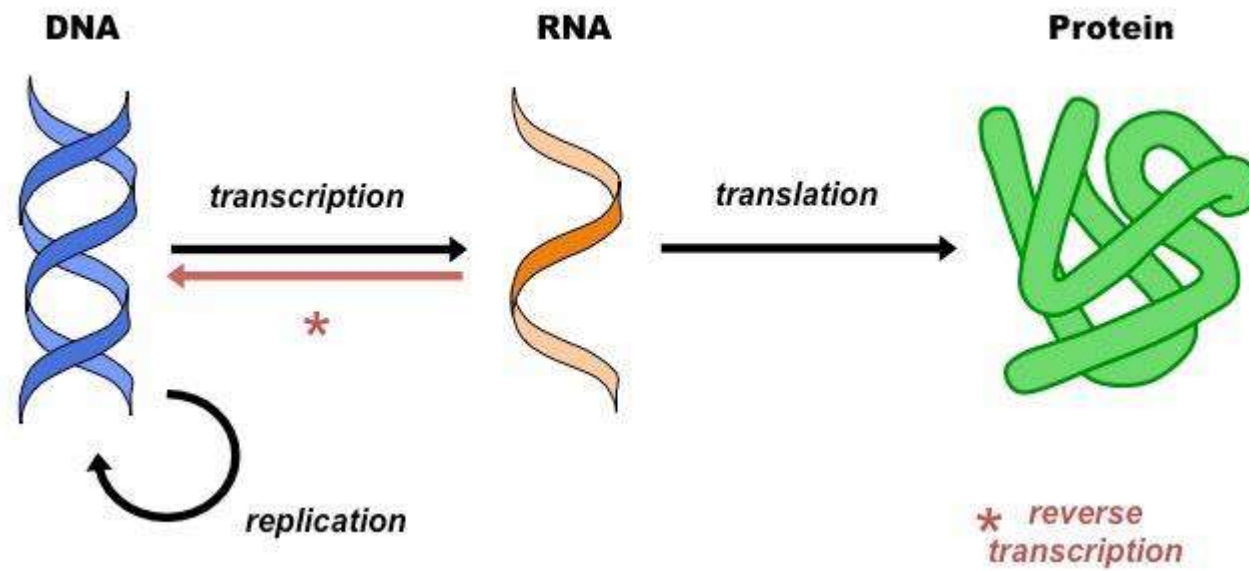


# Synthetic honey against en *Salmonella*



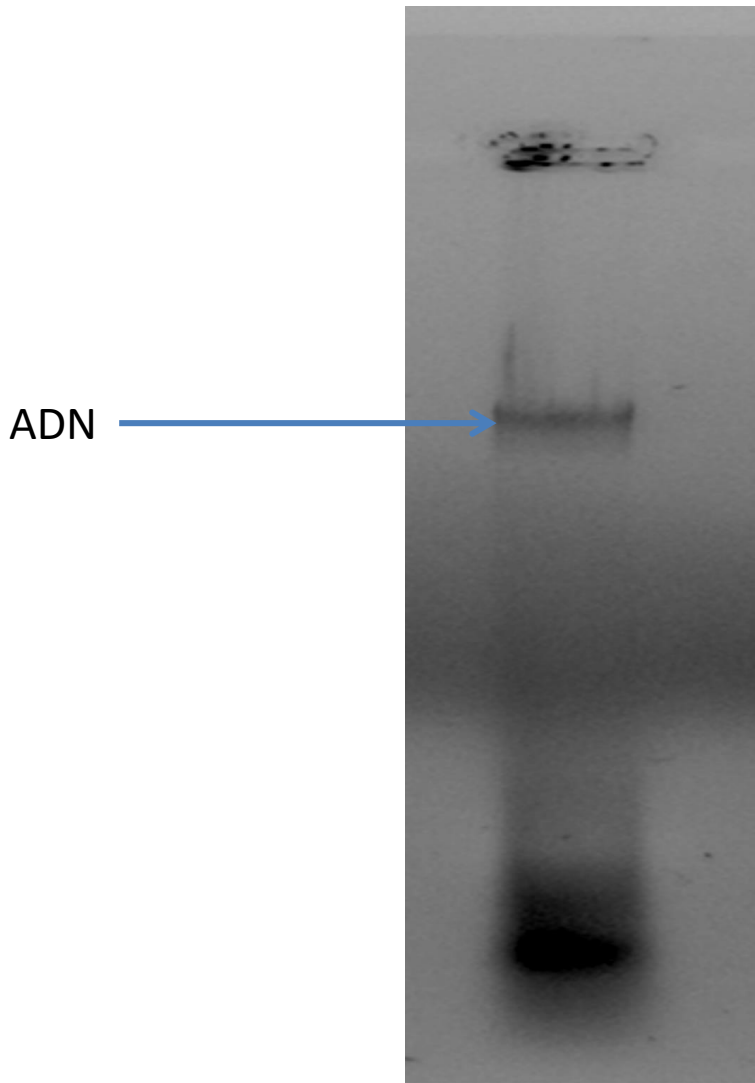
# Honey against *Salmonella* Typhimurium

Honey concentration	Type of honey	Authors
8% , 9%, 7% V/V	Apis, Melipona, Trigona	This study
18% V/V	Trebol (Apis)	Badawy et al., 2004
23% V/V	Avocado(Apis)	Toarmina et al., 2001
34% V/V	Acacia (Apis)	Al- Whari et al., 2013
16%,18%,17%,22% V/V	Conifera, Citricos,Tomillo, Multifloral(Apis)	Viodaruo et al.,2011
50% W/V	Manuka(Apis)	Mundo et al., 2004
10% V/V	<i>Melipona beecheii</i>	Morales et al., 2017
15% P/V	<i>T. carbonaria</i>	Boorn et al., 2009



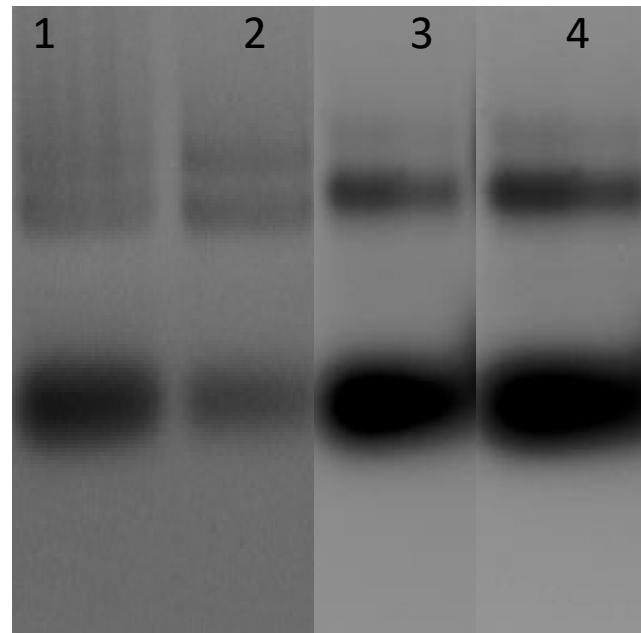


# DNA and RNA



DNA band

Sub-lethal 3% of honey



Carril 1 (control)

Carril 2 (trat. Apis)

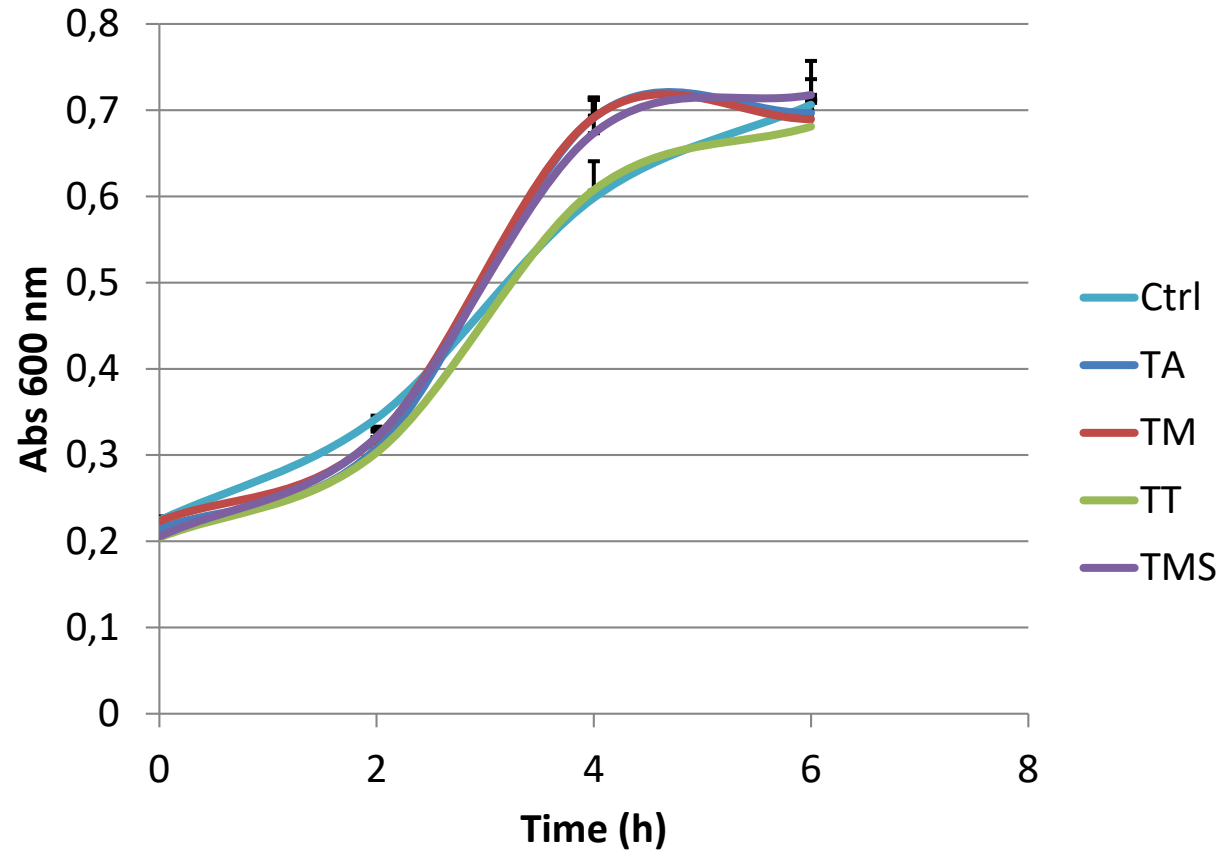
Carril 3 (trat. Melipona)

Carril 4 (trat. Trigona)

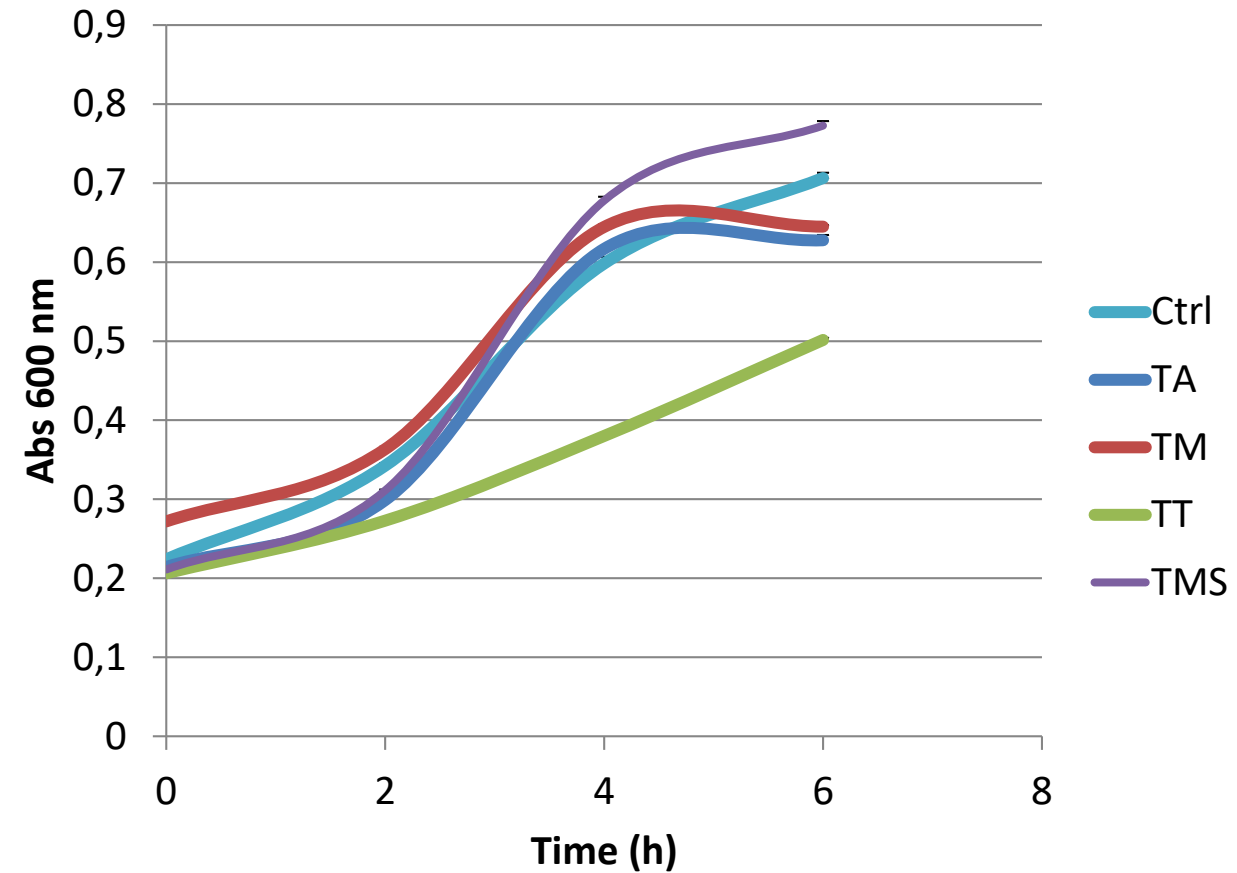
RNA

# Effects of honey on *S. Typhimurium* growth

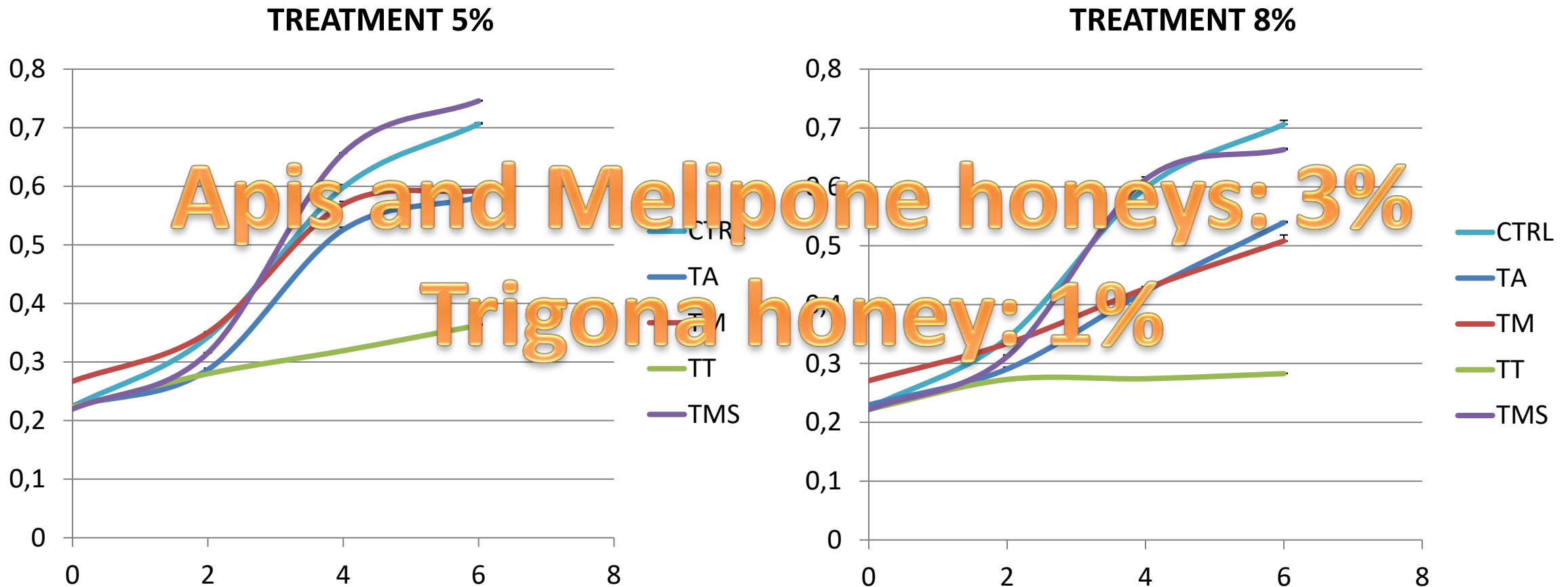
## TREATMENT 1%



## TREATMENT 3%



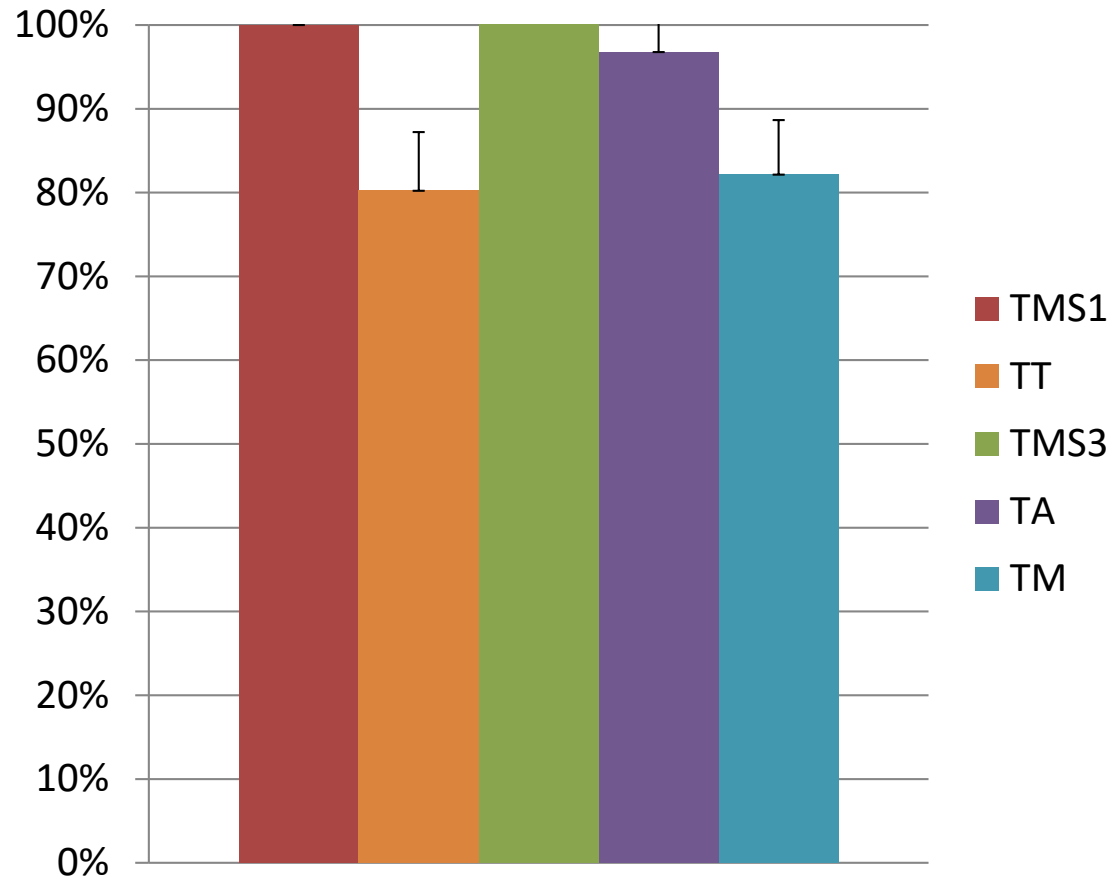
# Effectos of honey on *S. Typhimurium* growth



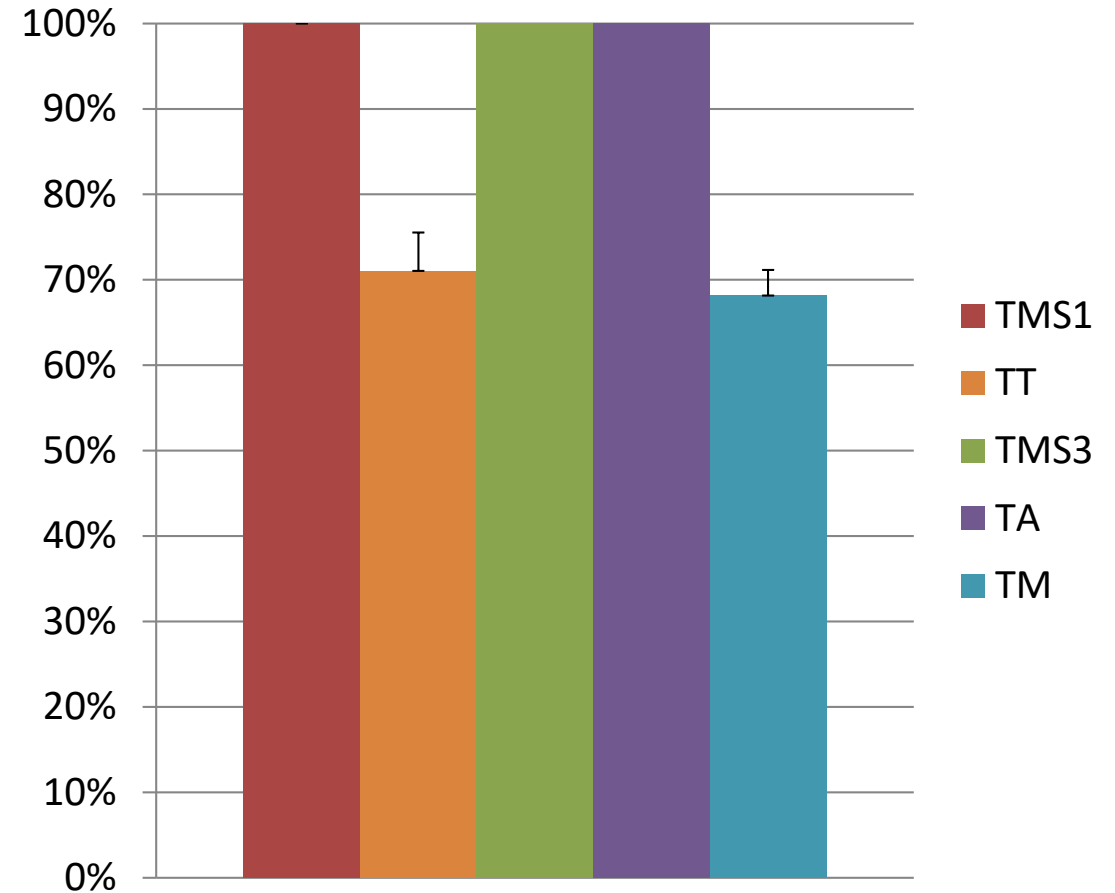


# Expression of virulence genes SPI-1

Gene expression of *sigD-lacZ*

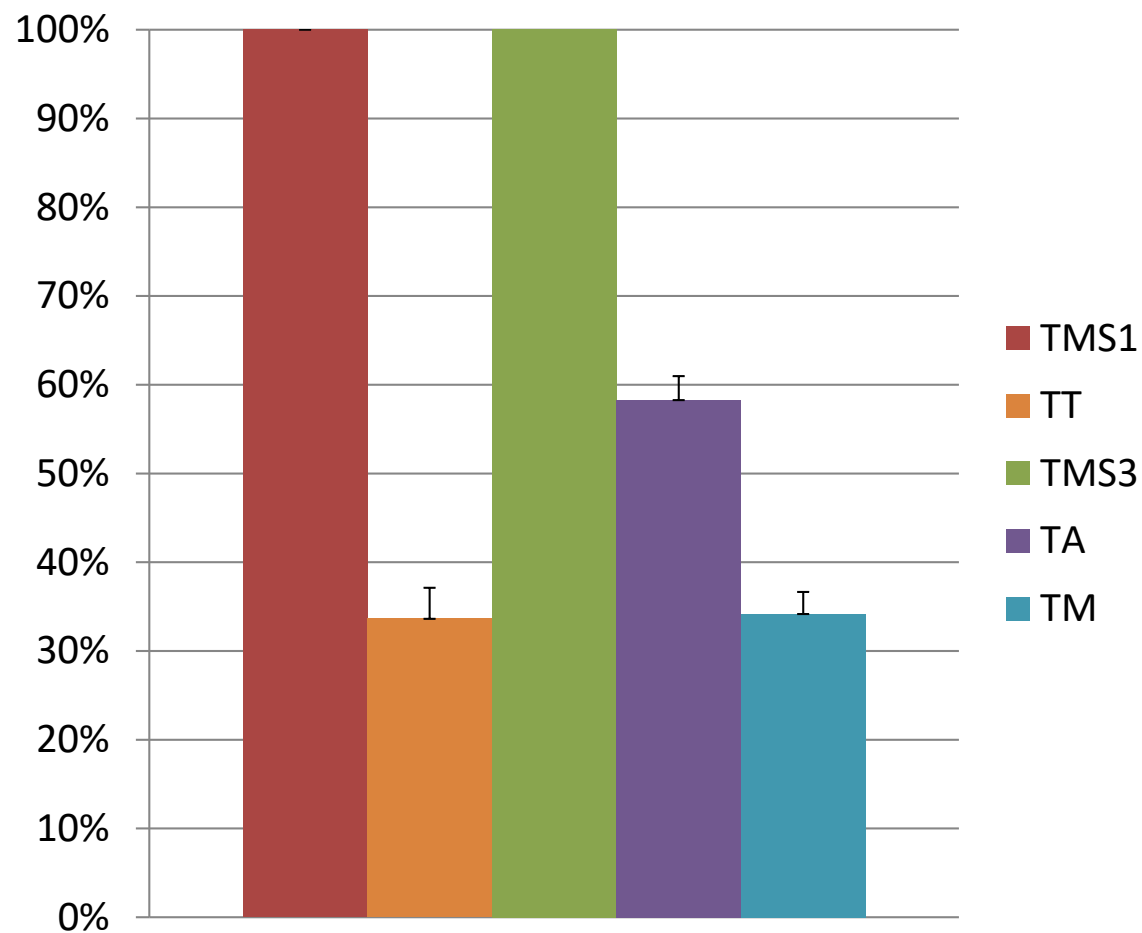


Gen expression of *invF-lacZ*

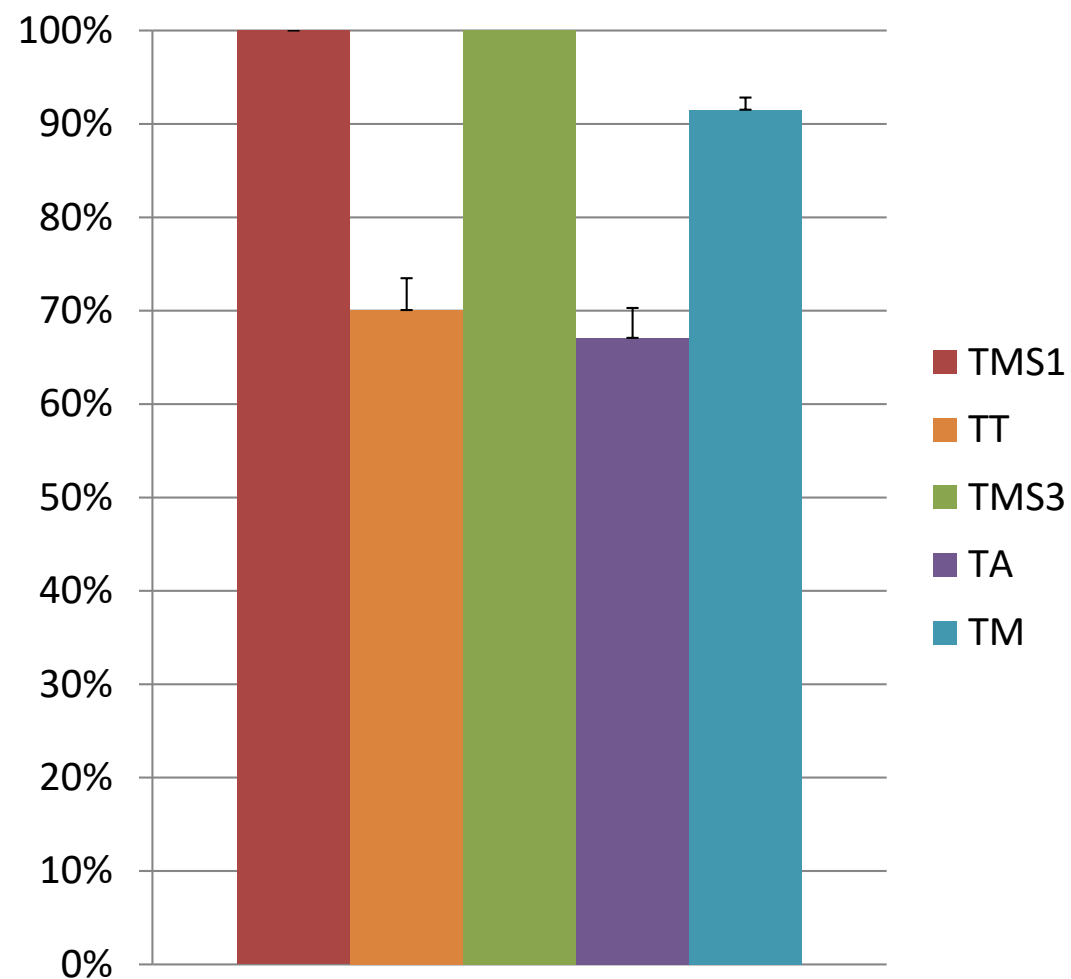


# Gene expresión (flagellum)

Gene expression *fliC-cat*



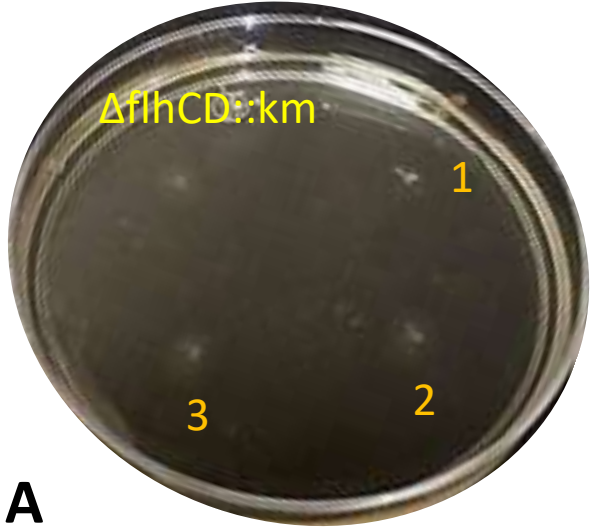
Gene expression *flhDC-cat*



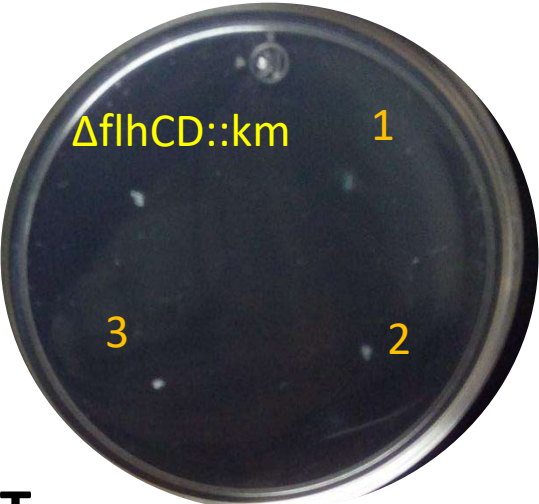
# Motility of *S. Typhimurium*



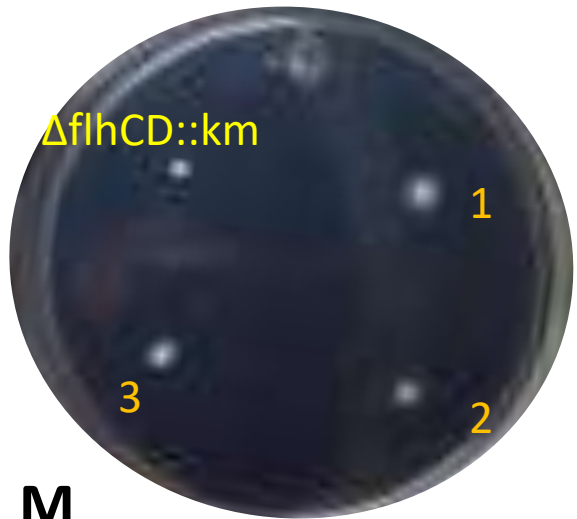
SH



A



T



M

TREATMENT	Diameter (mm)	% motility
Synthetic honey	25.7	100
APIS 3%	5	14
MELIPONA 3%	2	8
TRIGONA 1%	1	4



# Conclusions

- Apis, Melipona and Trigona (Frieseomelitta) honeys decreased the motility and gene expression of the virulence and flagellum proteins at 3% V/V, 3%V/V and 1%V/V, respectively.
- Trigona (Frieseomelitta) honey was the more efective against S. Typhimurium.