

CHARACTERIZATION OF PROBIOTIC POTENTIAL OF *Lactobacillus sp* ISOLATED FROM *Apis mellifera* BEE BREAD FOR DEVELOPMENT OF FUNCTIONAL FOOD

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GASTROINTESTINAL PROBLEM

**Pathogen
microorganisms**

Beneficial microorganisms

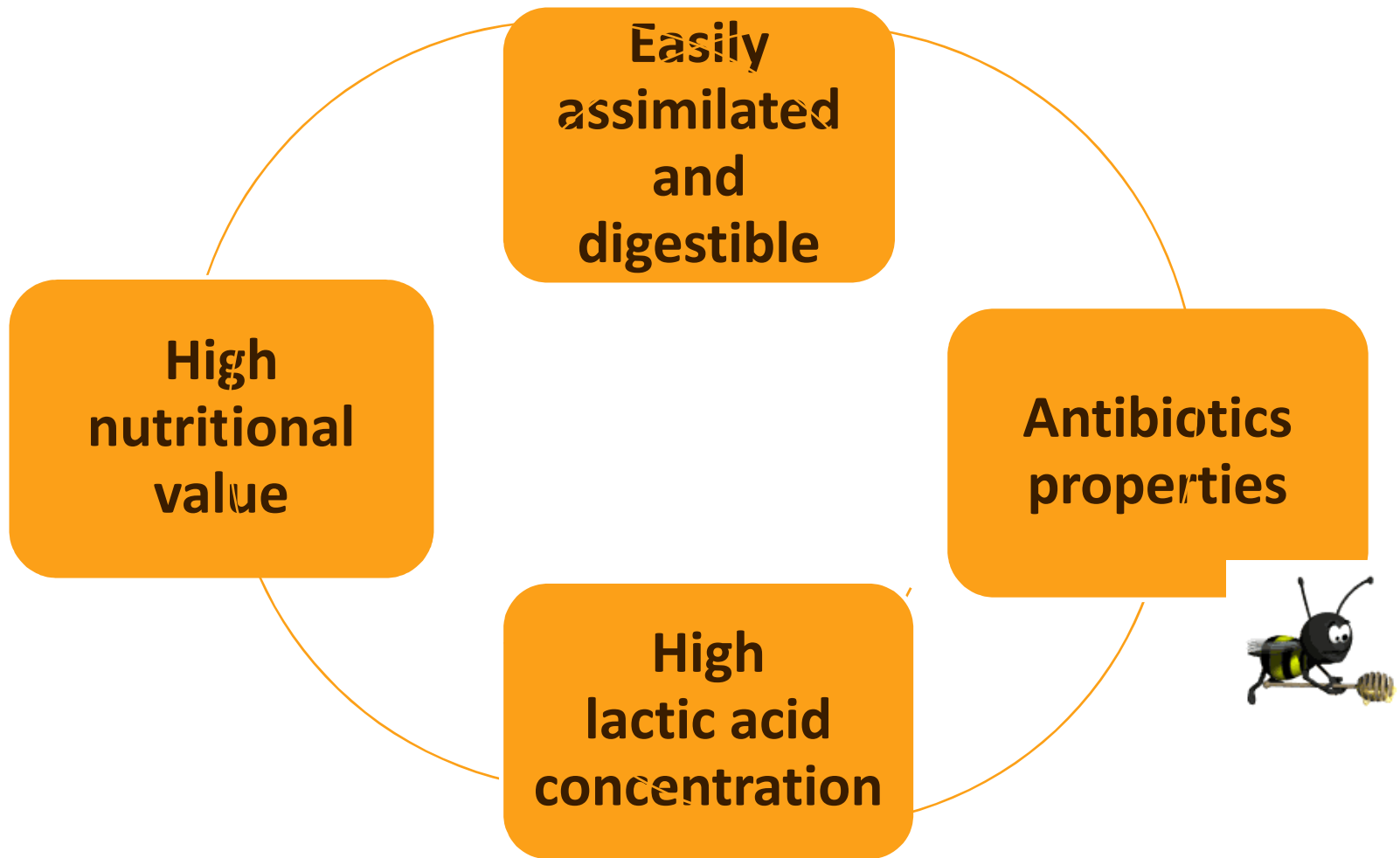
LACTIC ACID BACTERIA

Preventive therapy against microorganisms

GUIDELINES FOR THE EVALUATION OF PROBIOTICS

BEE PRODUCTS SOURCE OF MICROBIAL DIVERSITY

BEE BREAD



BEE BREAD

Microorganisms isolates

- ☐ *Lactobacillus*
- ☐ *Streptococcus*
- ☐ *Pseudomonas*
- ☐ *Eschericia*
- ☐ *Bacillus*
- ☐ Yeast (*Sacharomyces*)

LACTOBACILLUS

- **Domain: Bacteria**
- **Phylum: Firmicutes**
- **Class: Bacilli**
- **Order: Lactobacillales**
- **Family: Lactobacillaceae**
- **Genus: *Lactobacillus***

LACTOBACILLUS

MORPHOLOGICAL CHARACTERISTICS

- ***Bacillis have different sizes and thickness**
- ***Non-spore forming**
- ***No flagella**

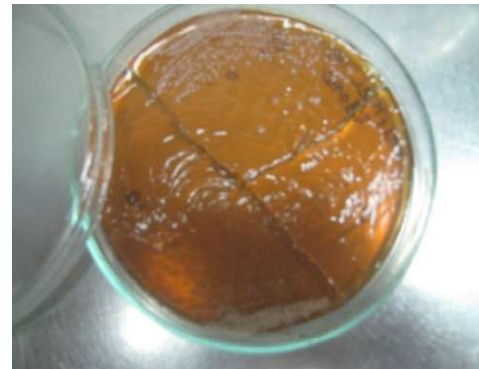
BIOCHEMICAL CHARACTERISTICS

- ***Catalase negative**
- ***Cytochrome oxidase negative**
- *** CO₂ Atmosphere**
- ***Growth at low pH and T° 30 – 40 °C**

OBJECTIVES

- Isolate and Characterize *Lactobacillus* from bee bread of *Apis mellifera*.
- Evaluate the probiotic potential of *Lactobacillus in vitro*.
- Compare the fermentative behavior of isolated strain vs commercial strain *Lactobacillus acidophilus* recognized as probiotic, in the natural substrate bee pollen for industrial application.

- **Culture media used. Man Rogosa Sharpe Agar (MRS A)**
- Honey 5%

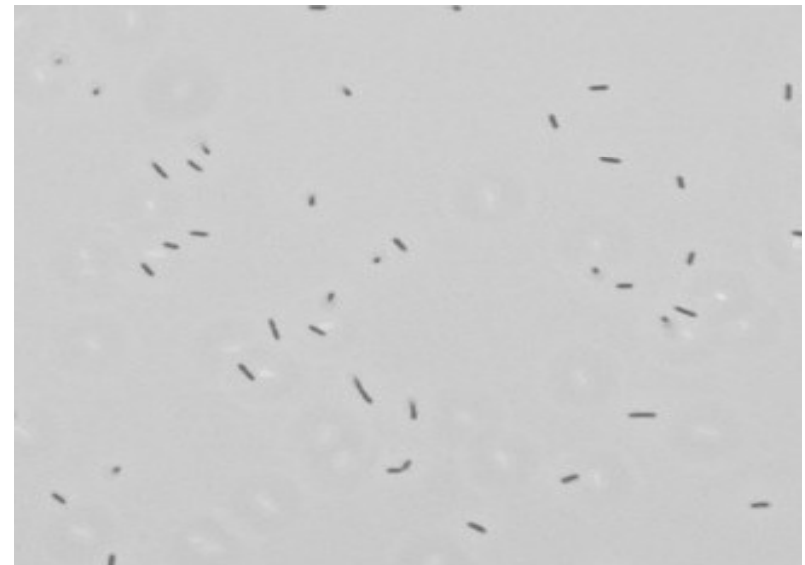


- Pollen 5%





- ***MICROBIAL IDENTIFICATION***

- **Gram Stain**



OBTAINING AND CONSERVATION

- Were obtained eleven strains
- Morfology 
 - Gram Stain
 - Spores Stain (Shaeffer Foulton)
- Biochemicals Test 
 - Catalase
 - Oxidase
- Conservation in MRS broth with 20 % Glycerol and maintained -20°C

***RESULTS OF MORFOLOGY AND
BIOCHEMICAL TEST***

ISOLATES	GRAM	SPORES	CATALASE	OXIDASE
1	BGP	NEGATIVE	NEGATIVE	NEGATIVE
2	BGP	NEGATIVE	NEGATIVE	NEGATIVE
3	CBGP	NEGATIVE	NEGATIVE	NEGATIVE
4	CBGP	NEGATIVE	NEGATIVE	NEGATIVE
5	CBGP	NEGATIVE	NEGATIVE	NEGATIVE

***RESULTS OF MORFOLOGY AND
BIOCHEMICAL TEST***

ISOLATES	GRAM	SPORES	CATALASE	OXIDASE
6	CBGP	NEGATIVE	NEGATIVE	NEGATIVE
7	CBGP	NEGATIVE	NEGATIVE	NEGATIVE
8	BGP	NEGATIVE	NEGATIVE	NEGATIVE
9	CBGP	NEGATIVE	NEGATIVE	NEGATIVE
10	CBGP	NEGATIVE	NEGATIVE	NEGATIVE
11	CBGP	NEGATIVE	NEGATIVE	NEGATIVE

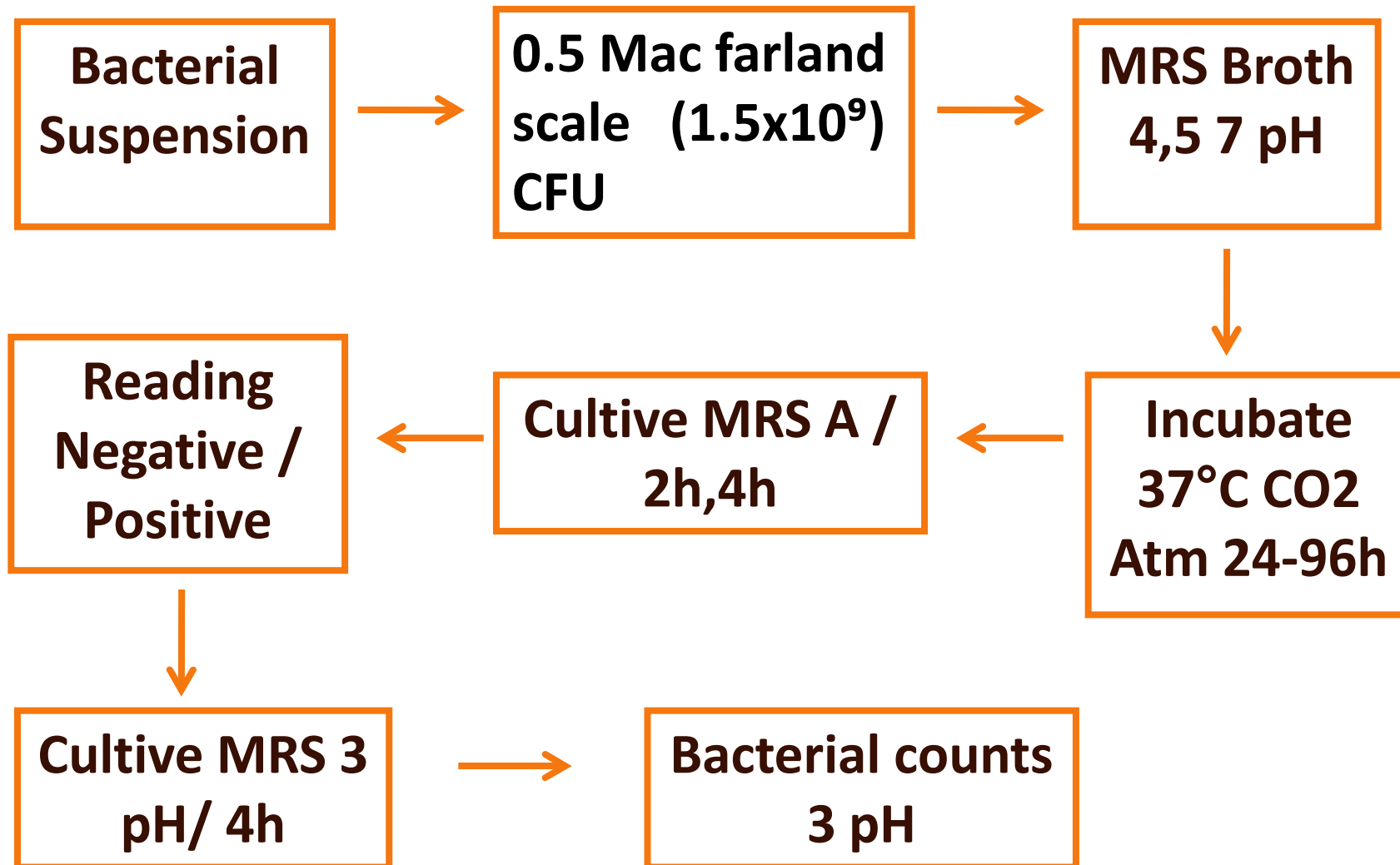
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ASSESSMENT OF PROBIOTIC POTENTIAL

- Acid pH tolerance (3,4 and 5)
- Growth in bile (0.3, 0.5 and 1.0%)
- Hemolytic activity
- Antibacterial activity against reference strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*)
- Sensitivity to antibiotics
- Molecular Identification

Acid pH Tolerance



Results Acid pH Tolerance

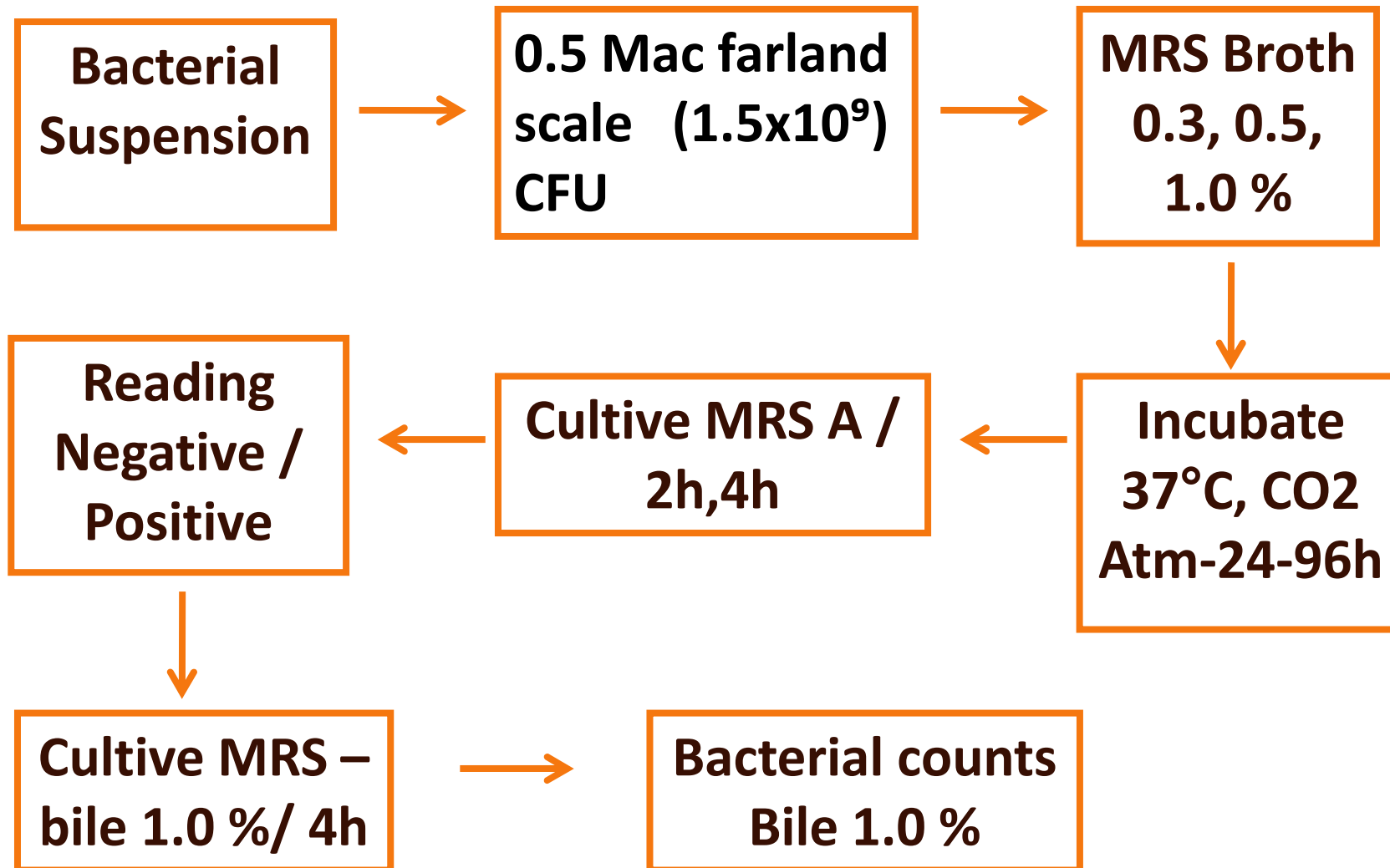
(positive/negative) 4h / bacterial counts CFU/mL

STRAIN	pH 3.0	pH 4.0	pH 5.0	pH 7.0
1-7	POSITIVE	POSITIVE	POSITIVE	POSITIVE
8	NEGATIVE	POSITIVE	POSITIVE	POSITIVE
9-11	POSITIVE	POSITIVE	POSITIVE	POSITIVE

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Growth in bile



Results Growth in bile

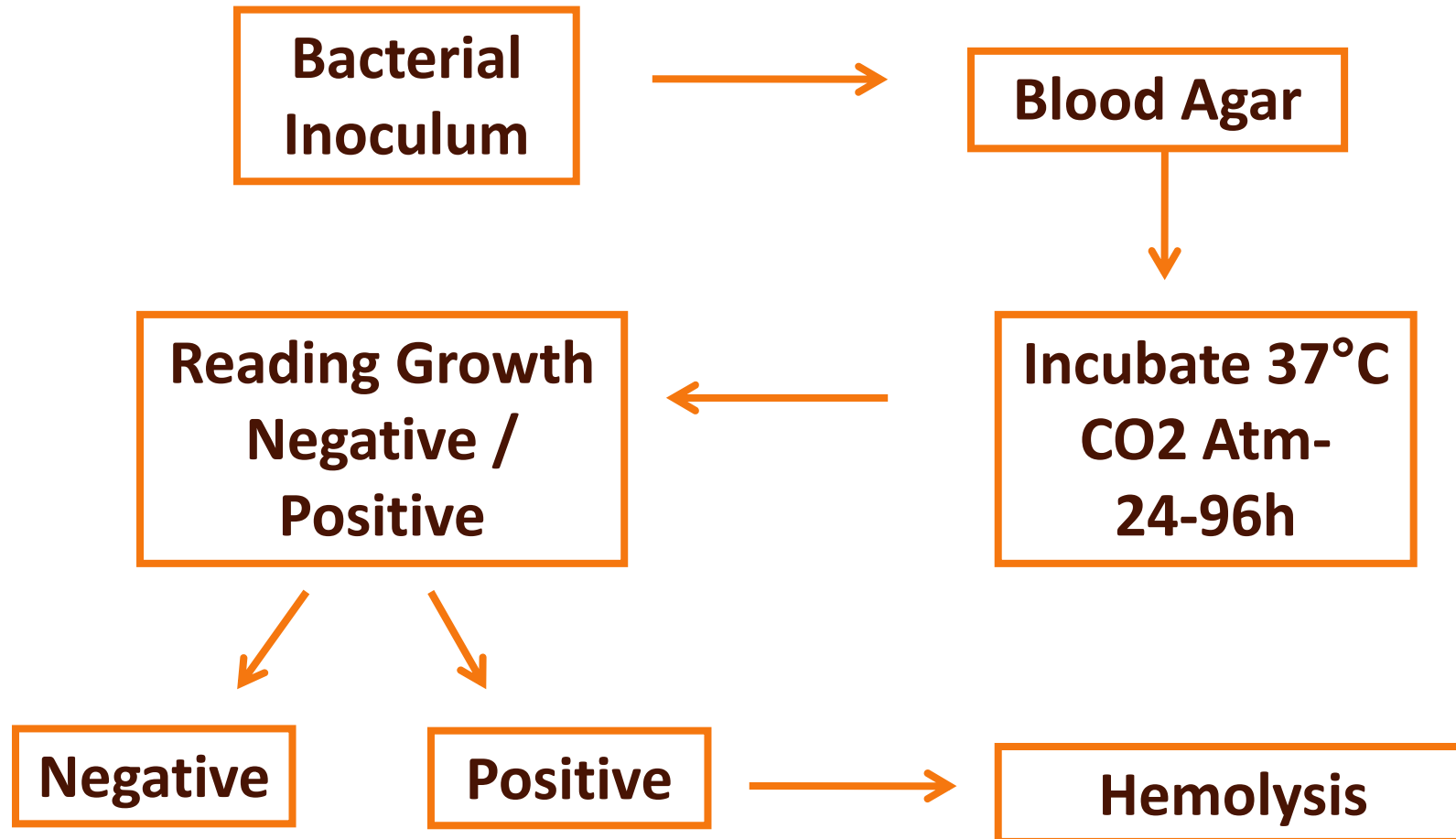
(positivo/negativo) 4h

AI SLADOS	0.3%	0.5%	1.0 %
1-7	POSITIVO	POSITIVO	POSITIVO
8	POSITIVO	POSITIVO	NEGATIVO
9	NEGATIVO	NEGATIVO	NEGATIVO
10	POSITIVO	POSITIVO	POSITIVO
11	POSITIVO	POSITIVO	POSITIVO

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Hemolytic activity



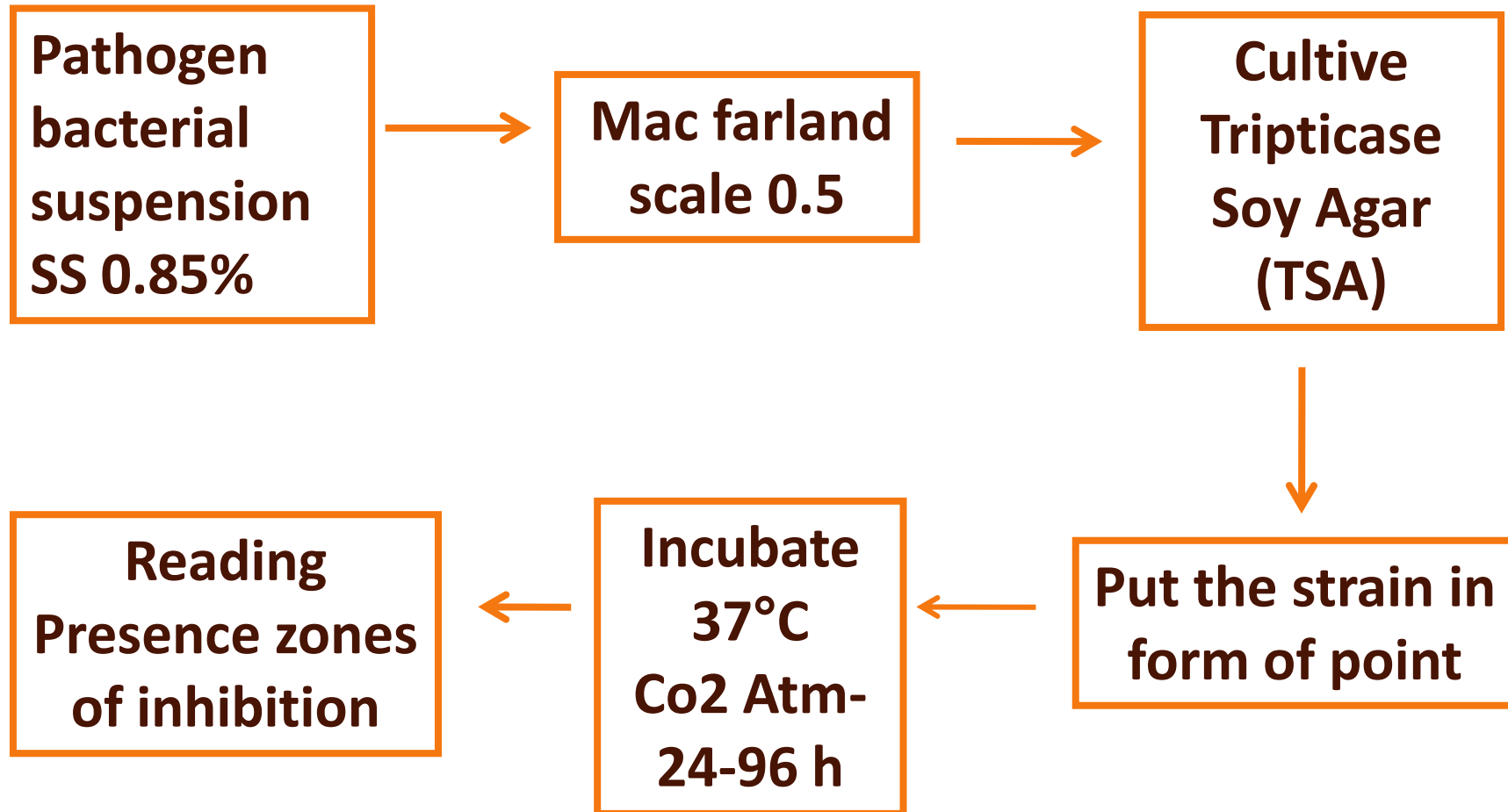
Results Hemolytic activity

ISOLATES	GROWTH	HEMOLYSIS
1-4	NEGATIVE	
5	POSITIVE	δ
6	POSITIVE	δ
7	POSITIVE	δ
8	NEGATIVE	
9	NEGATIVE	
10	POSITIVE	δ
11	POSITIVE	δ

ASSESSMENT OF PROBIOTIC POTENTIAL

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Antibacterial activity



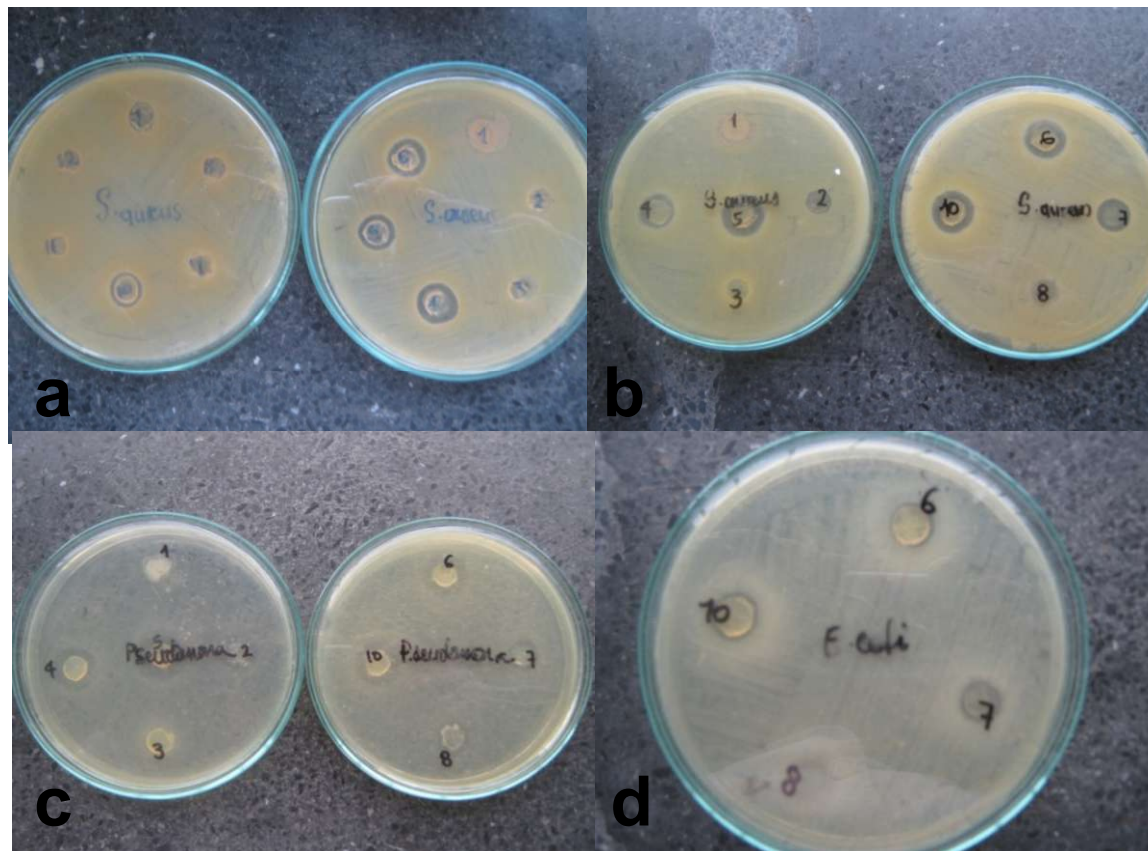
Results Antibacterial activity

Reference strain Isolated	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>B. subtilis</i>
1	Si	Si	No	No
2	Si	Si	No	No
3	Si	No	No	No
4	Si	Si	No	No
5	Si	Si	No	No

Results Antibacterial activity

Reference strain Isolated	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>B. subtilis</i>
6	Si	No	No	No
7	Si	Si	Si	No
8	No	No	No	No
9	No	Si	No	No
10	Si	Si	Si	No
11	Si	Si	Si	No

Results Antibacterial activity



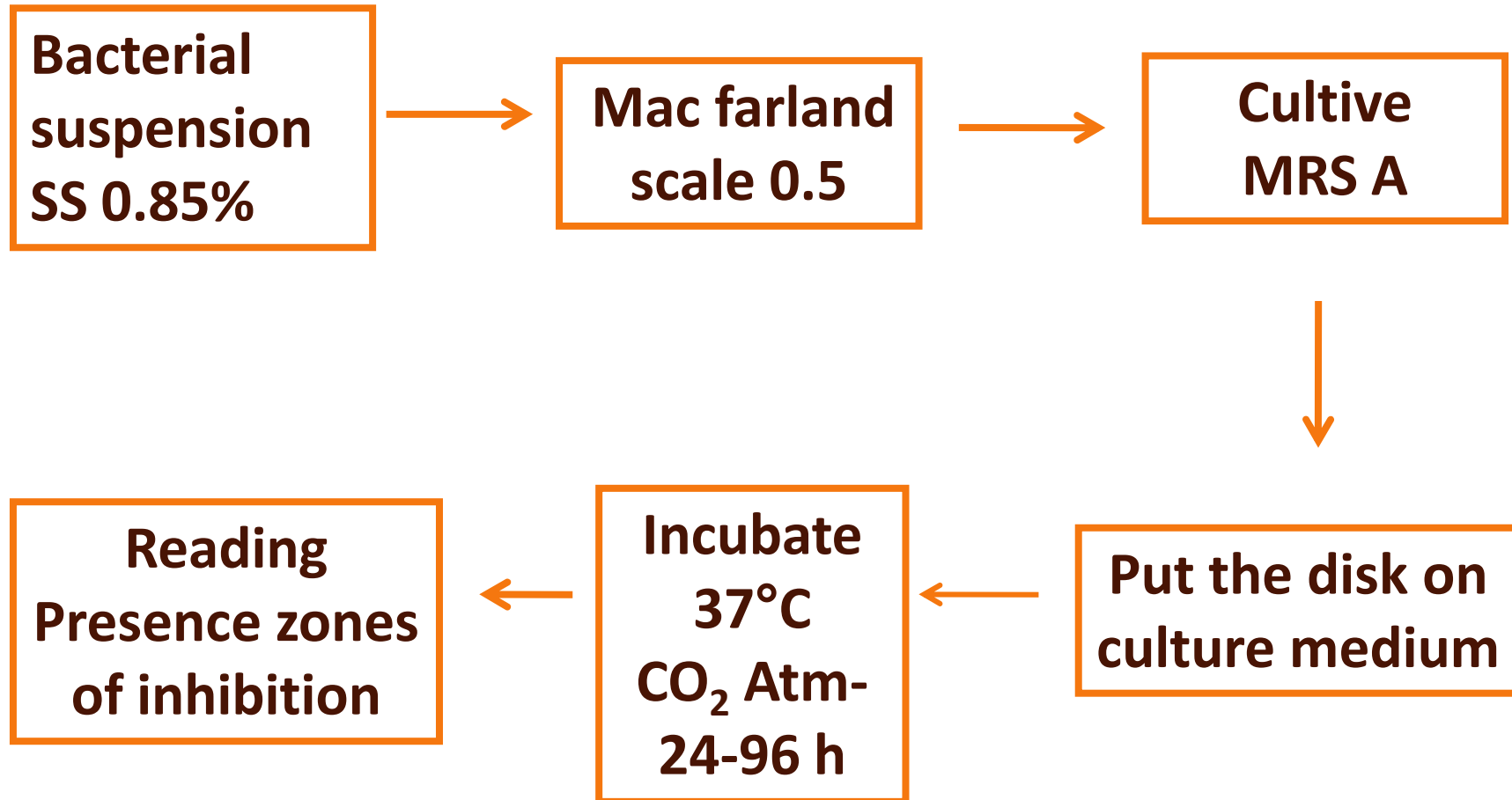
Antibacterial activity. **a.** y **b.** vs *S. aureus*,
c. vs *P. aeruginosa* **d.** vs *E. coli*

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Sensitivity to antibiotics

(disk diffusion method)



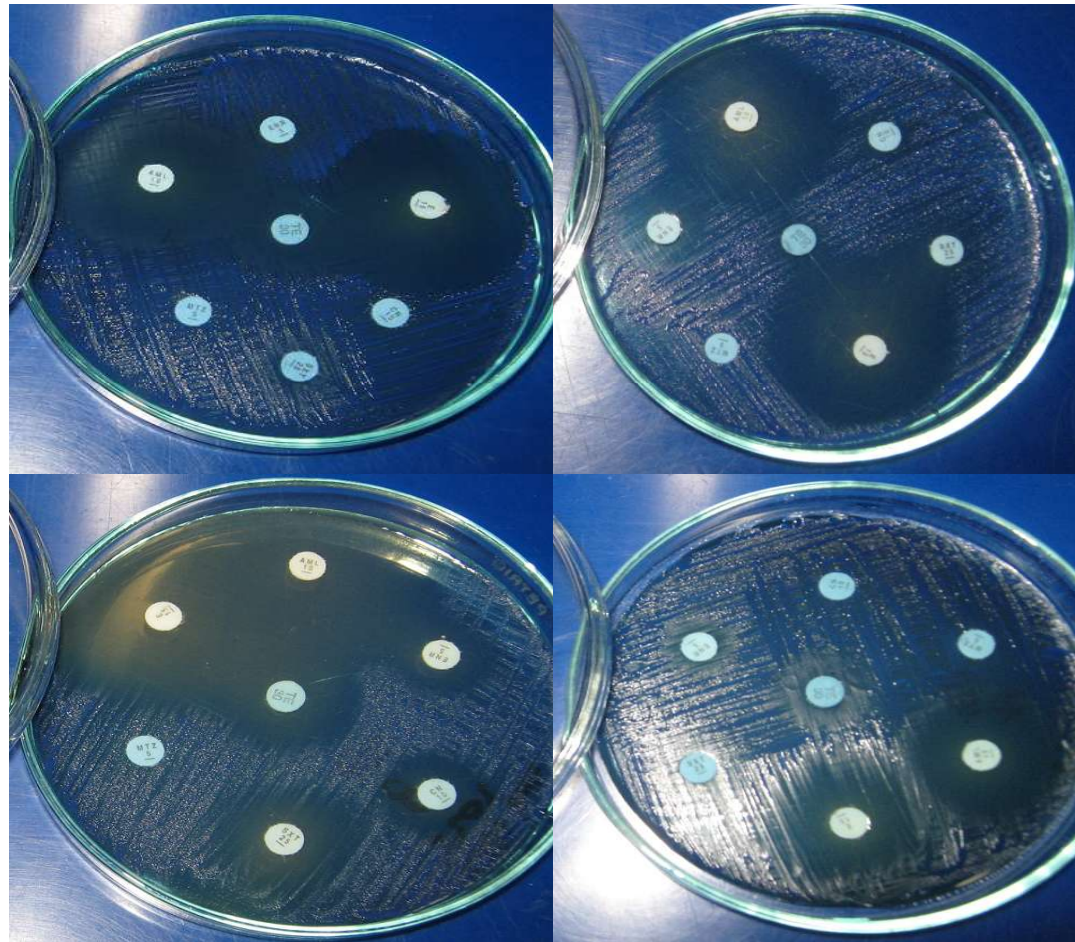
Sensitivity to antibiotics

(disk diffusion method)

ZONES OF INHIBITIONS							
STRAIN	E (15µg)	CN (10 µg)	TE (30 µg)	AML (10 µg)	SXT (25 µg)	MTZ (5 µg)	ENR (5 µg)
5	S (20)	R	S (10)	S (22)	R	R	S (6)
6	S (32)	R	R	S (30)	S (6)	R	S (10)
7	S (36)	S (10)	S (18)	S (40)	S (16)	R	S (8)
10	S (24)	R	S (12)	S (22)	R	R	S (8)
11	S (28)	R	S (12)	S (30)	R	R	R

Sensitivity to antibiotics

(disk diffusion method)



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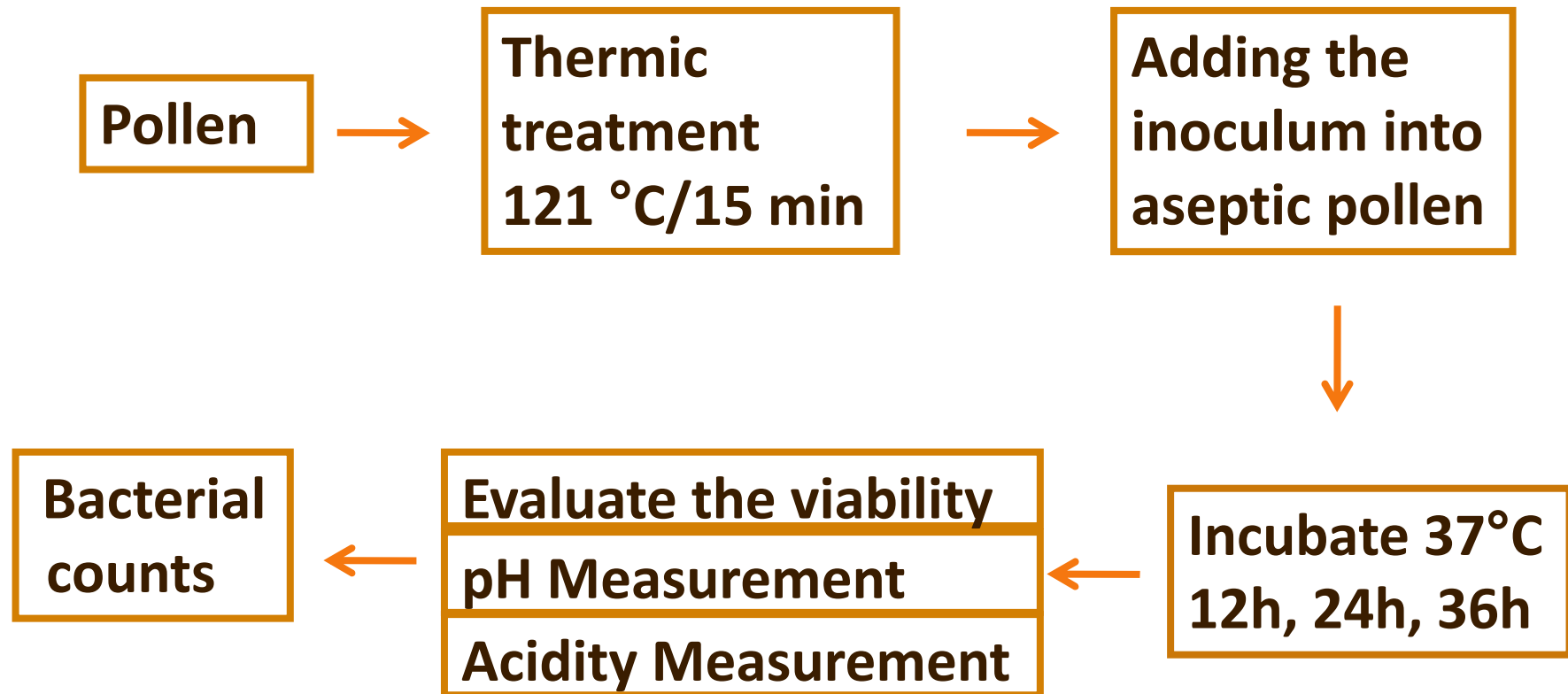
MOLECULAR IDENTIFICATION

- SPECIE-SPECIFIC PCR
- TTTTATGCTTGCATAAATGATTTTTGGATTCTGGAGCGAGTGG
CGAACTGGTGAGTAACACGTGGGTAACTGCCCCGAAGCG
GGGGATAACATTTGGAAACAAGTGCTAATACCGCATAATTA
GTTGGAACCGCATGGTTCCAACCTTGAAAGATGGCTCTGCTAT
CACTTTGGGATGGACCCGCGCCGTATTAGTTAGTTGGTGAG
ATAAAAGCCACCAAGACGATGATACGTAGCCGACCTGAGA
GGGTAATCGGCCACATTGGGACTGAGACACGGCC
- *Lactobacillus kunkeei* gene for 16S rRNA, partial
sequence, strain: F20-1

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FERMENTATION TEST



(Fuenmayor. et al.2009),

FERMENTATION TEST

(L. kunkey / L. acidophilus)

STRAIN	PH	ACIDITY	VIABILITY
5	LOWER	LOWER	PRESENT
6	LOWER	LOWER	PRESENT
10	LOWER	LOWER	PRESENT
11	LOWER	LOWER	PRESENT

CONCLUSION

- The incorporation of honey and pollen to culture medium doesn't provides advantages in the bacterial isolated in comparison with other culture medium
- There aren't many species of *Lactobacillus* in Colombian bee bread
- The isolated *L. kunkeei* has a higher survival in adverse conditions than other species found

CONCLUSION

- *L. Kunkeei* presents a lower performance compared with *L. acidophilus*, but it should be further investigated.
- Obtaining of strains with probiotic potential from plants sources and incorporated into matrices of the same species show a functional food alternative in beekeeping.



THANK YOU