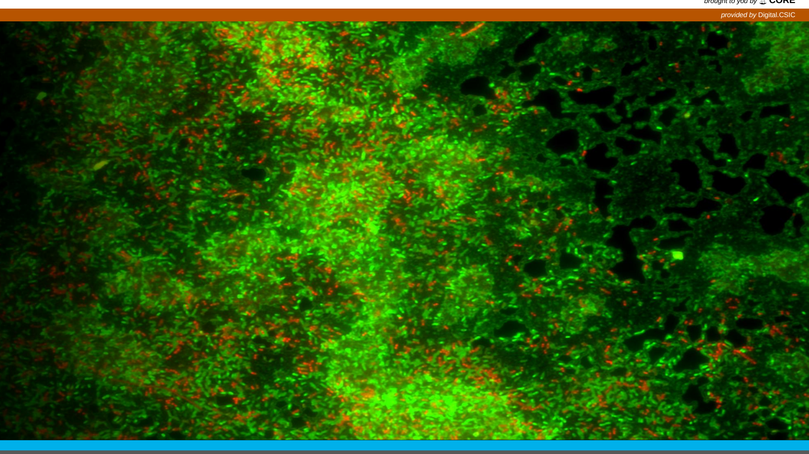
3D morphological features and antibiotic resistance in *L. monocytogenes* – *Pseudomonas sp.* biofilms



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» BACKGROUND «

- *Listeria monocytogenes* has been one of the foodborne pathogens of major concern in the last years since it can produce human listeriosis with a 30 % increment of incidence in Europe regarding previous data (1).
- This pathogen can be found in food industry premises as biofilms associated with a wide variety of microorganisms (2, 3) such as *Pseudomonas spp*. which confers the biofilm particular morphological features and increased resistance to antibiotics (4).

» OBJECTIVE «

1. To investigate the **VARIATIONS IN 3D MORPHOLOGIES** in three different **MIXED – SPECIES** *L. monocytogenes – Pseudomonas sp.* biofilms grown on stainless steel (SS) using Confocal Laser Scanning Microscopy (CLSM).
2. To assess the **RESISTANCE** of these biofilms to **ANTIBIOTICS** if such resistance can be related with their **TRIDIMENSIONAL** structure.

» METHODS «

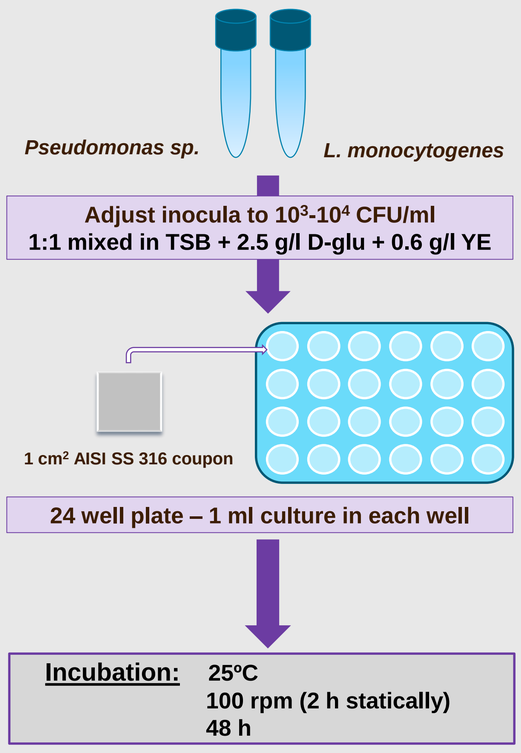
1. MIXED – SPECIES BIOFILMS

L. monocytogenes L34 + *P. aeruginosa* CECT 110

L. monocytogenes L34 + *P. putida* CECT 324

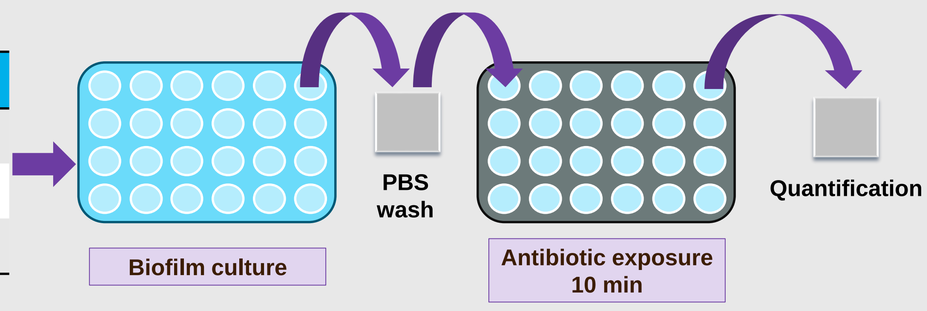
L. monocytogenes L34 + *P. fluorescens* B52

2. DUAL SPECIES BIOFILMS SETUP



3. ANTIBIOTIC EXPOSURE

Antibiotic	Concentration (µg/ml)
Ampicillin	0.125, 0,5, 2
Ciprofloxacin	0.5, 1, 2, 12.5
Gentamicin	0.125, 0,5, 2



Agar plating

Lethal dose and maximum inhibition value determination

$$E(\%) = K\left(\frac{1}{1+e^{r(LD_{50}-D)}} - \frac{1}{e^{rLD_{50}+1}}\right)$$

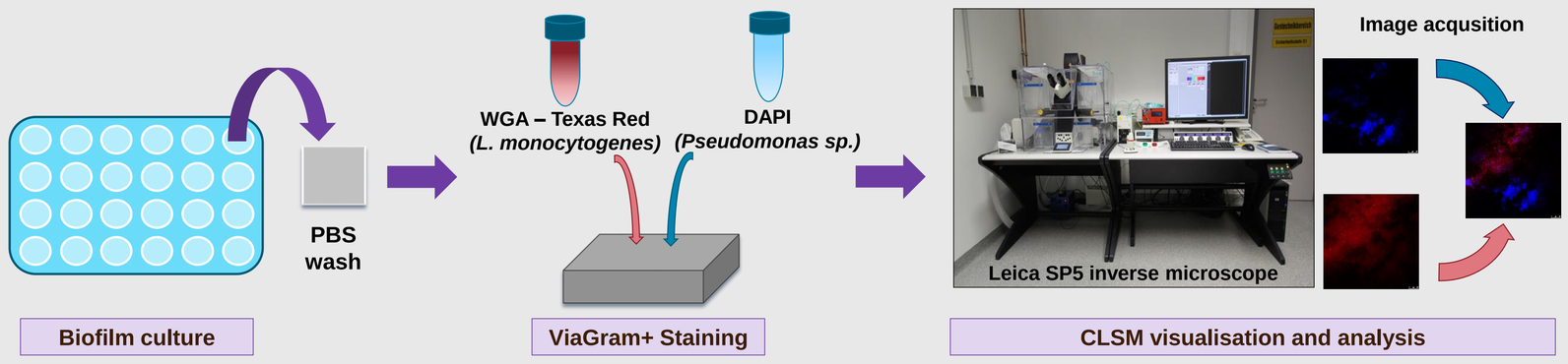
According to a logistic model (5) where:

LD_{50}: Dose that kills 50 % of population

K: Maximum inhibition value

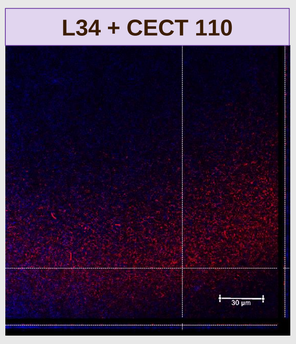
r: Specific inhibition coefficient

4. CONFOCAL LASER SCANNING MICROSCOPY (CLSM)

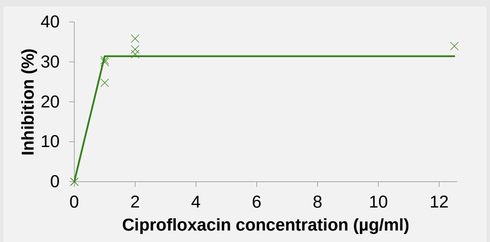


» RESULTS «

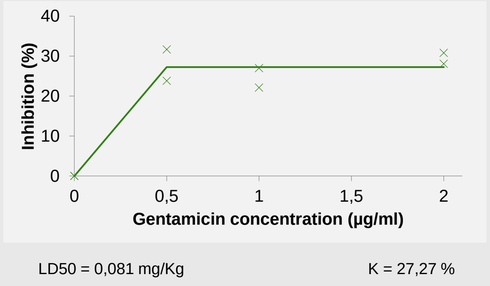
L34 + CECT 110



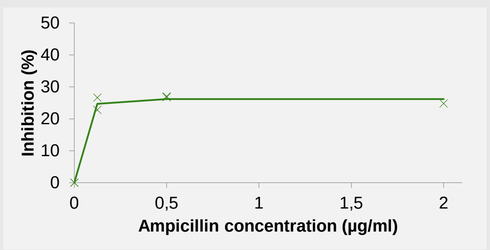
- **No clusters present**
- **Max. thickness: 8 µm**
- **Low antibiotic resistance**



LD50 = 0,081 mg/Kg K = 31,42 %

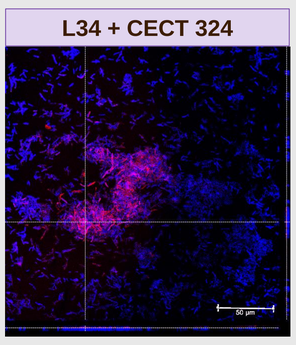


LD50 = 0,081 mg/Kg K = 27,27 %

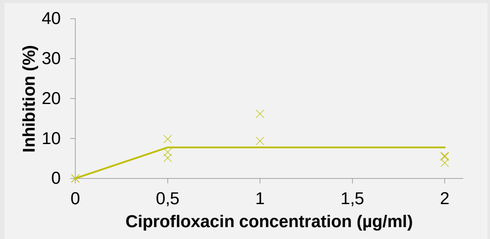


LD50 = 0,081 mg/Kg K = 26,20 %

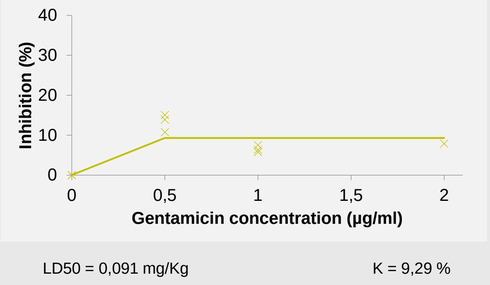
L34 + CECT 324



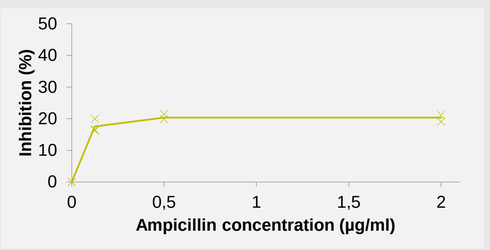
- **Small clusters present**
- **Max. thickness: 10 µm**
- **Highest antibiotic resistance especially with ciprofloxacin and gentamicin**



LD50 = 0,091 mg/Kg K = 7,77 %

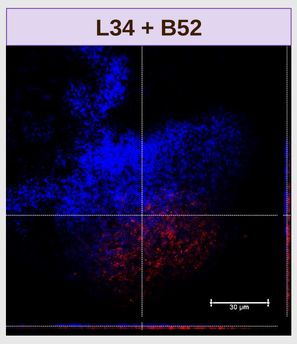


LD50 = 0,091 mg/Kg K = 9,29 %

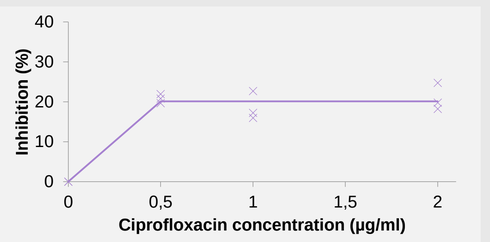


LD50 = 0,103 mg/Kg K = 20,35 %

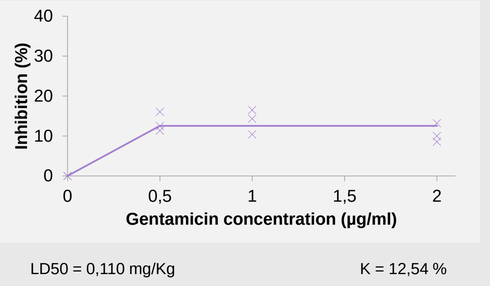
L34 + B52



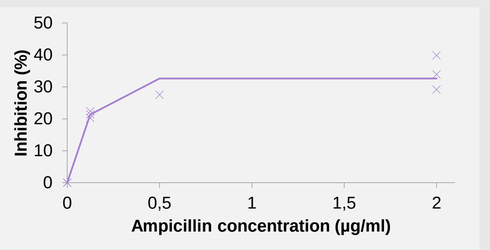
- **Large clusters present**
- **Max. thickness: 14 µm**
- **Moderate antibiotic resistance**



LD50 = 0,110 mg/Kg K = 20,11 %



LD50 = 0,110 mg/Kg K = 12,54 %



LD50 = 0,138 mg/Kg K = 32,62 %

» CONCLUSIONS «

1. Accompanying strain strongly influenced the final tridimensional structure of the mixed – species biofilm. In all cases *L. monocytogenes* occupied the bottom layers of the biofilm.
2. L34 + CECT 324 presented the highest resistance to gentamicin and ciprofloxacin, followed by L34 + B52 and L34 + CECT 110. This indicated that regardless of size, cluster presence may help to *L. monocytogenes* to resist higher antibiotic concentrations.

» REFERENCES «

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» ACKNOWLEDGMENTS «

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