



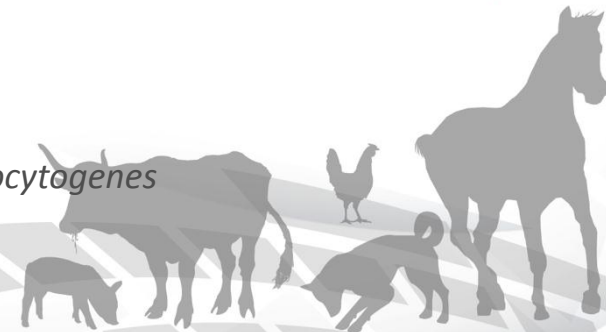
**IZSAM G. CAPORALE
TERAMO**


**Studio delle caratteristiche di adesività ed invasività di alcuni ceppi di
Listeria monocytogenes isolati da alimenti e dall'uomo**

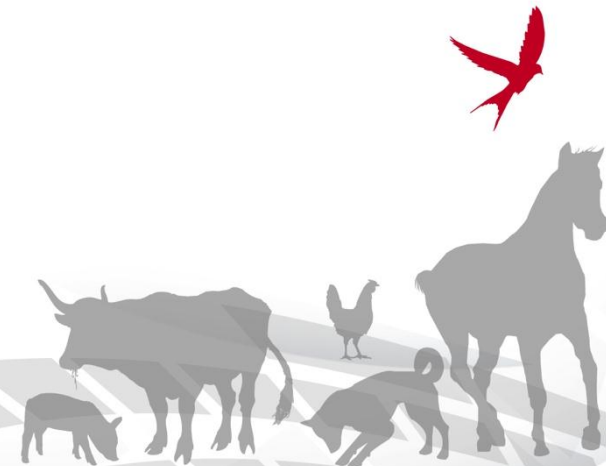
Loredana Zocchi

Laboratorio Nazionale di Riferimento per *Listeria monocytogenes*

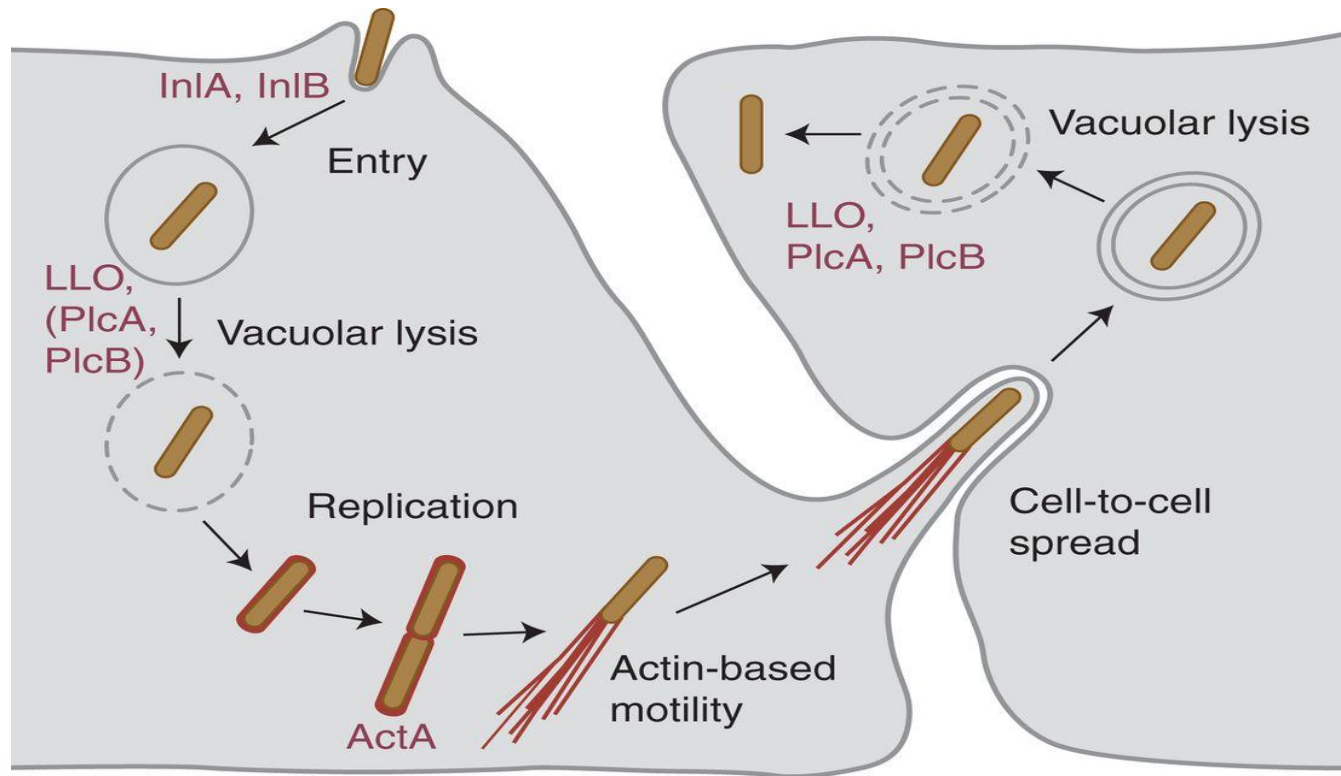
Teramo 12-13 dicembre 2013



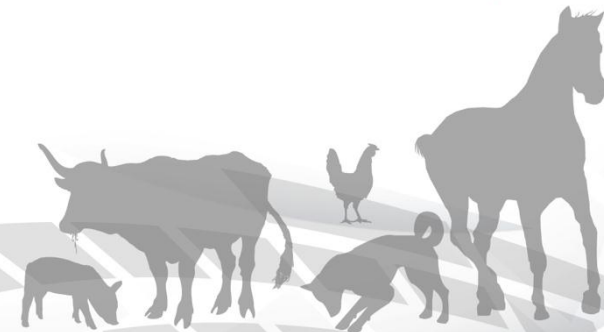
- 
- Introduzione relativa all'adesione ed internalizzazione di *Listeria monocytogenes* in cellule epiteliali di mammifero
 - Descrizione degli esperimenti preliminari e della metodica utilizzata per ricreare *in vitro* parte del ciclo intracellulare di *L.monocytogenes*
 - Risultati ottenuti dall'analisi dei ceppi selezionati
 - Conclusioni



Ciclo intracellulare di *Listeria monocytogenes*



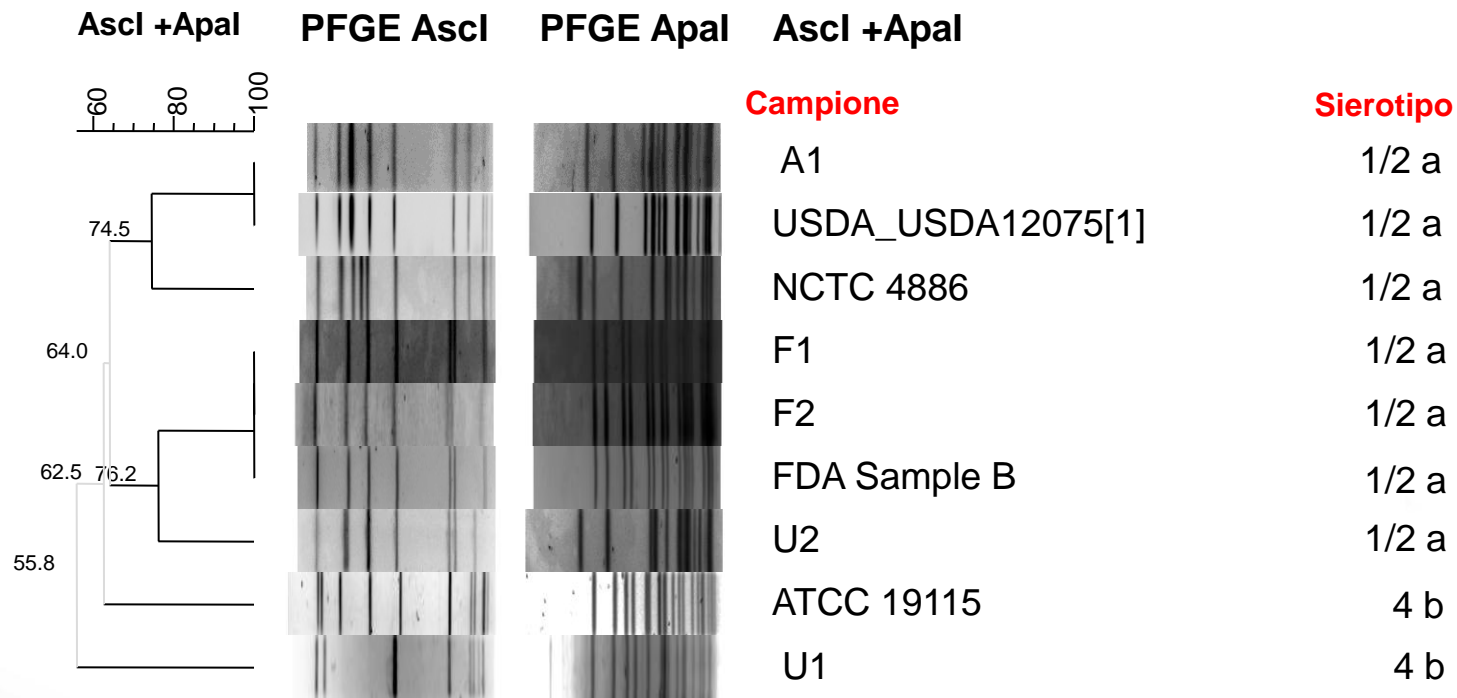
Pizarro-Cerdá J et al. Cold Spring Harb
Perspect Med 2012;2:a010009



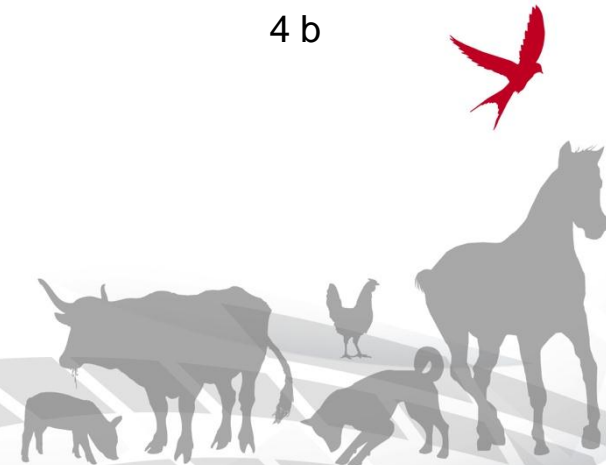
- Valutare le caratteristiche di adesività ed invasività *in vitro* di alcuni ceppi di *Listeria monocytogenes* selezionati, con la finalità di utilizzare tali metodiche per l'ulteriore caratterizzazione di ceppi prescelti



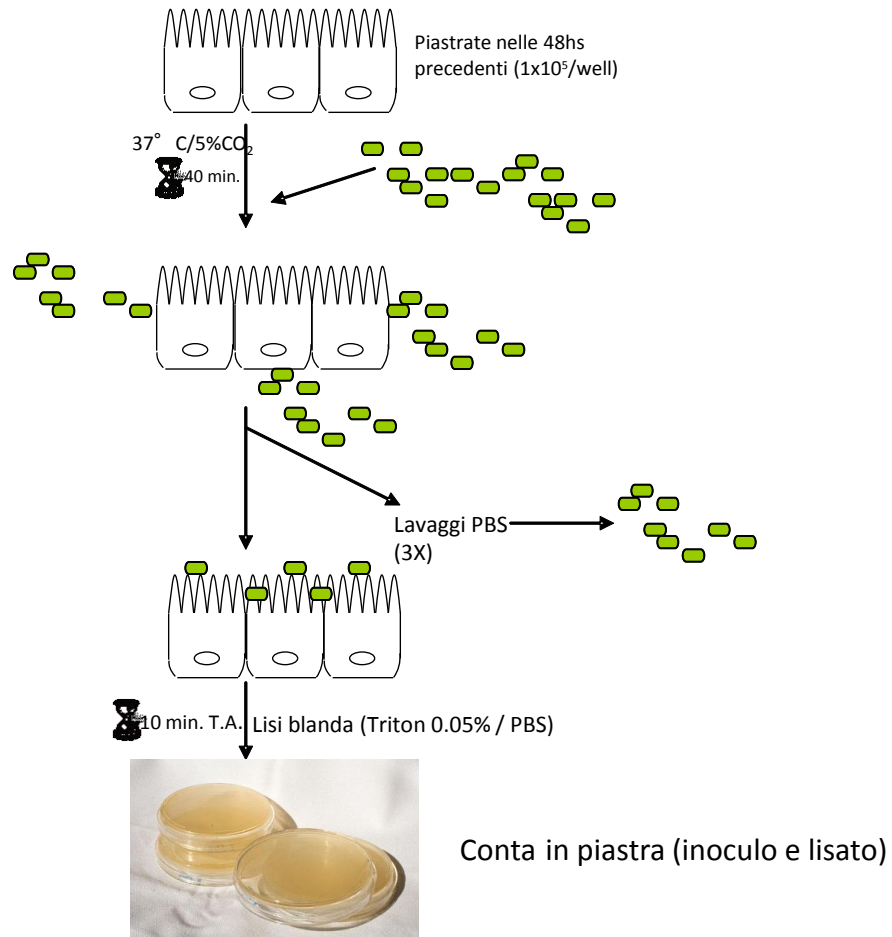
PFGE & Sierotipo dei ceppi selezionati




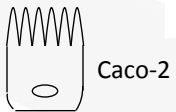
F=formaggio
U=uomo
A=ambientale



Saggio di adesione

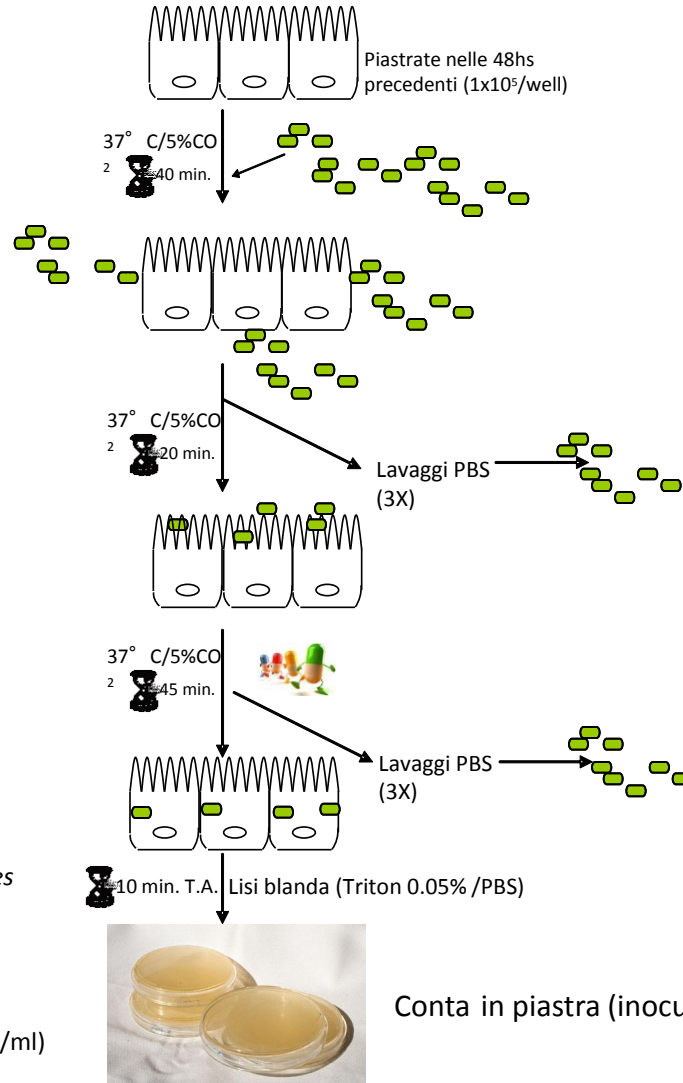





 *Listeria monocytogenes*



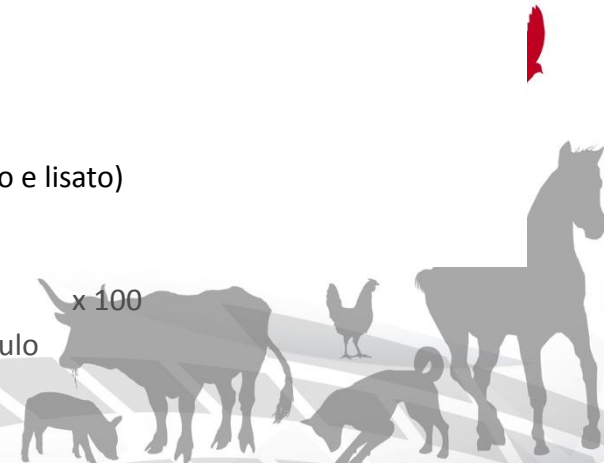
$$\% \text{ Adesione} = \frac{\text{numero di colonie di } L. \text{ monocytogenes}}{\text{numero di colonie di } L. \text{ monocytogenes dell'inoculo}} \times 100$$





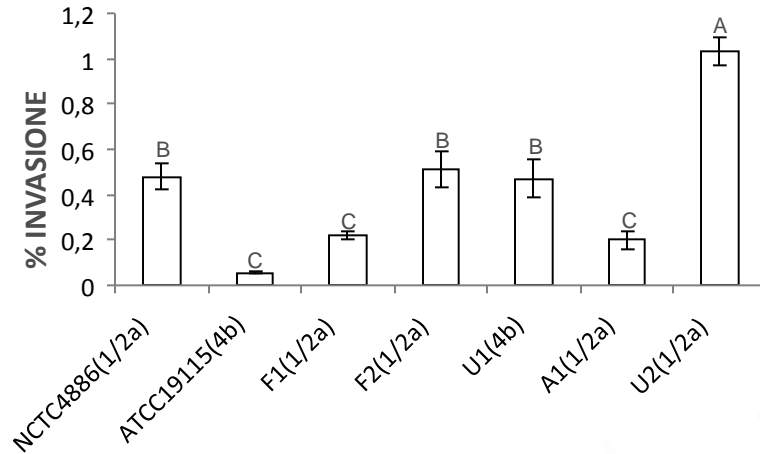
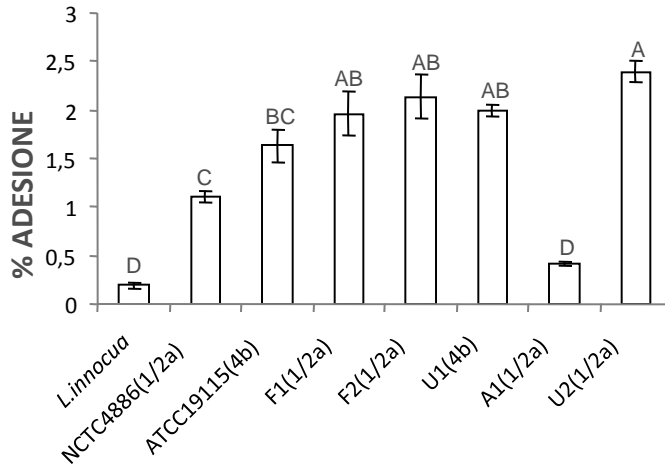
-  *Listeria monocytogenes*
-  Caco-2
-  Gentamicina (150 ug/ml)

$$\% \text{ Invasione} = \frac{\text{numero di colonie di } L. \text{ monocytogenes}}{\text{numero di colonie di } L. \text{ monocytogenes dell'inoculo}} \times 100$$



Saggi di adesione ed invasione

Numerazione



ANOVA: one way P values < 0,0001; F di Fisher= 40.877 ;
Test per comparazioni multiple per la variabile CEPPO Tukey (HSD)

ANOVA: one way P values < 0,0001; F di Fisher=37.745 ;
Test per comparazioni multiple per la variabile CEPPO Tukey (HSD)

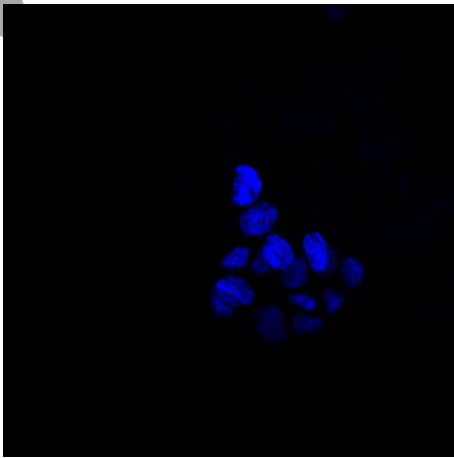


Risultati

Saggi di invasione

Immunofluorescenza

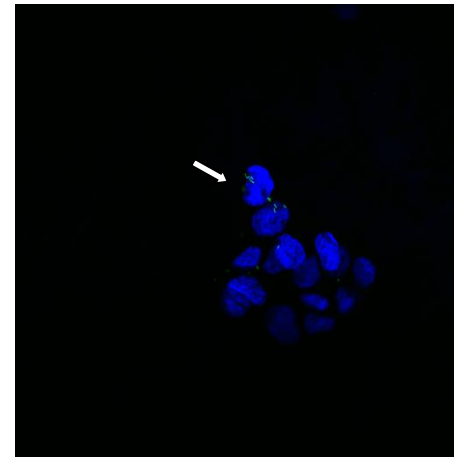
DAPI



Listeria monocytogenes



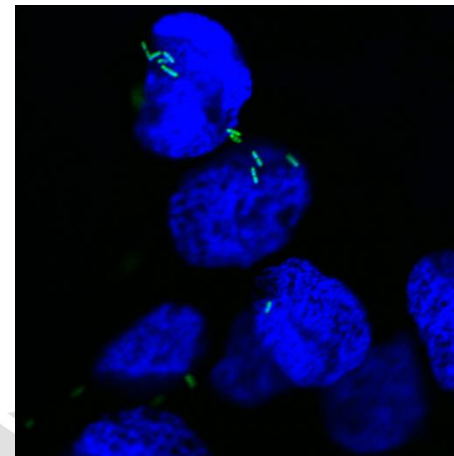
MERGE



NCTC4886
(1/2a)

63x (25%)

63X(100%)

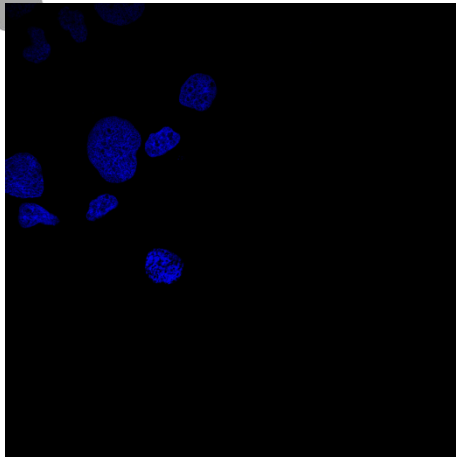


Risultati

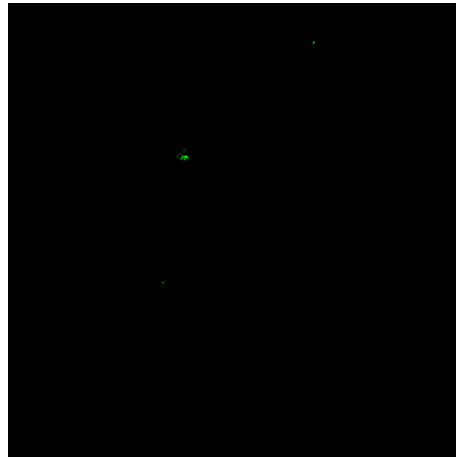
Saggi di invasione

Immunofluorescenza

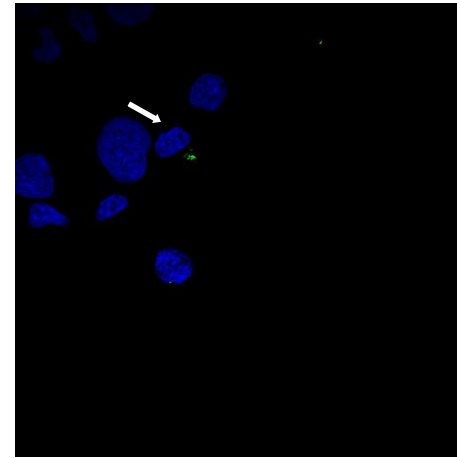
DAPI



Listeria monocytogenes



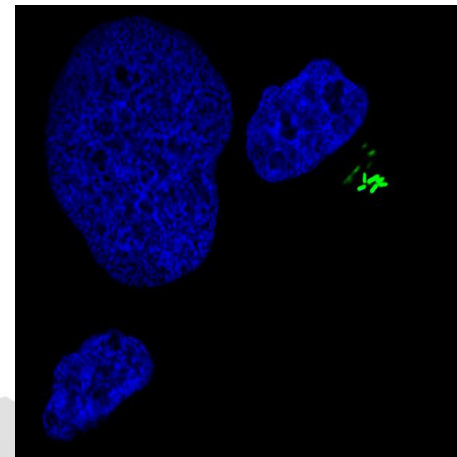
MERGE



ATCC19115
(4b)

63x (25%)

63X(100%)

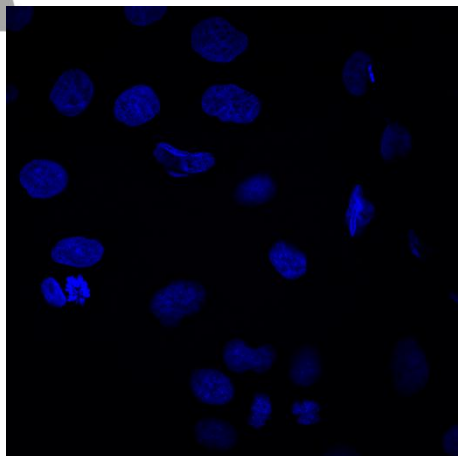


Risultati

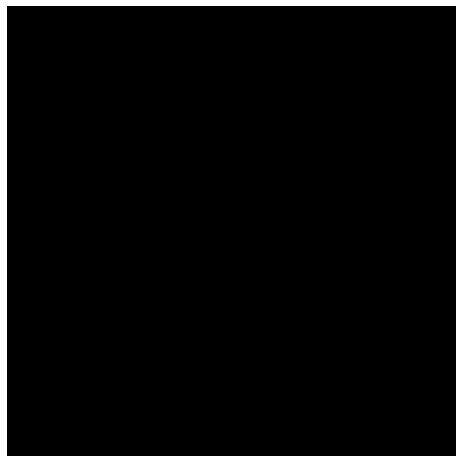
Saggi di invasione

Immunofluorescenza

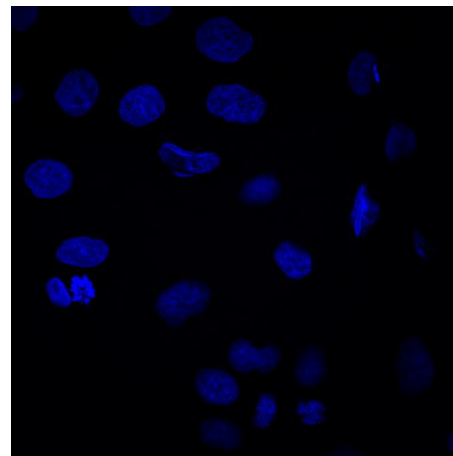
DAPI



Listeria innocua



MERGE



L. innocua

63x (25%)

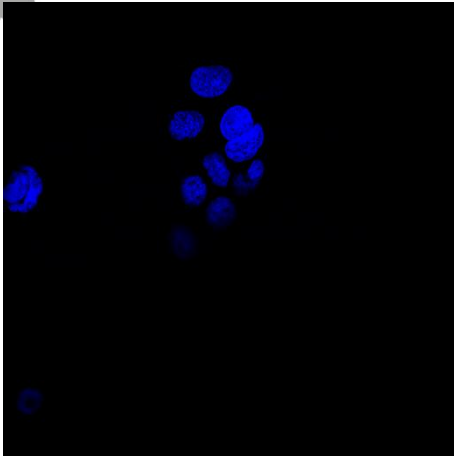


Risultati

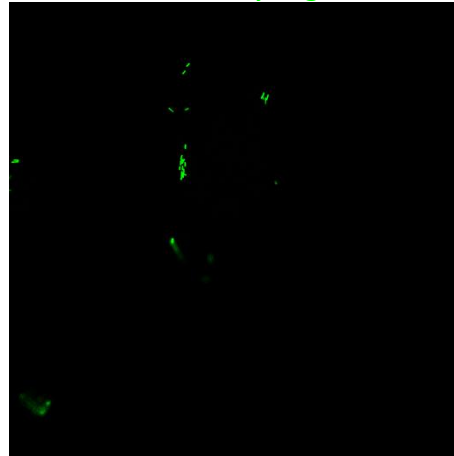
Saggi di invasione

Immunofluorescenza

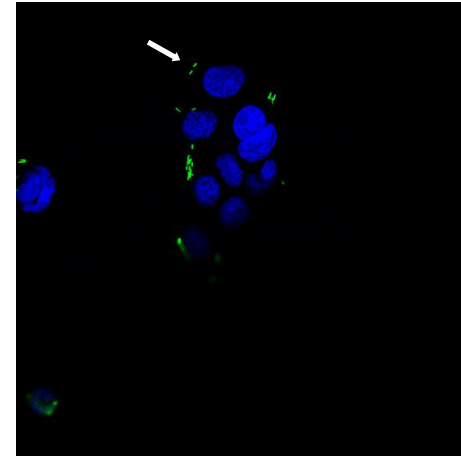
DAPI



Listeria monocytogenes



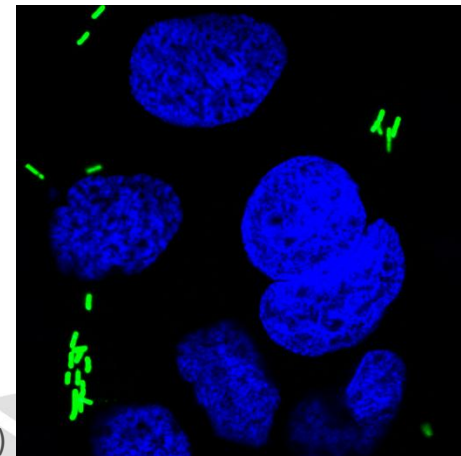
MERGE



F1(1/2a)

63x (25%)

63X(100%)

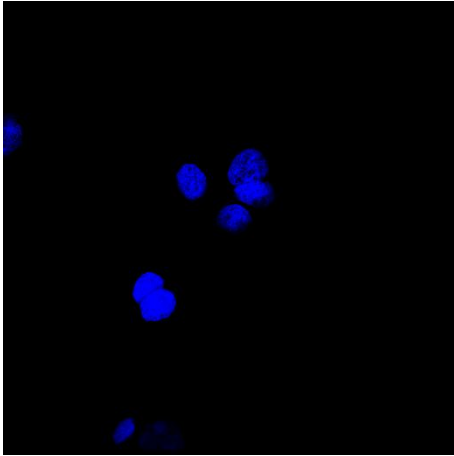


Risultati

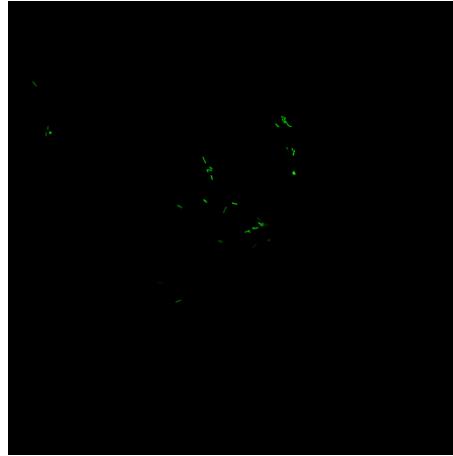
Saggi di invasione

Immunofluorescenza

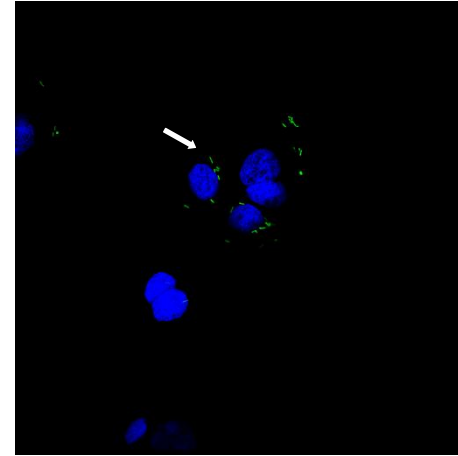
DAPI



Listeria monocytogenes



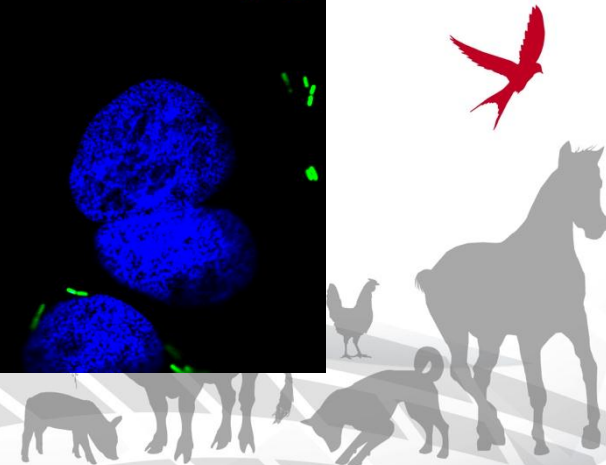
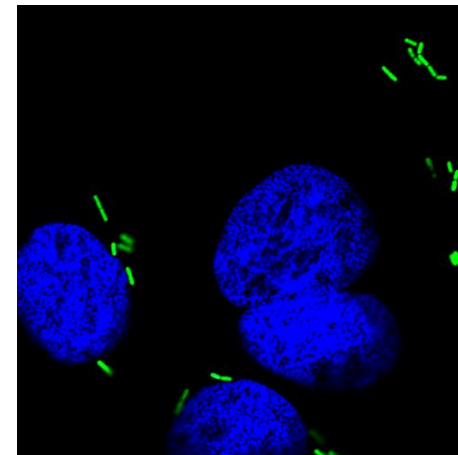
MERGE



F2 (1/2a)

63x (25%)

63X(100%)

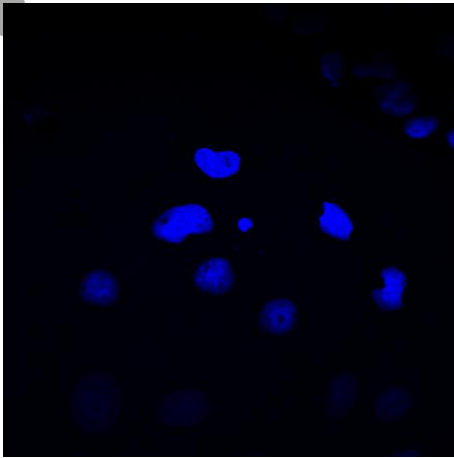


Risultati

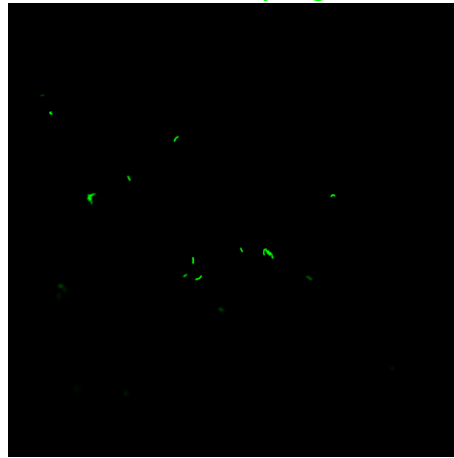
Saggi di invasione

Immunofluorescenza

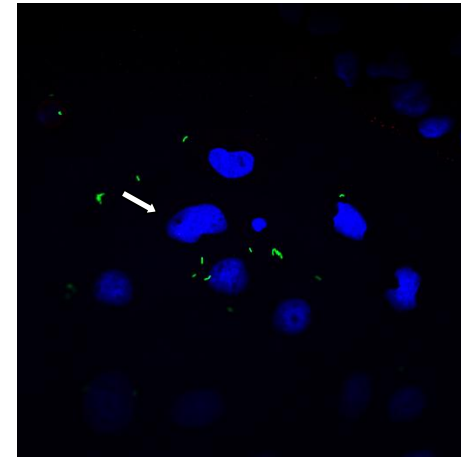
DAPI



Listeria monocytogenes



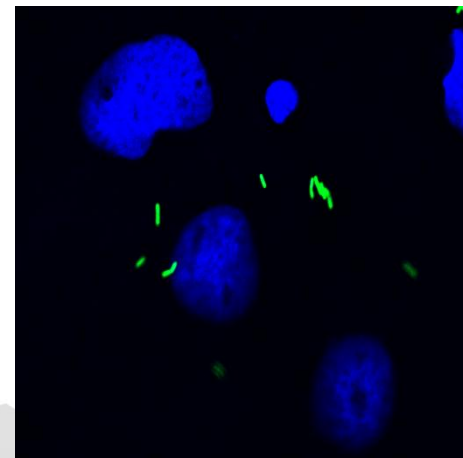
MERGE



A1

63x (25%)

63X(100%)

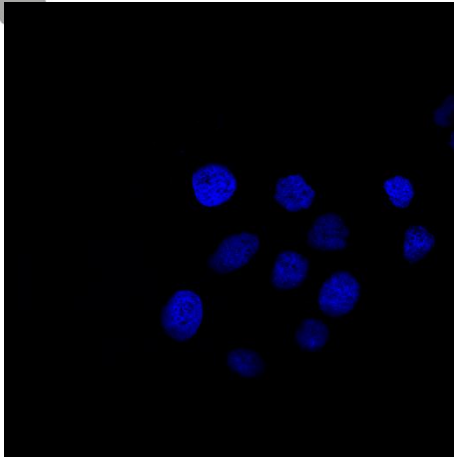


Risultati

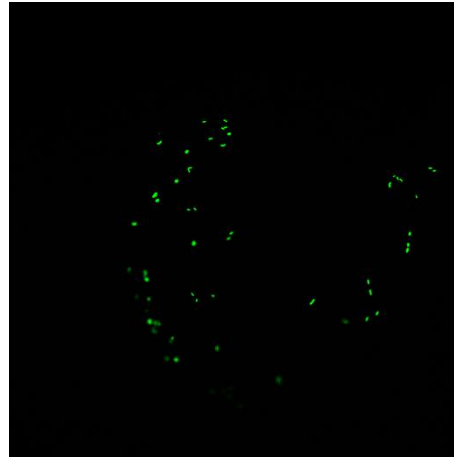
Saggi di invasione

Immunofluorescenza

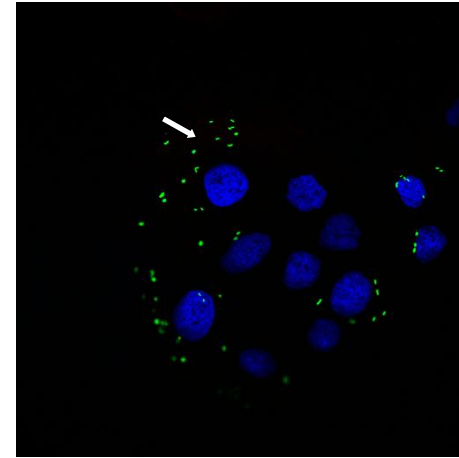
DAPI



Listeria monocytogenes

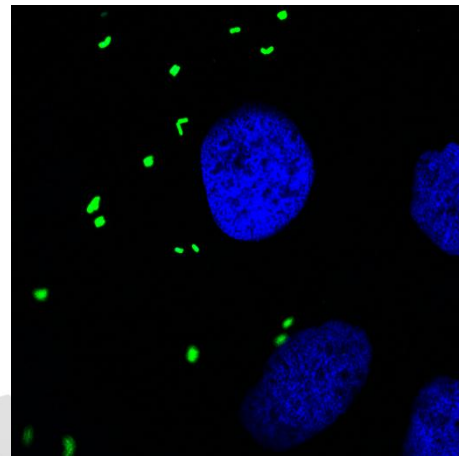


MERGE



U1(4b)

63x (25%)



63X(100%)

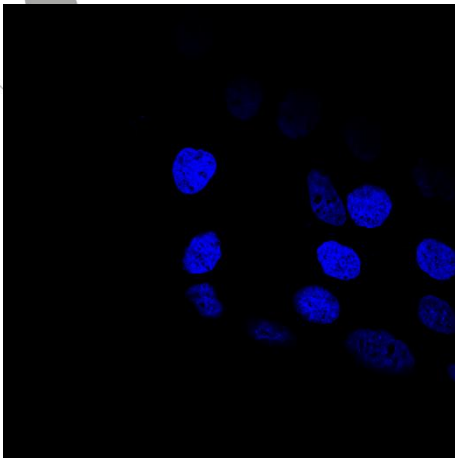


Risultati

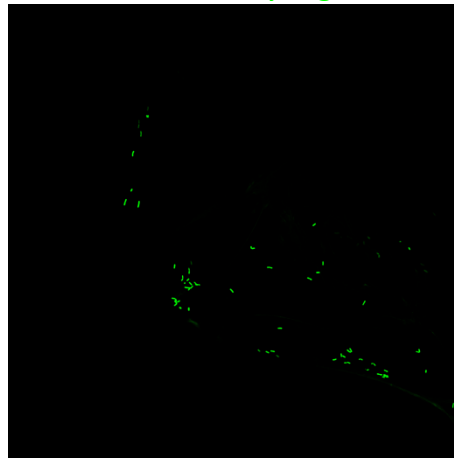
Saggi di invasione

Immunofluorescenza

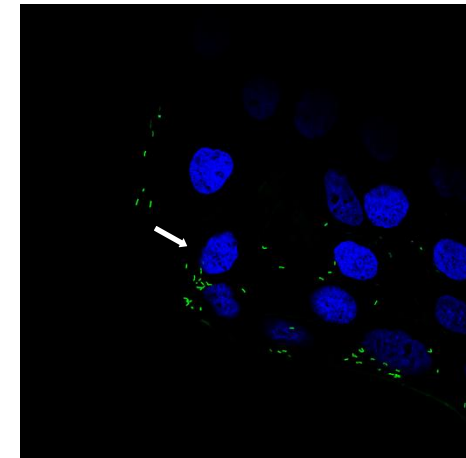
DAPI



Listeria monocytogenes



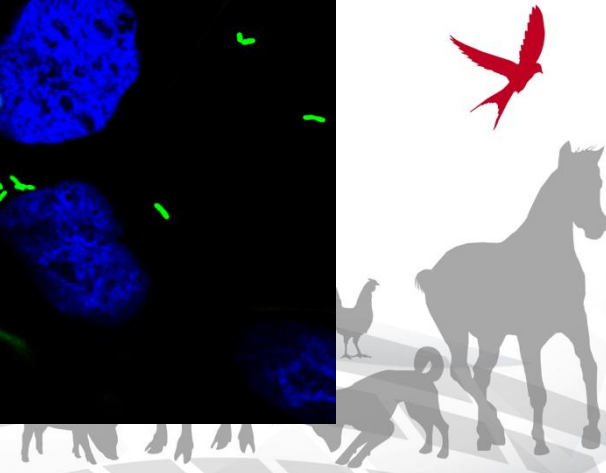
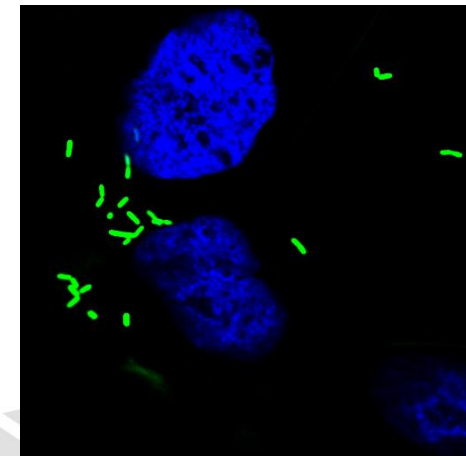
MERGE



U2(1/2a)

63x (25%)

63X(100%)



Risultati

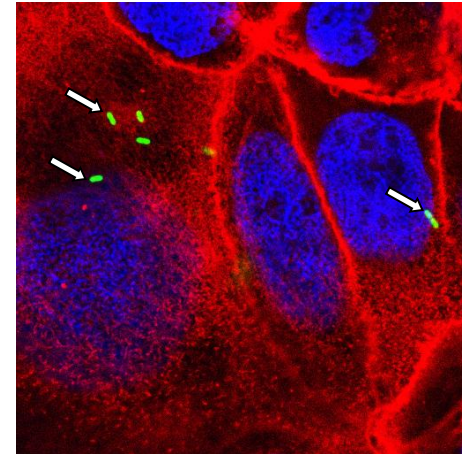
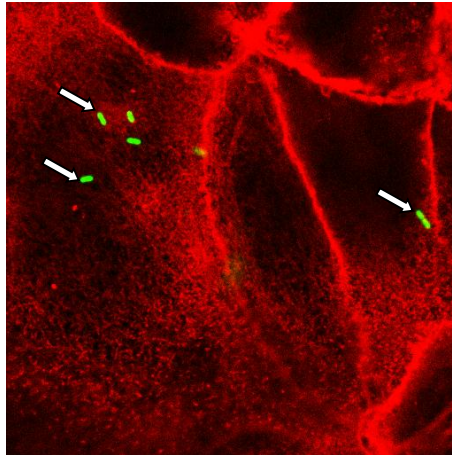
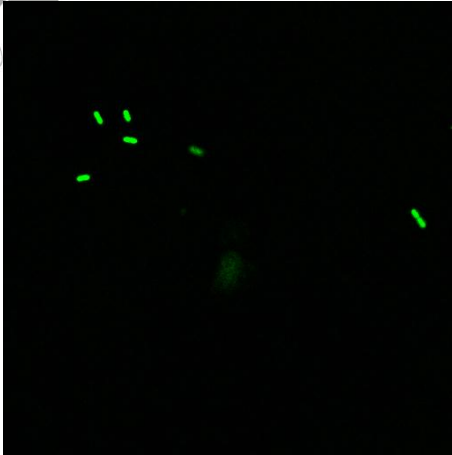
Saggi di invasione

Immunofluorescenza

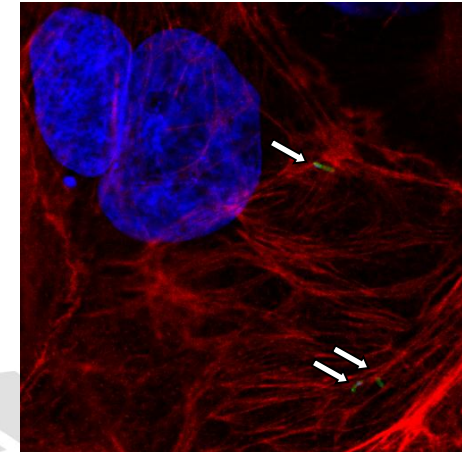
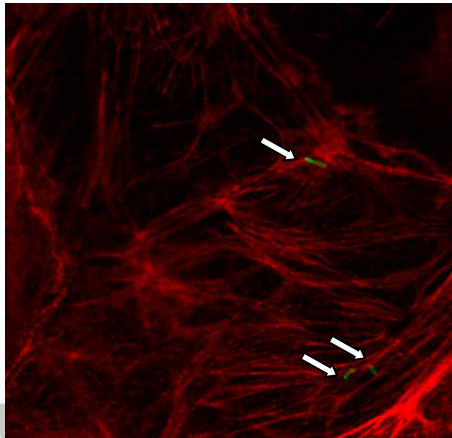
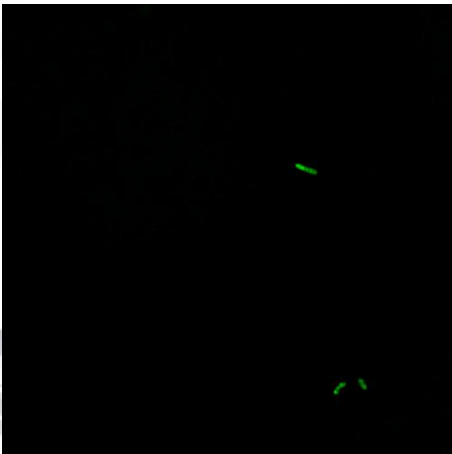
Listeria monocytogenes

F-ACTINA

MERGE



NCTC 4886
(1/2a)



ATCC 19115
(4b)



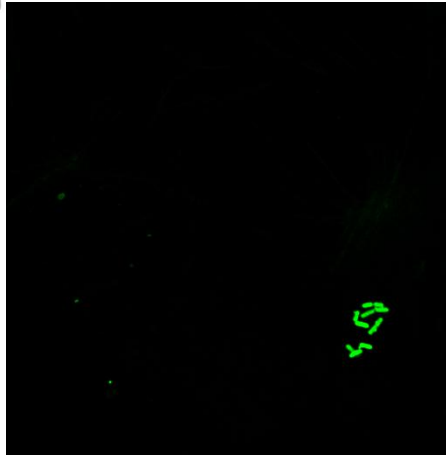
Risultati

Saggi di invasione

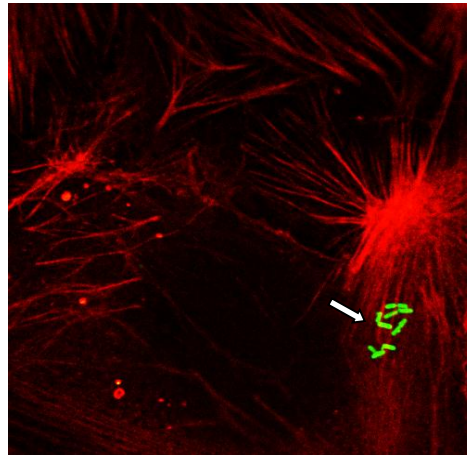
Immunofluorescenza



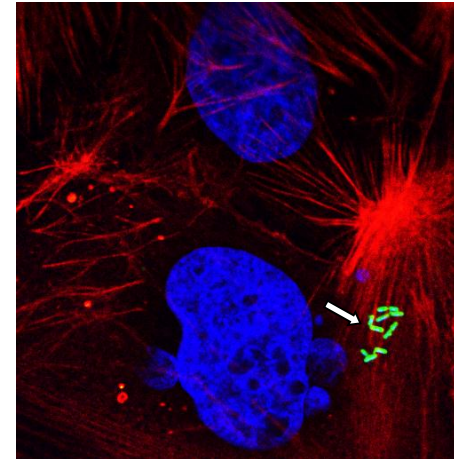
Listeria monocytogenes



F-ACTINA

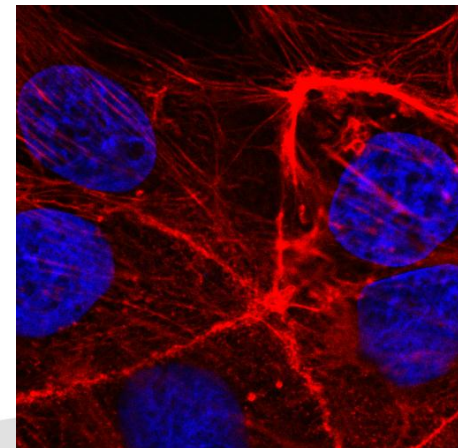
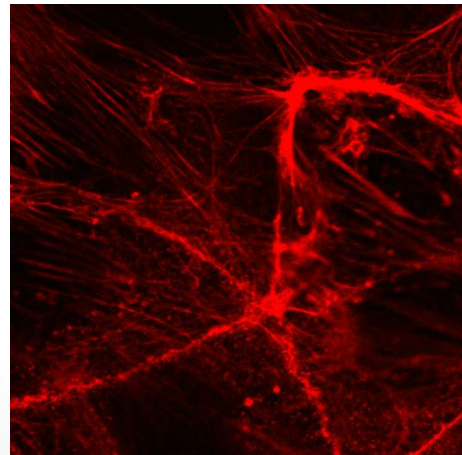
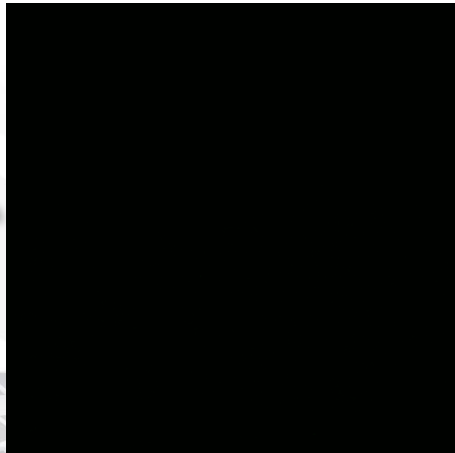


MERGE



A1
(1/2a)

Listeria innocua



Risultati

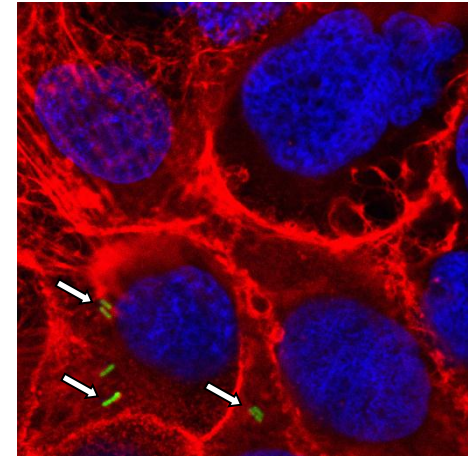
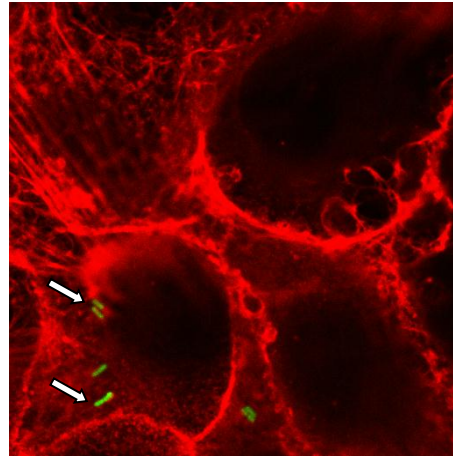
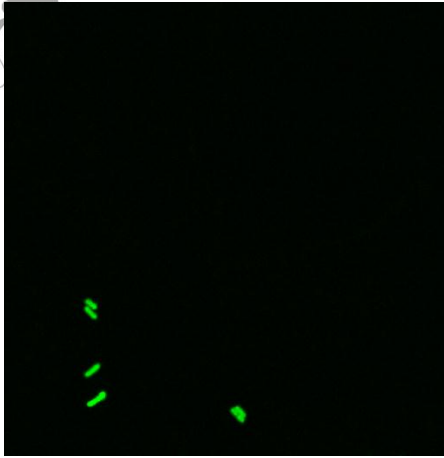
Saggi di invasione

Immunofluorescenza

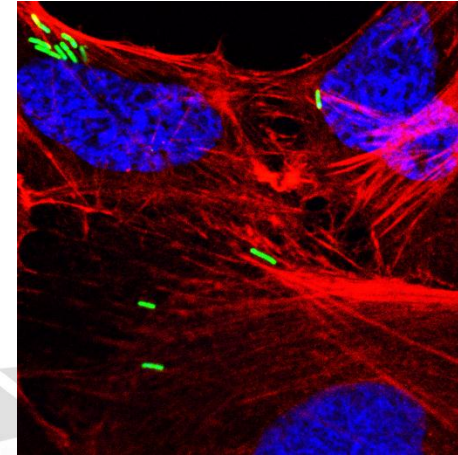
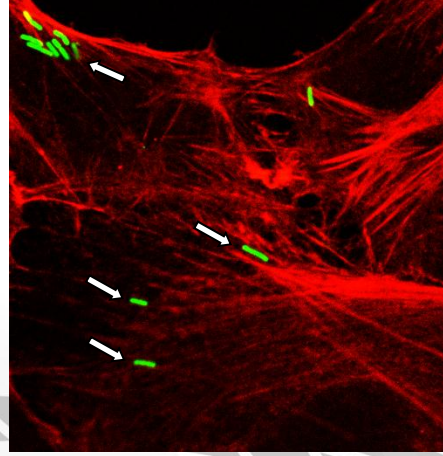
Listeria monocytogenes

F-ACTINA

MERGE



F1
(1/2a)



F2
(1/2a)

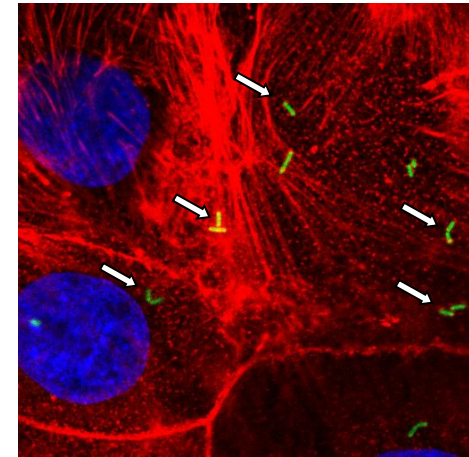
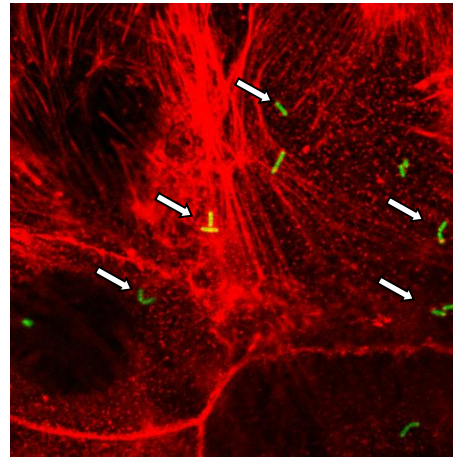
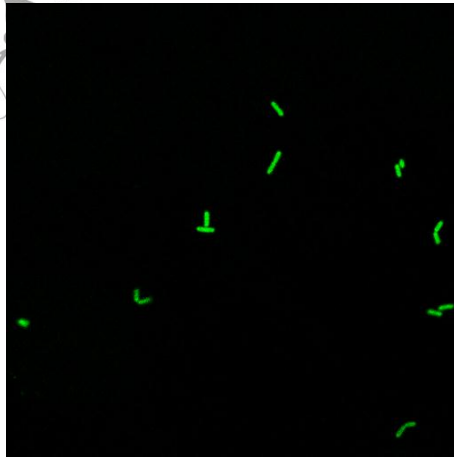


Risultati Saggi di invasione

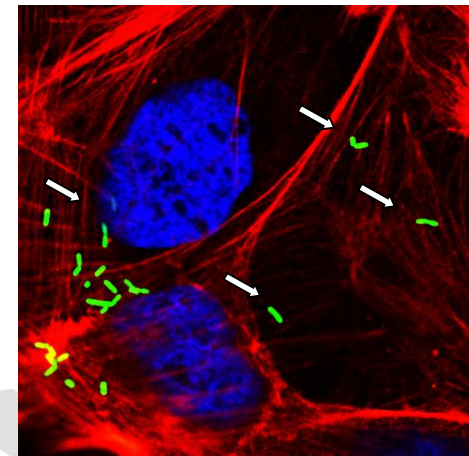
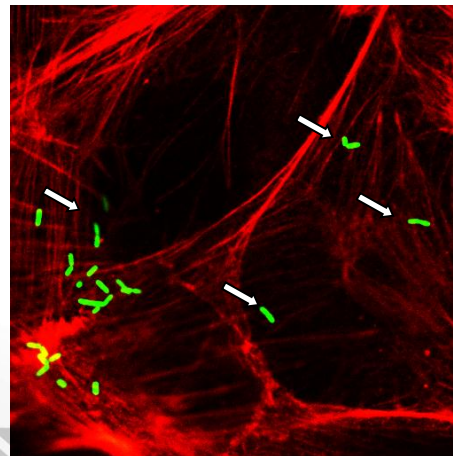
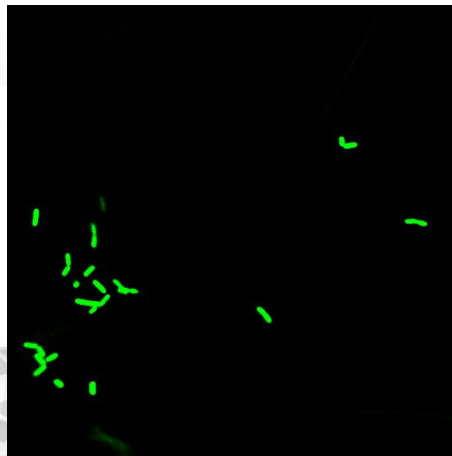
Immunofluorescenza MERGE

Listeria monocytogenes

F-ACTINA



U1(4b)



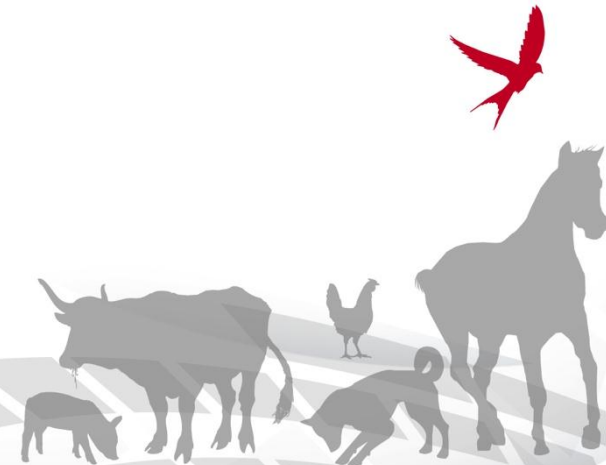
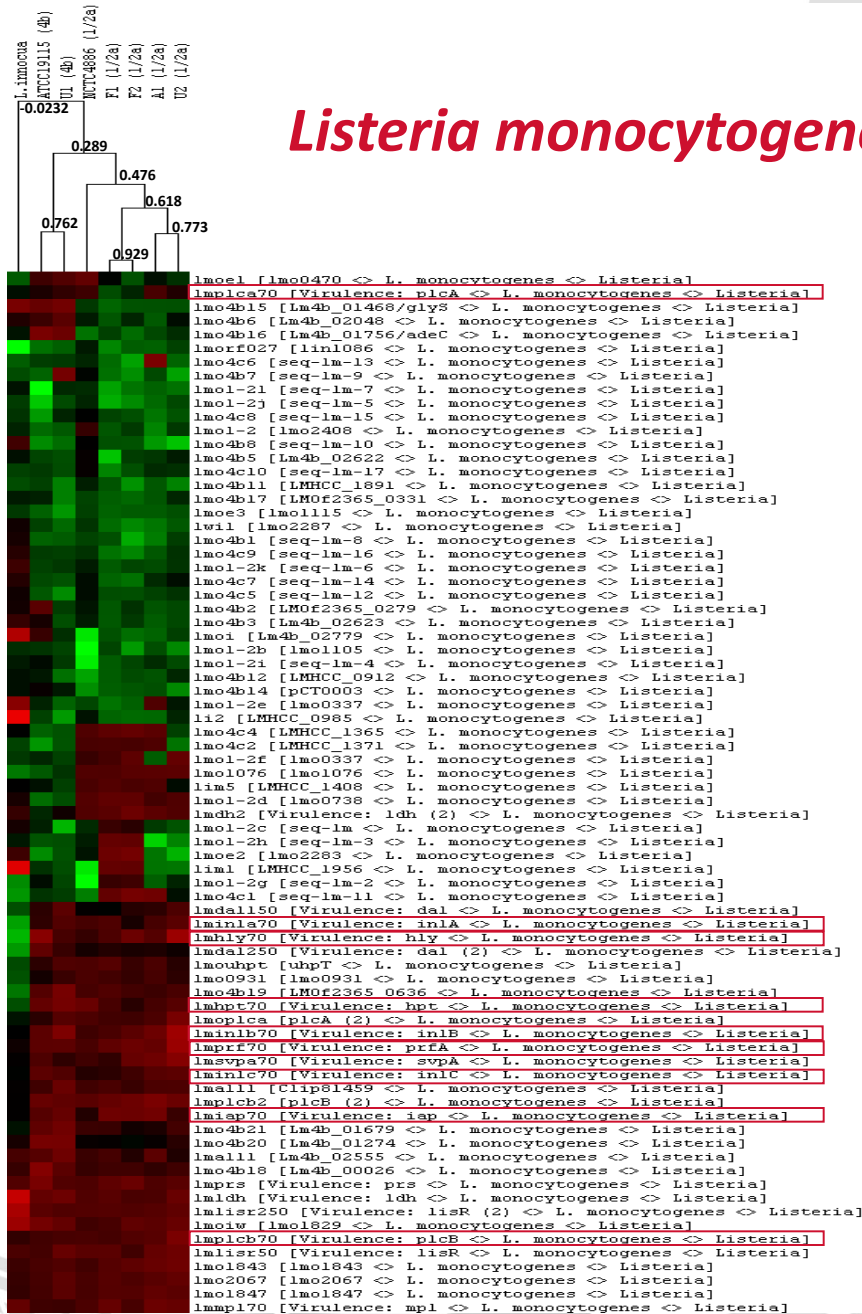
U2(1/2a)






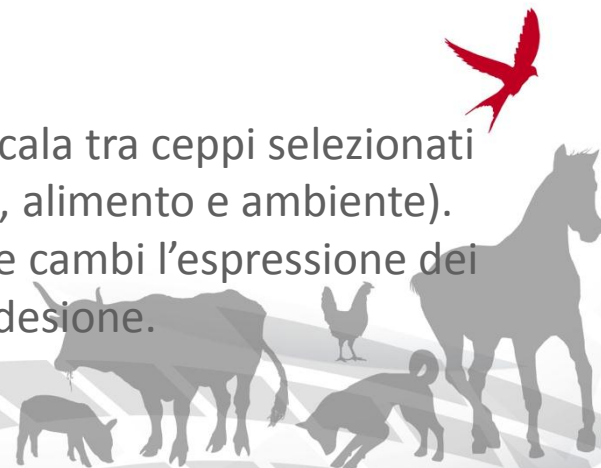
Microarray dei ceppi di

Listeria monocytogenes utilizzati nello studio



Conclusioni & Prospettive Future

- 
- Messa a punto di saggi di adesione/invasione *in vitro* volti a valutare alcune delle caratteristiche di patogenicità dei ceppi di *Listeria monocytogenes*
 - Comparazione dei ceppi di riferimento e di campo, seppure limitata per il numero dei campioni analizzati, ha messo in evidenza una certa eterogeneità nelle capacità di adesione ed invasione dei campioni analizzati
 - L'analisi microarray, svolta sui ceppi selezionati, ha consentito una prima caratterizzazione molecolare. Il sequenziamento del genoma dei ceppi utilizzati consentirà una più fine analisi dei geni d'interesse di questi ed altri ceppi aggiuntivi
 - Utilizzo di tali saggi per la comparazione su più larga scala tra ceppi selezionati di *Listeria monocytogenes* di differente origine (uomo, alimento e ambiente). Successivamente sarà interessante studiare se e come cambi l'espressione dei geni di virulenza durante il processo di invasione ed adesione.



Dott.Vincenza Prencipe

Reparto di Igiene e delle Tecnologie Alimentari

Laboratorio di Riferimento Nazionale per *Listeria monocytogenes*

Dott. Anna Rita D'Angelo
(microscopia)

Dott. Luca Candeloro
(statistica)

**Dott. Vicdalia Acciari/
Dott. Marina Torresi**
(PFGE/ceppi *Listeria m.*)

Dott. Patrizia Centorame
(microarray)

