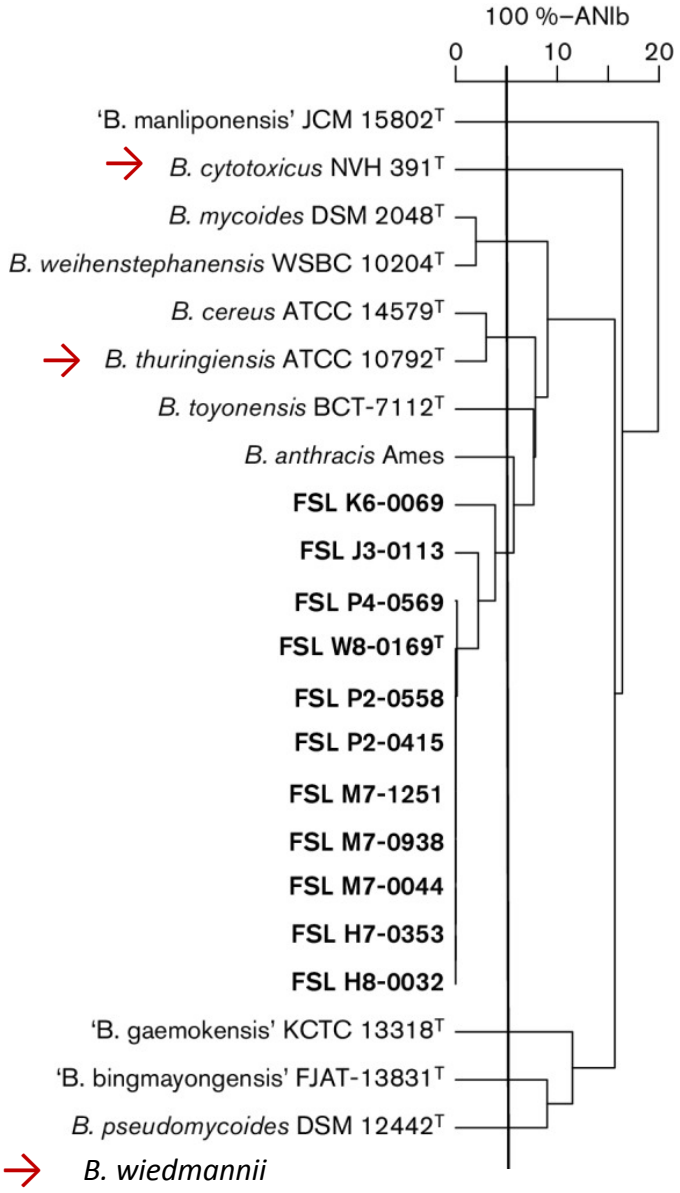
GIORNATA STUDIO SULLE PROBLEMATICHE ATTUALI DEL SETTORE LATTIERO-CASEARIO

Facoltà di Medicina Veterinaria, Via dell'Università n. 1, Lodi, Aula Magna
Sabato 1 dicembre 2018

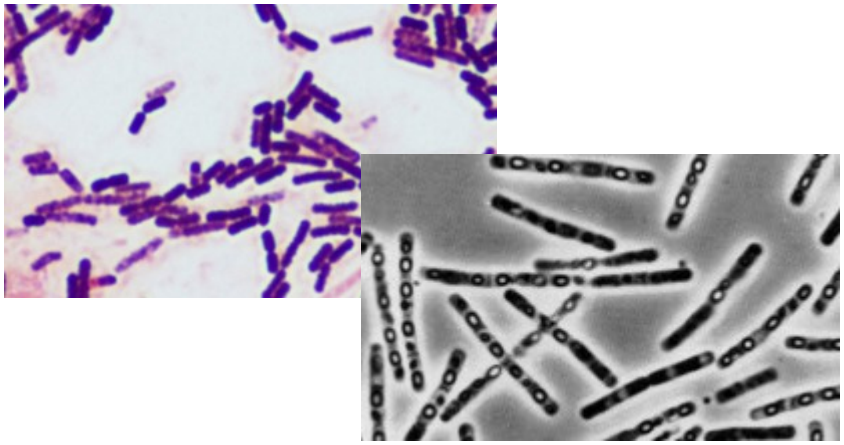
Bacillus cereus: tassonomia, fattori di patogenicità e problematiche nell'uomo

Prof.ssa Emilia Ghelardi
Dipartimento di Ricerca Traslazionale NTMC,
Università di Pisa

IL GRUPPO *BACILLUS CEREUS*

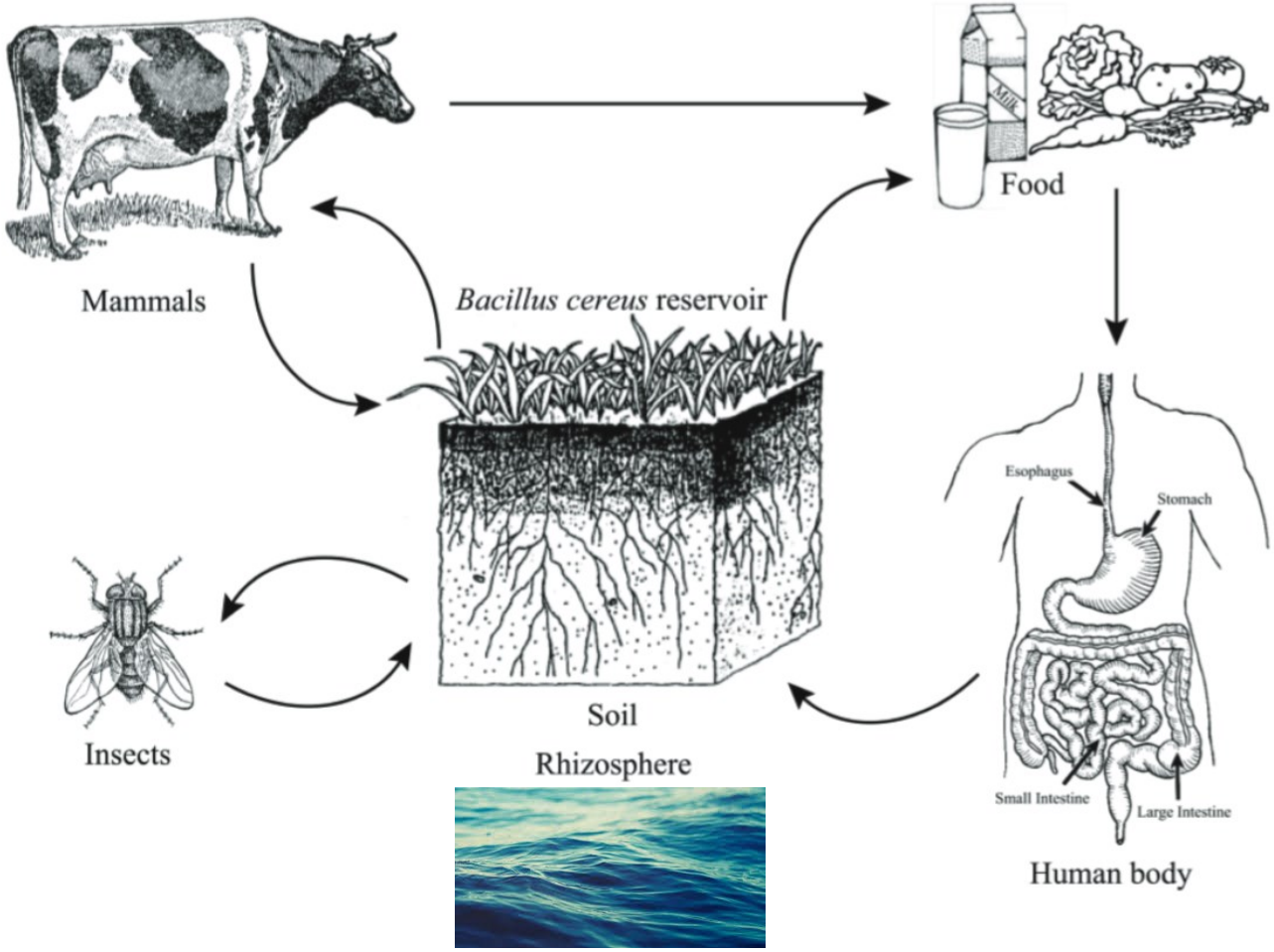


(Miller et al. Int J Syst Evol Microbiol 2016)



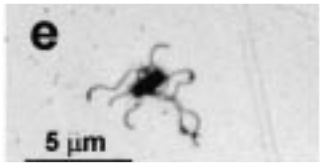
- Bastoncelli Gram-positivi, aerobi o aerobi facoltativi, sporigeni
- Ampiamente diffusi nell'ambiente sotto forma di spore
- Specie strettamente correlate a livello genetico ma con diversi ecotipi
- Patogene e non patogene

BACILLUS CEREUS: DIFFUSIONE E ISOLAMENTO

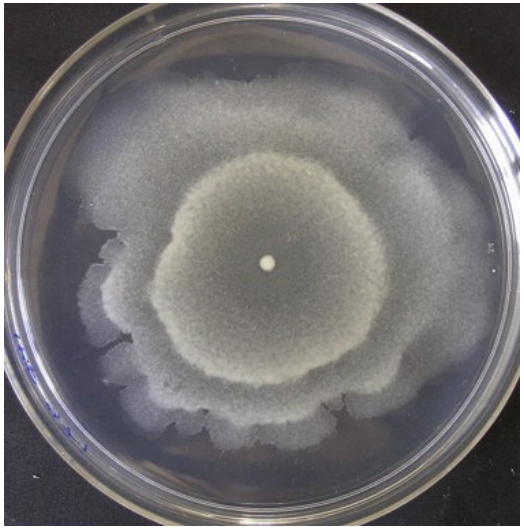


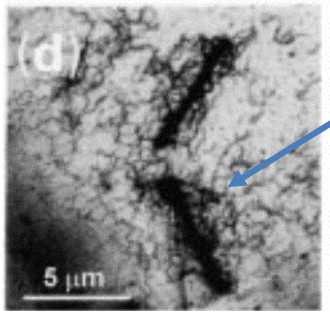
BACILLUS CEREUS: MOTILITÀ

- Mobile per flagelli peritrichi



- Sciama su superfici solide o semisolide

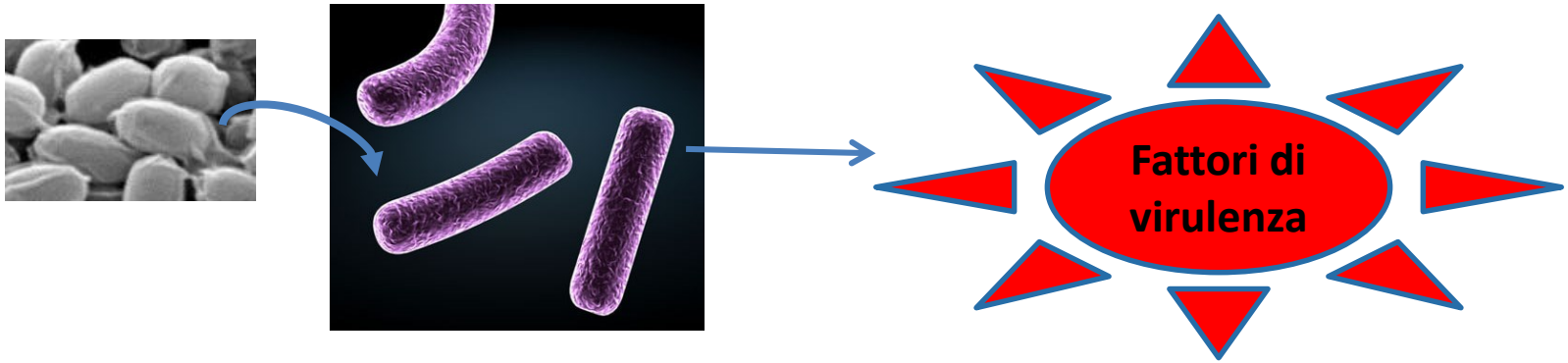




Cellula *swarm*

Maggiore adesività a superfici biotiche e abiotiche
Incrementata sintesi e secrezione di alcuni fattori di virulenza

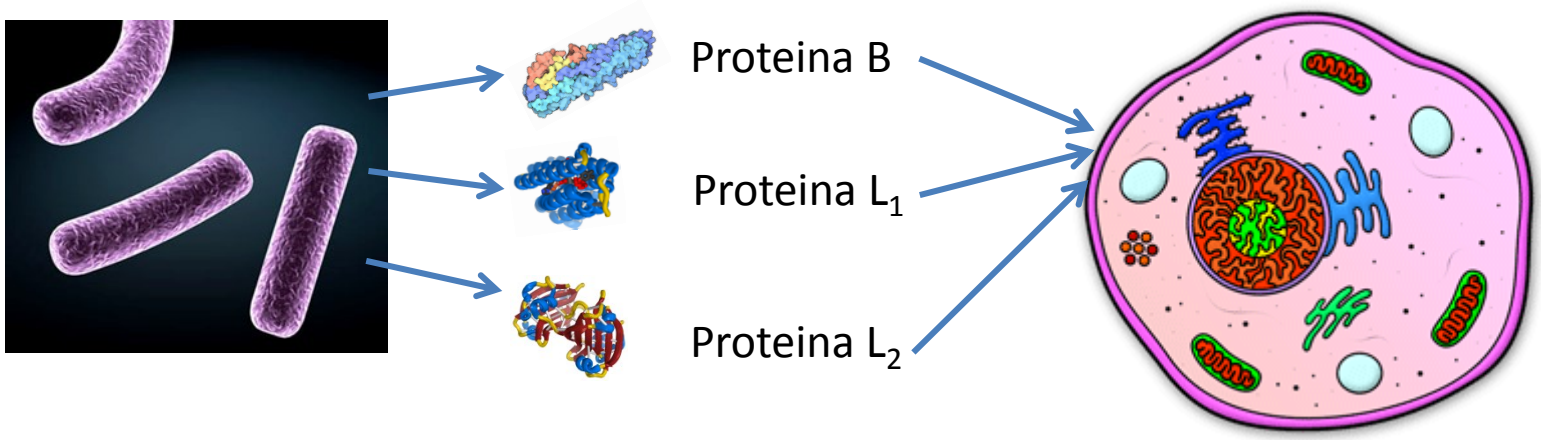
BACILLUS CEREUS: VIRULENZA



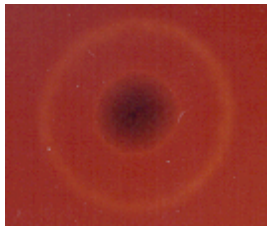
- Due complessi enterotossici:
  emolisina BL (HBL) e enterotossina non-emolitica (NHE)
- Varie emolisine/citolisine:
  HlyI (cereolisina O); HlyII; HlyIII, HlyIV(citossina K CytK)
- Enterotossine minori (Ent FM; Ent Bcet…)
- Diverse **fosfolipasi-C**
- Collagenasi
- Tossina **emetica**

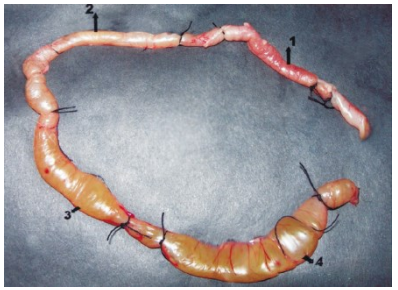
EMOLISINA BL HBL

- Prodotta da circa il 60% dei ceppi di *B. cereus*



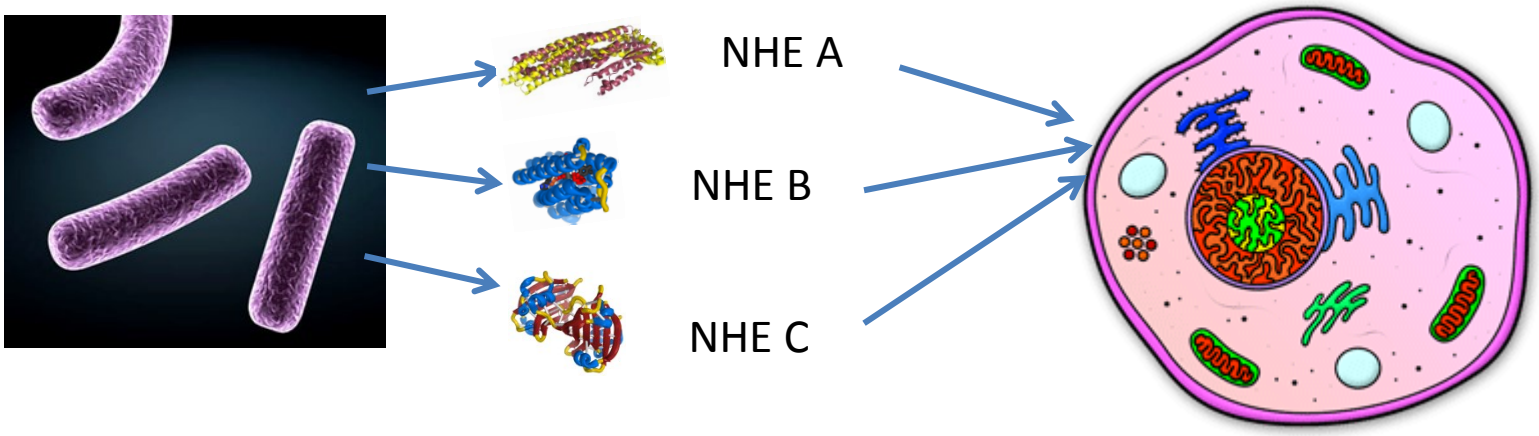
- Attività
  - **emolitica**
  - dermonecrotica
  - incremento permeabilità vascolare
  - necrosi oculare
  - **enterotossica**





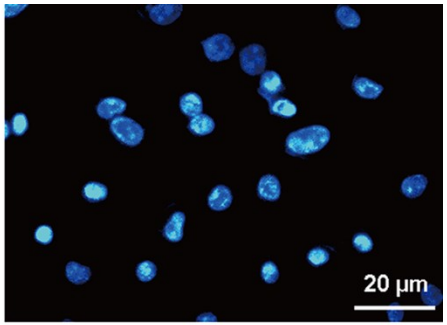
ENTEROTOSSINA NON EMOLITICA NHE

- Prodotta da circa il 100% dei ceppi di *B. cereus*



- Attività enterotossica?
  apoptosi (Liu et al., Cell Microbiol, 2016)
  cellule epiteliali

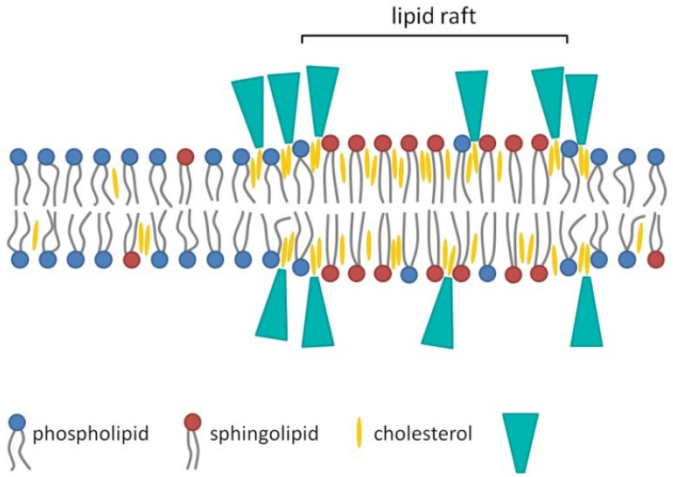
Nhe →



HLY I, HLY II, HLY IV

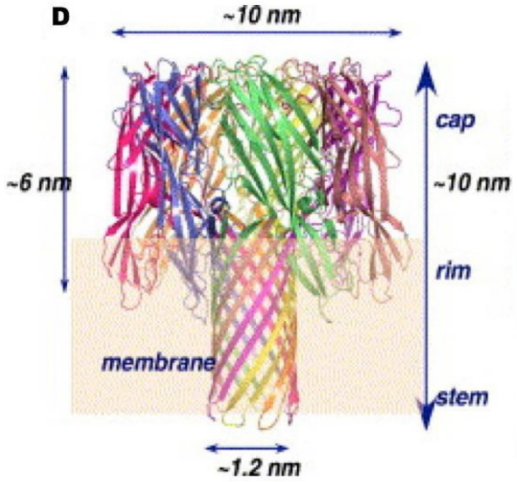
Hly I Cereolisina O

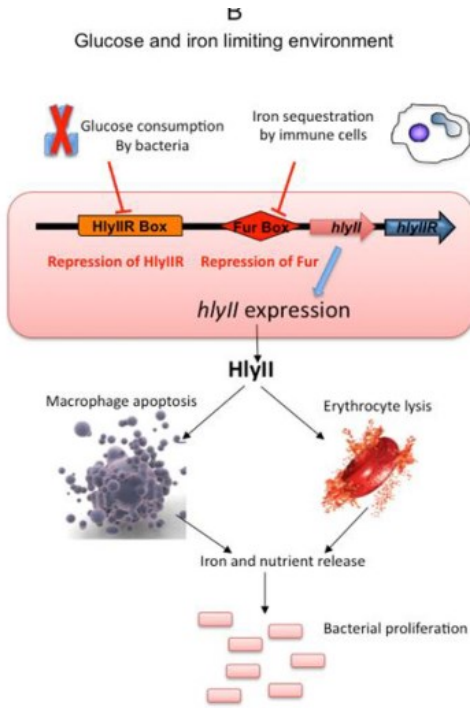
Tossina termolabile la cui attività è inibita dal colesterolo e che viene neutralizzata da un siero anti streptolisina-O



Hly II

Azione simile alla α-tossina di *S. aureus*





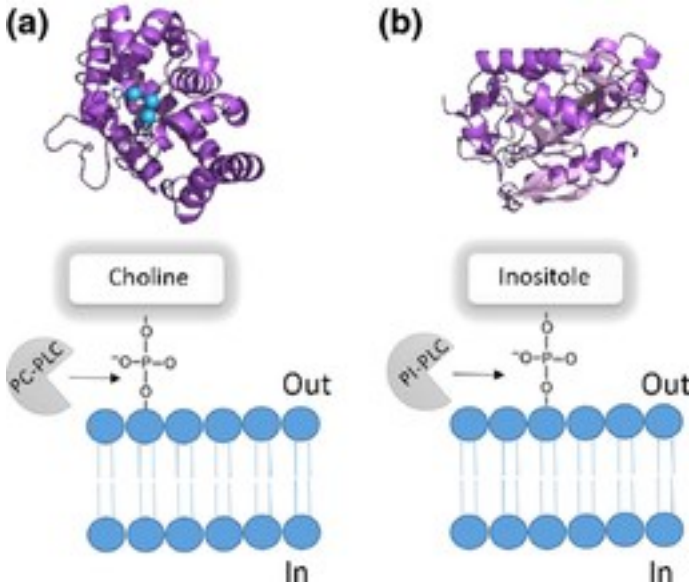
Hly IV CytK

Grave epidemia Francia 1998
NVH 391-98 (*B. cytotoxicus*)
CytK-1 e CytK-2
Tossica per cellule epiteliali (Caco-2 e Vero) ed emolitica
Simile alla leucocidina di *S. aureus*

FOSFOLIPASI C

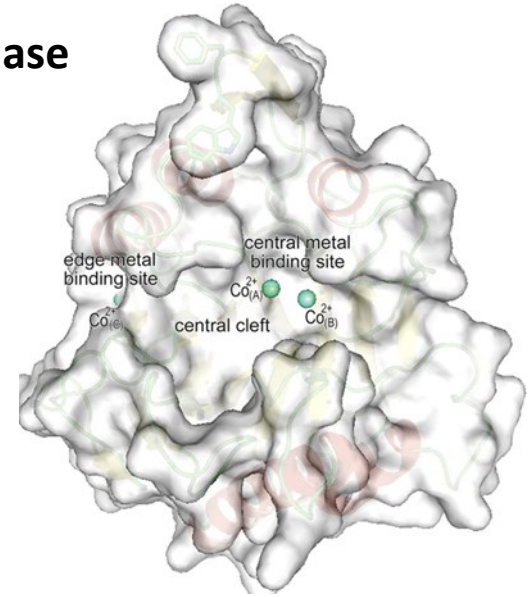
Attive su diversi tipi cellulari
Ruolo determinante nelle infezioni sistemiche sostenute da *B. cereus*

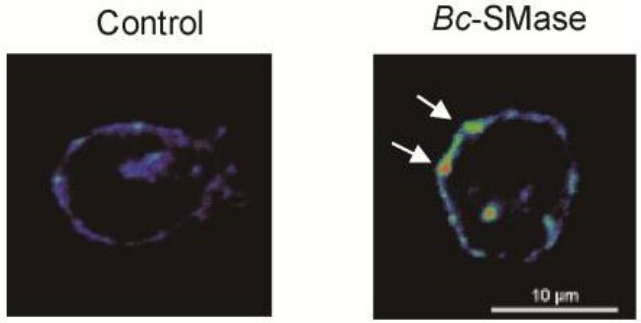
PC-PLC **PI-PLC**



(Celandroni et al., FEMS Microbiol Lett 2014)

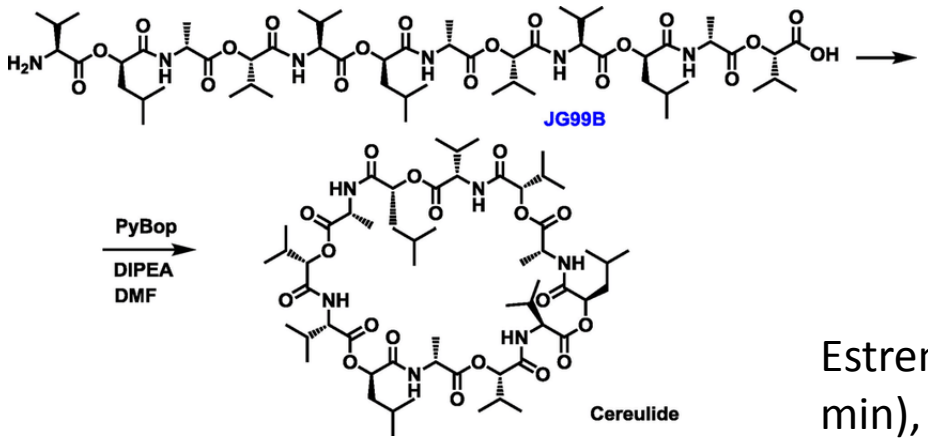
SMase





Macrofagi

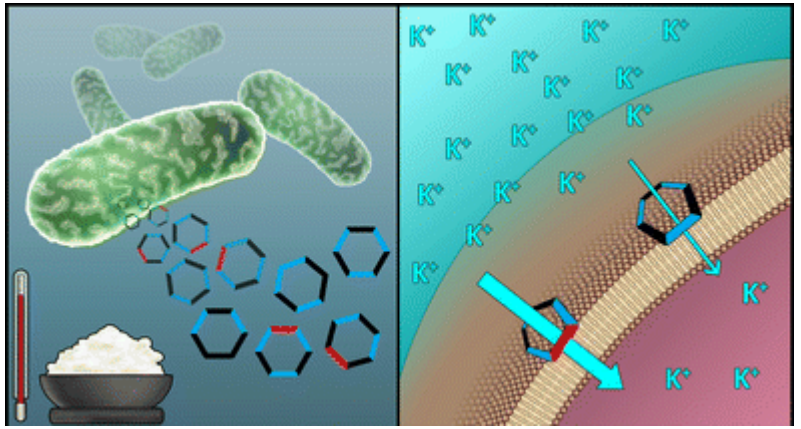
TOSSINA EMETICA CEREULIDE



Dodecadepsipeptide (D-*O*-Leu–D-Ala–L-*O*-Val–L-Val)$_3$ sintetizzato da un complesso di sintetasi peptidiche non-ribosomiali (NRPS) dette cereulide sintetasi e codificate dal cluster di geni *ces*

Estremamente termostabile (120°C per 90 min), stabile ad un ampio range di pH (2-11) e resistente agli enzimi proteolitici

- Agisce come ionoforo del K^+
- Alterazione del gradiente elettrochimico
- Alterazione mitocondriale



TOSSINA EMETICA CEREULIDE

a

cereulide: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)]

isocereulide A : [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(D-*O*-Leu-D-Ala-L-*O*-Leu-L-Val)]

isocereulide B: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(D-*O*-Val-D-Ala-L-*O*-Val-L-Val)]

isocereulide C: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(D-*O*-Leu-D-Ser-L-*O*-Val-L-Val)]

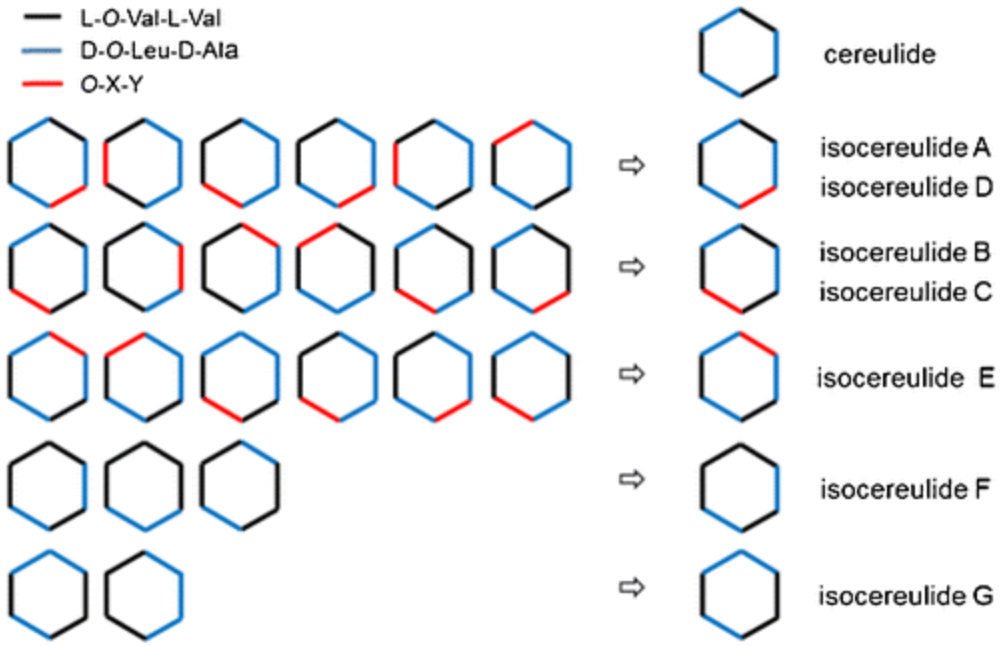
isocereulide D: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(D-*O*-Leu-D-Ala-L-*O*-Val-L-Ala)]

isocereulide E: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(D-*O*-Leu-D-Ala-D-*O*-Ile-D-Ala)]

isocereulide F: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(L-*O*-Val-L-Val-L-*O*-Val-L-Val)]

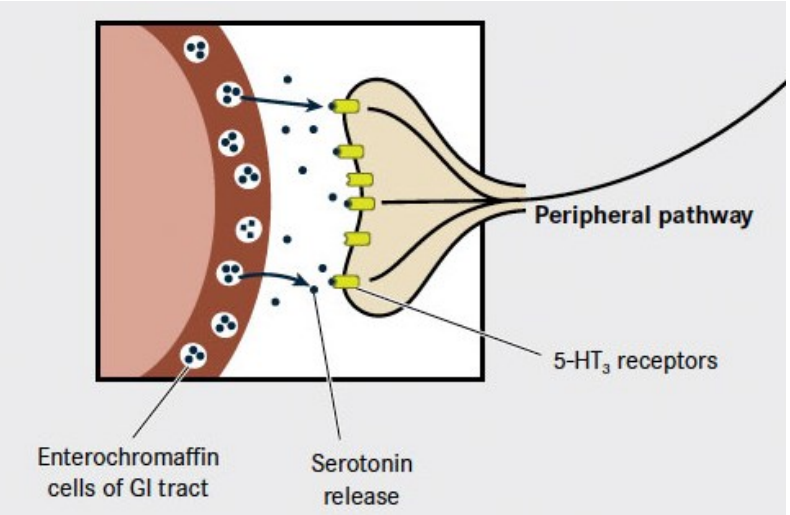
isocereulide G: [(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_2$(L-*O*-Val-L-Val-D-*O*-Leu-D-Ala)]

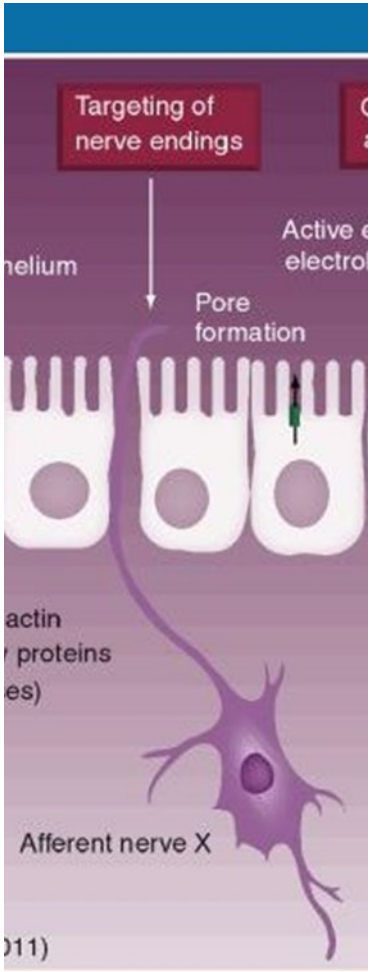
b



EFFETTO EMETICO DELLA CEREULIDE

Dipendente dalla stimolazione dei recettori 5-HT_3 sui neuroni vagali afferenti





MALATTIE NON GASTROINTESTINALI

Varietà di infezioni locali e sistemiche
Infezioni cutanee, batteriemie, meningitis, endoftalmiti…….

Species	Total samples(n. of strains)	Deep body sites(n. of strains)	Superficial body sites(n. of strains)
B. cereus	33.3% (25)	32.0% (16)	36.0% (9)
B. pumilus	18.7% (14)	22.0% (11)	12.0% (3)
B. subtilis	14.7% (11)	10.0% (5)	24.0% (6)
B. licheniformis	6.7% (5)	10.0% (5)	0.0% (0)
B. megaterium	6.7% (5)	6.0% (3)	8.0% (2)
B. simplex	6.7% (5)	6.0% (3)	8.0% (2)
B. mycoides	4.0% (3)	2.0% (1)	8.0% (2)
B. flexus	4.0% (3)	4.0% (2)	4.0% (1)
P. glucanolyticus	2.7% (2)	4.0% (2)	0.0% (0)
P. amylolyticus	1.3% (1)	2.0% (1)	0.0% (0)
P. lautus	1.3% (1)	2.0% (1)	0.0% (0)
Total	100% (75)	100% (50)	100% (25)

doi:10.1371/journal.pone.0152831.t002

(Celandroni et al., PLOSone 2016)

MALATTIE NON GASTROINTESTINALI

Species(n. of strains)	Toxins/enzymes				Virulence genes					
	Hemolysins	HBL	PC-PLC	Proteases	*plca*	*sph*	*cytK*	*nheA*	*nheB*	*nheC*
B. cereus (25)	100	84.0	88.0	56.0	40.40.0	52.0	24.0	60.0	56.0	56.0
B. pumilus (14)	92.8	0.0	0.0	100	0.0	0.0	0.0	71.4	50.8	57.1
B. subtilis (11)	0.0	0.0	0.0	100	0.0	0.0	0.0	18.2	0.0	0.0
B. licheniformis (5)	0.0	0.0	0.0	60.0	0.0	0.0	0.0	0.0	0.0	0.0
B. megaterium (5)	0.0	0.0	0.0	100	0.0	0.0	0.0	20.0	0.0	0.0
B. simplex (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. mycoides (3)	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0
B. flexus (3)	0.0	0.0	0.0	100	0.0	0.0	0.0	33.3	0.0	33.3
P. glucanolyticus (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. amylolyticus (1)	0.0	0.0	0.0	100	0.0	0.0	0.0	100	0.0	100
P. lautus (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total (75)	50.6	28.0	29.3	69.3	13.3	17.3	8.0	40.0	28.0	32.0

doi:10.1371/journal.pone.0152831.t004

(Celandroni et al., PLOSone 2016)

SINDROME DIARROICA

*Diarrheal syndrome is due to the **production of heat labile enterotoxins during growth of vegetative cells in the small intestine** of the host and the **infective dose is 10^4–10^9 cells per gram of food**. This syndrome is mild and primarily manifested by **abdominal cramps and diarrhea** following an **incubation period of 8 to 16 h** and lasting for 6 to 12 h. Diarrhea may be mild or profuse and watery.*

SINDROME EMETICA

The emetic syndrome is more severe and acute than diarrheal syndrome and is referred to as "short-incubation" or emetic form of the disease. Emetic syndrome is characterized by ***nausea and vomiting and abdominal cramps****. The toxin responsible for this syndrome is a small cyclic heat-stable peptide which causes vomiting after* ***1 to 6 h of ingestion (average 2 to 5 h)****. The toxin* ***is preformed and indigested with food****. In emetic type of illness, the dose is about* 10^5–10^8 *cells per gram in order to produce sufficient toxin. It resembles* Staphylococcus aureus *food poisoning in its symptoms and incubation period.*

Bacillus cereus:
a flexible human pathogen