Inability of *Escherichia coli* to Resuscitate from the Viable but Nonculturable State

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Viable but nonculturable (VBNC) state (1)

Intact and metabolically active but nonculturable bacterial cells

Stochastic cellular deterioration? (2)

Programmed cellular deterioration? (3)

Persistence strategy? (4)

RESUSCITATION OR NO RESUSCITATION?, "THAT IS THE QUESTION"

Recovery of growth capacity in true VBNC cells **or** Growth of few remaining culturable cells

Aim of the work

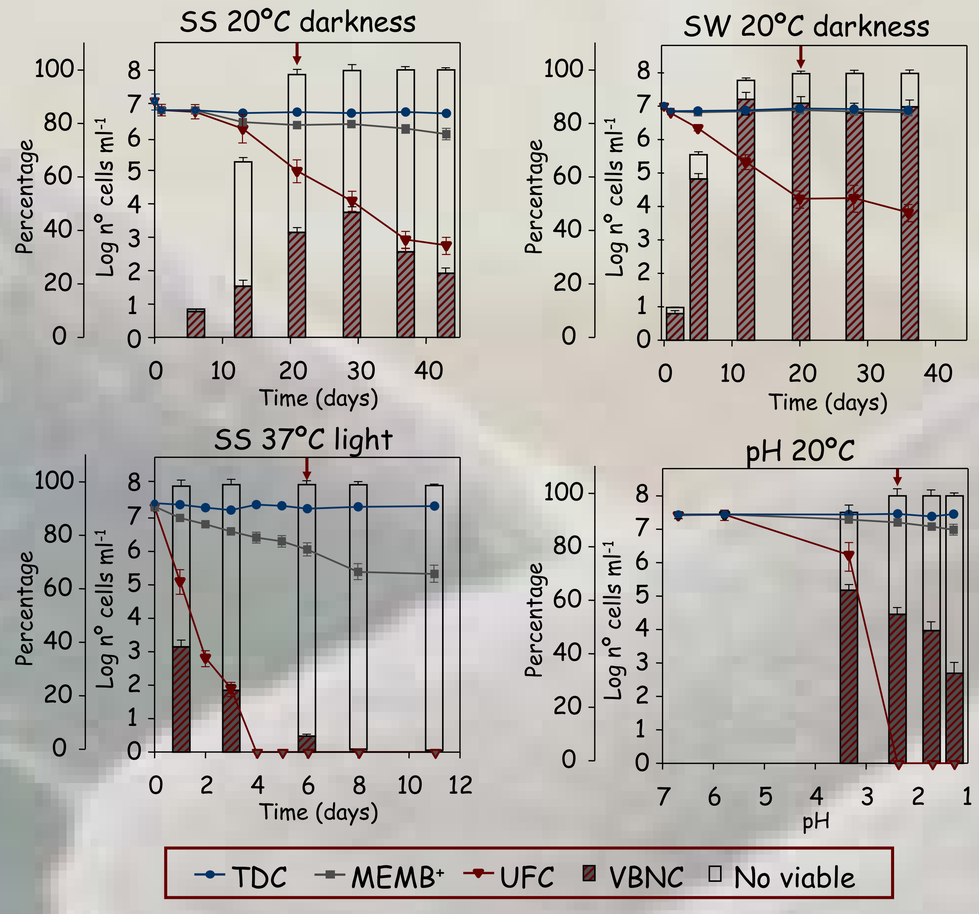
To study the influence of abiotic and biotic factors on the resuscitation of VBNC populations of *Escherichia coli*

RESULTS AND DISCUSSION

Formation of nonculturable *E. coli* populations

A pattern of response to adverse factors is obtained: a drop in density of culturable cells and the formation of culturable and nonculturable subpopulations.

From these results - The beginning of the loss of culturability and the percentages of different subpopulations formed are stress-dependent.



MATERIAL AND METHODS

Bacterial strains

Origin of VBNC populations: *Escherichia coli* STCC 416 (Spanish Type Culture Collection)

Origin of supernatants: *Escherichia coli* STCC 416, *Pseudomonas fluorescens* CHA0 (5) and *Enterococcus faecalis* pMV158GFP (6)

Bacterial counts

Total number of bacteria (TDC) (7)

Viable bacteria with intact cytoplasmic membranes (MEMB$^+$) (8)

Culturable bacteria (CFU)

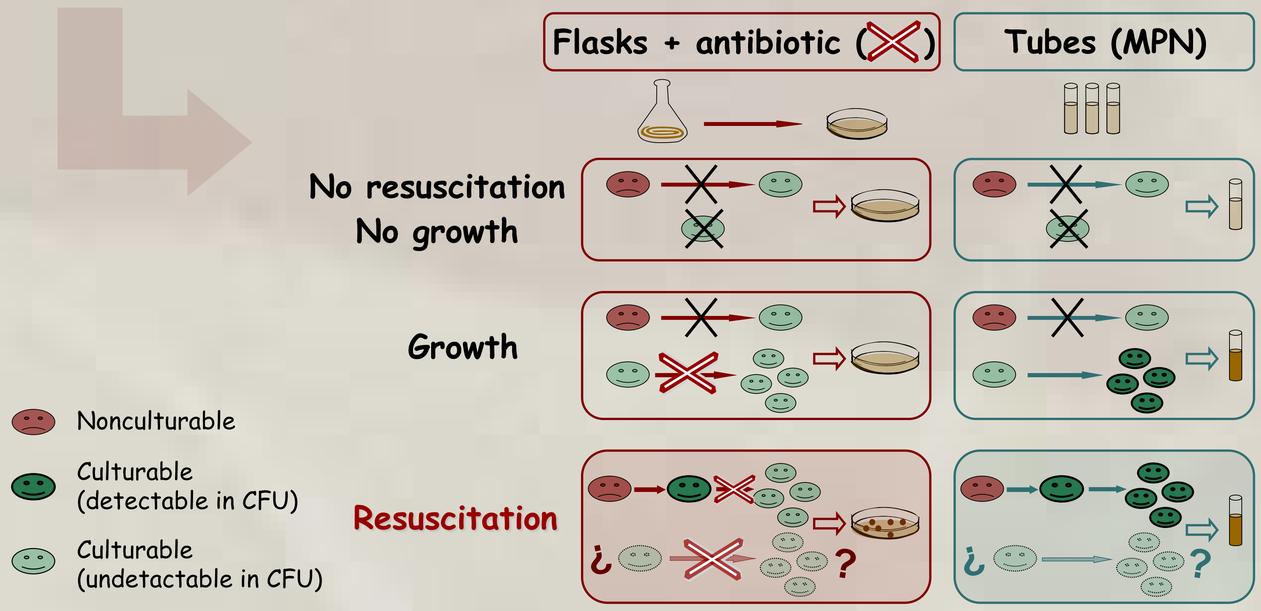
Preparation of nonculturable *E. coli* suspensions

Origin of nonculturable cells suspensions (VBNC + no viable cells): *E. coli* populations exposed to adverse conditions (starvation, visible radiation, seawater, acid environment, hydrogen peroxide).

Resuscitation procedures

Culture media (supplemented or not with catalase) or supernatants from growth curves of the three bacterial strains. In some cases, antibiotics (streptomycin and/or ciprofloxacin) or lisozyme were added..

A working protocol was designed combining two experimental procedures (flasks and MPN method) (see diagram). Working protocol allows distinguishing unequivocally between regrowth and resuscitation.



RESULTS AND DISCUSSION

Resuscitation of nonculturable *E. coli* populations

Three kind of resustation factors are analyzed:

1. Removal of environmental stress and prevention of additional oxidative stress (i.e addition of catalase).
2. Repairing of damage and activation of replicative functions by means of peptydoglycan cleavage (i.e. addition of lisozyme).
3. Signal molecules to stimulate the process (i.e. addition of supernatants)

Repeated resuscitation attempts indicate that the *E. coli* strain used is not able to resuscitate from the VBNC state. The behaviour of *E. coli* should not be necessarily a standard for other bacteria.

It is well known that VBNC cells release organic substances into the surrounding medium (9). The nutrients left over or contributed by the VBNC cells could serve to aid the survival of the persisting culturable cells.

What is the role of the VBNC cells?

The formation of a VBNC subpopulation could be seen as an adaptive process, designed for the benefit of the population as a whole.

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Aknowledges

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