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Influence of the carbon source on expression of sporulation gene *SpoIIE* in *Bacillus species*

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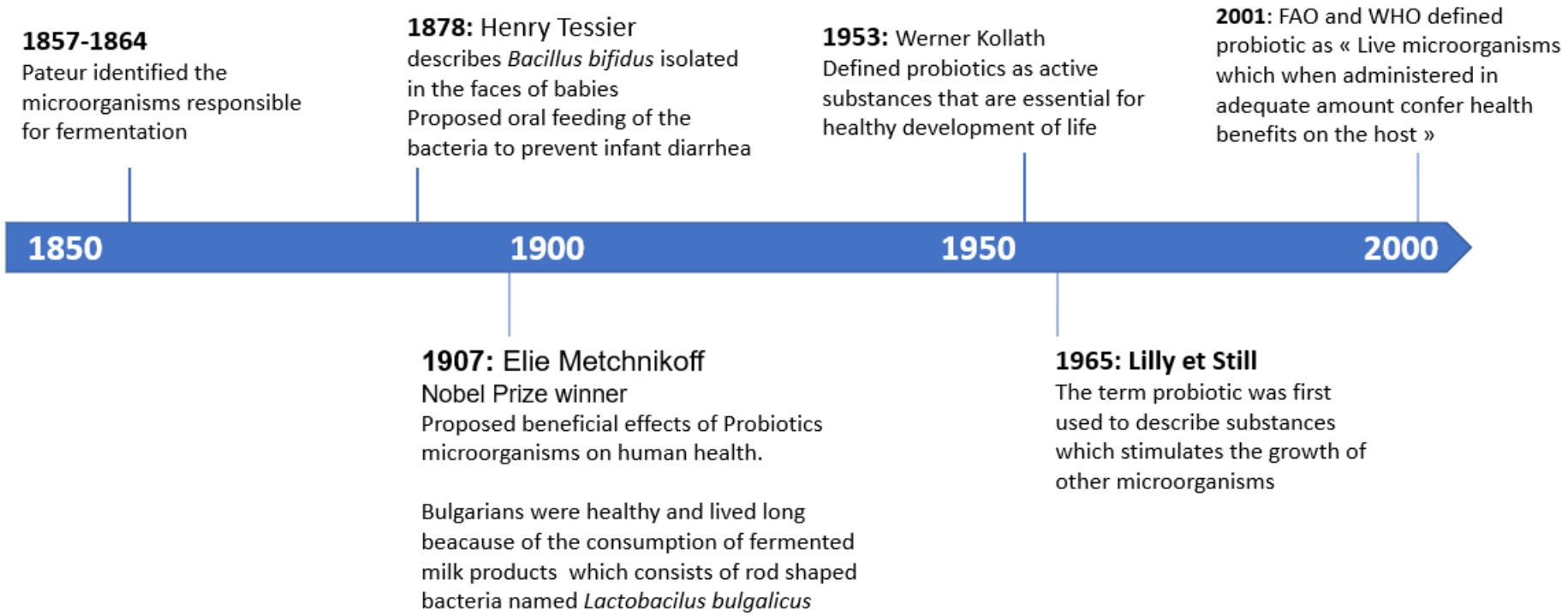
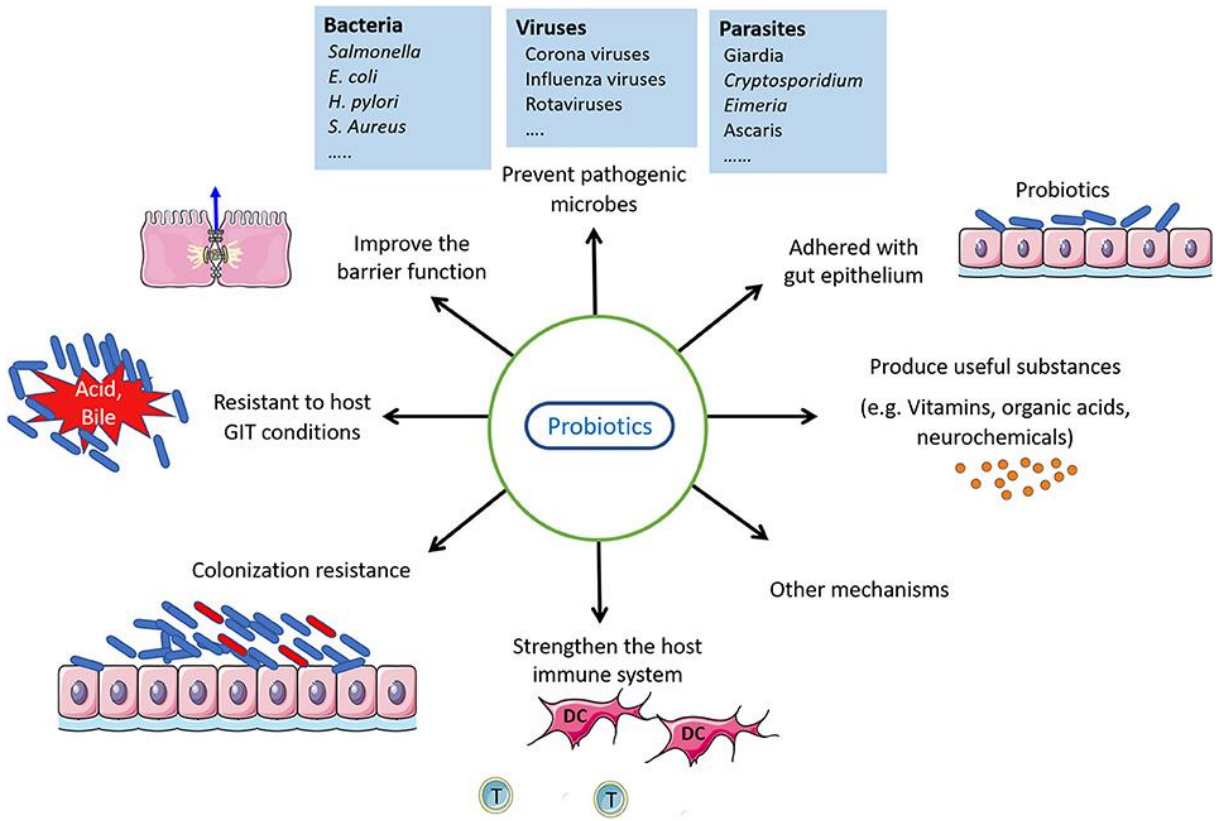
I. Introduction

Historic



doi: 10.1097/MCG.0000000000000697

Mode of action of probiotics



https://doi.org/10.3389/fimmu.2021.616713

Types of probiotics

- Probiotics products may contain one or more selected microbial strains
  - Human probiotic microorganisms belong mostly to the following Genius:

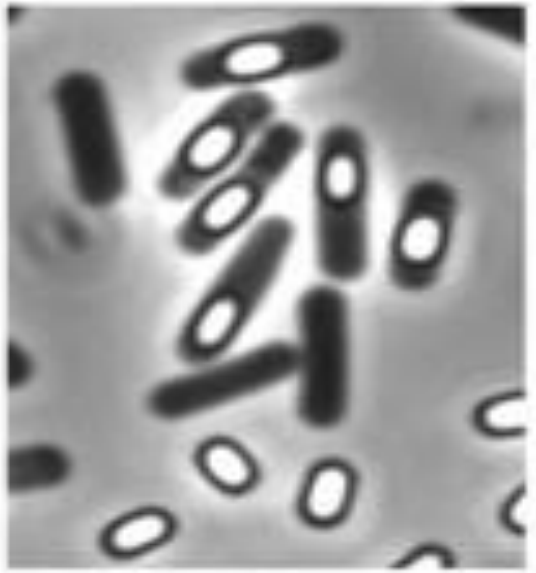
    Lactobacillus, *Bifodobaterium*, *Lactococcus*, *Streptococcus*, *Enterococcus*

  - Gram positive strains belong to Bacillus Genus : *B. coagulans*, *B. subtilis*, *B. licheniformis*, *B. clausii*

  - Yeast strains belong to Saccharomyces Genus: *S. boulardii* is commonly used in probiotic products

Interest of sporulating bacilli as probiotics

- *Bacillus* species have advantage over *Lactobacillus* species :
  - They have an enhanced tolerance and survivability under gastrointestinal tract hostile environment
  - They are stable during processing and storage of food and pharmaceutical preparations
  - Ease maintaining storage without loss of viability
  - They are able to revitalize and growth quickly to the maximum concentration in a simple fermentation medium



Endospores of *B. subtilis*

https://schaechter.asmblog.org/schaechter/2008/01/subtle-bacill-1.html

Objectives

- Follow the expression of *spoIIE* gene by using gene encoding GFP under the control of the promoter of interest.
  - Construction of plasmid carrying the fluorescence reporter gene downstream P*spoIIE* promoter (P*spoIIE-gfp)*.
  - Plasmid will be harboured by *B. licheniformis* and *B. subtilis* and sporulation followed in presence of different sugars.

III. Results

Plasmid construction

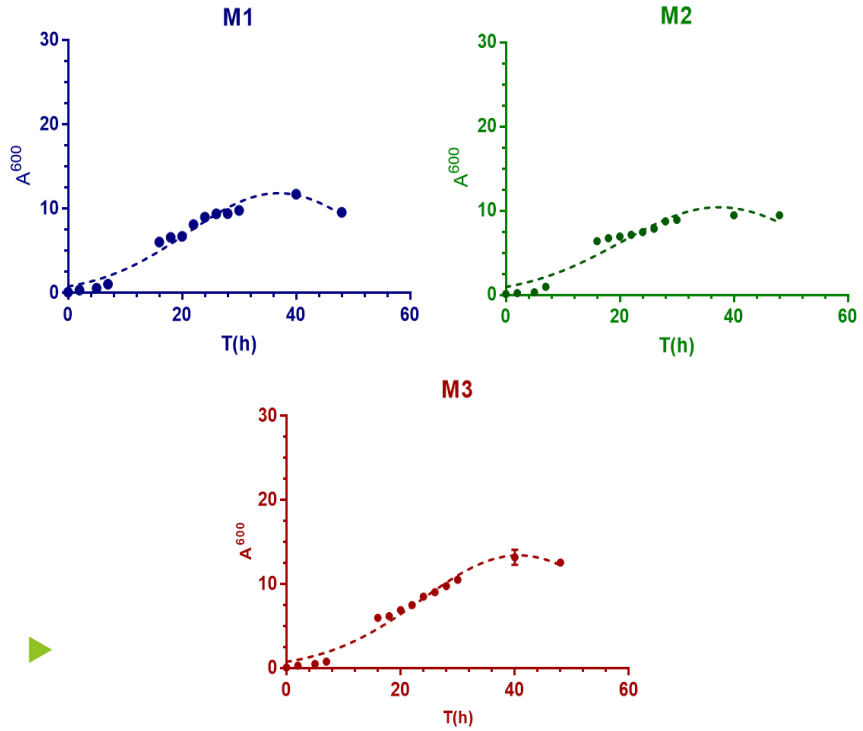
Plasmid	Organism of origin	Transformation in
pUlg_PspoIIE_gfp_*B.licheniformis*	*B. licheniformis*	*B. licheniformis*
pUlg_PspoIIE_gfp_*B. subtilis*	*B. subtilis*	*B. subtilis*

Following the expression of *spoIIE gene*

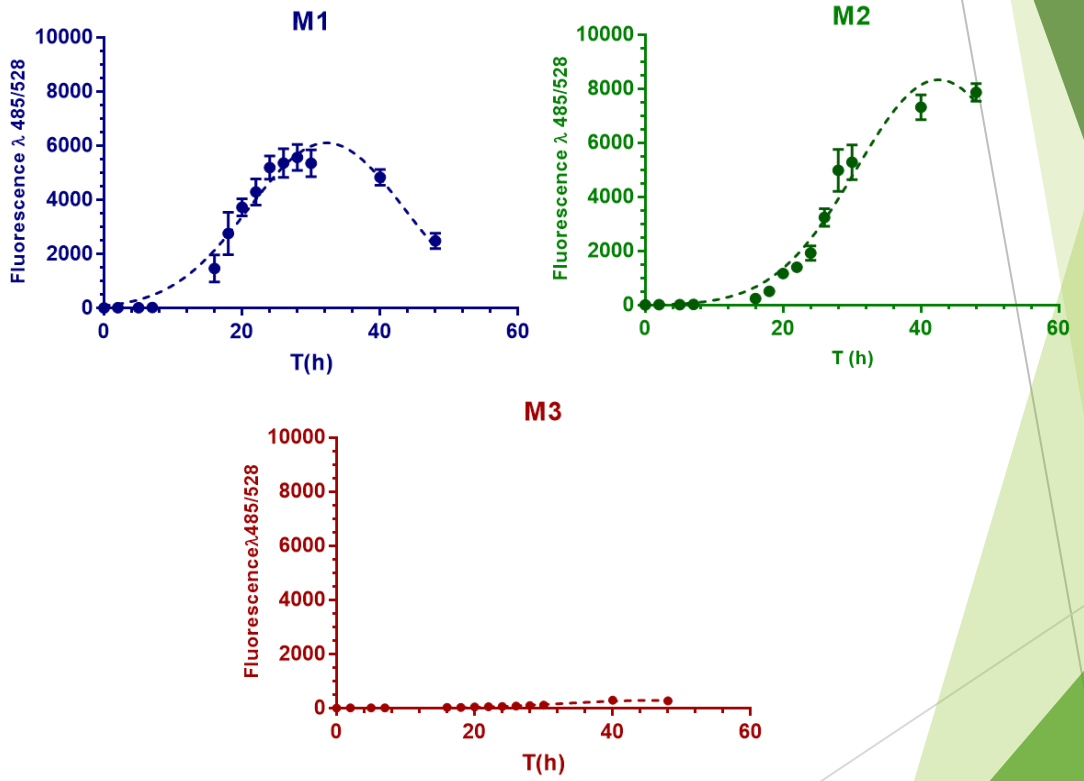
- Both in *B. licheniformis* and *B. subtilis*, cells were grown at 37°C in:
  - M1 medium (without added sugar)
  - M2 medium (supplemented with 0.5% of glucose)
  - M3 medium (supplemented with 0.5% of xylose) .
- After inoculation, samples were collected at different time points and the pH, absorbance and fluorescent intensity were recorded.
- The expression of *spoIIE* gene was examined by measuring the fluorescent intensity of *gfp* (λ^{exc} = 485 nm and λ^{emi} 528 nm).
- The number of spores and total floral were count in both species.

Expression of *spoIIE* in *B. licheniformis* P_{spoIIE}-*gfp*

- Absorbance (600 nm)



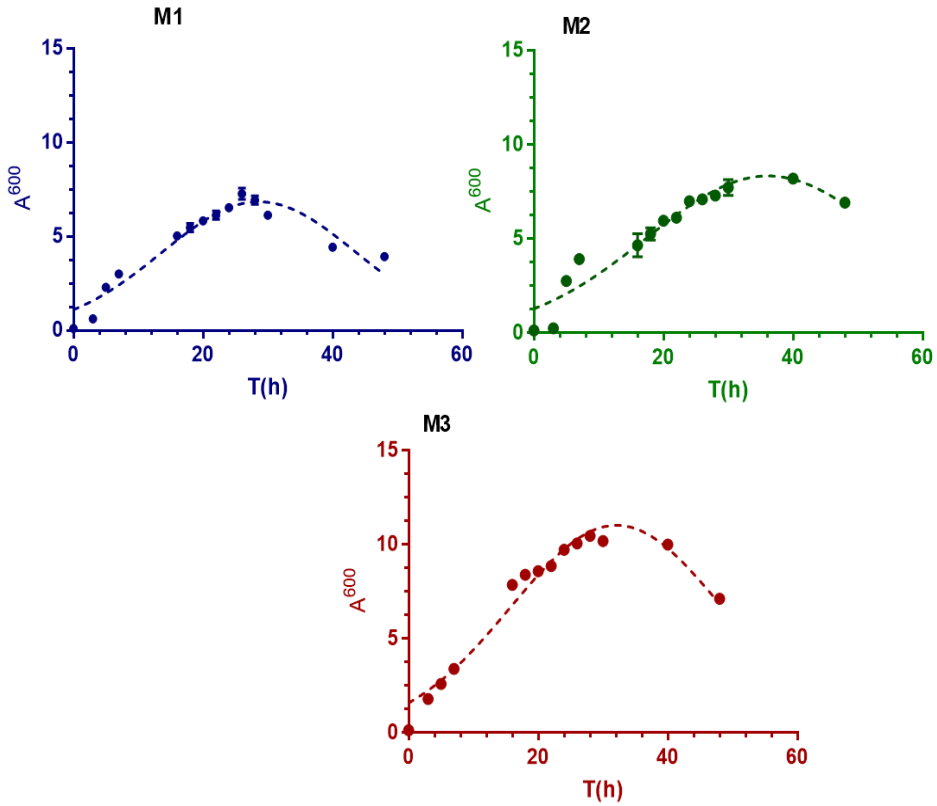
- Fluorescence (λ485/528 nm)



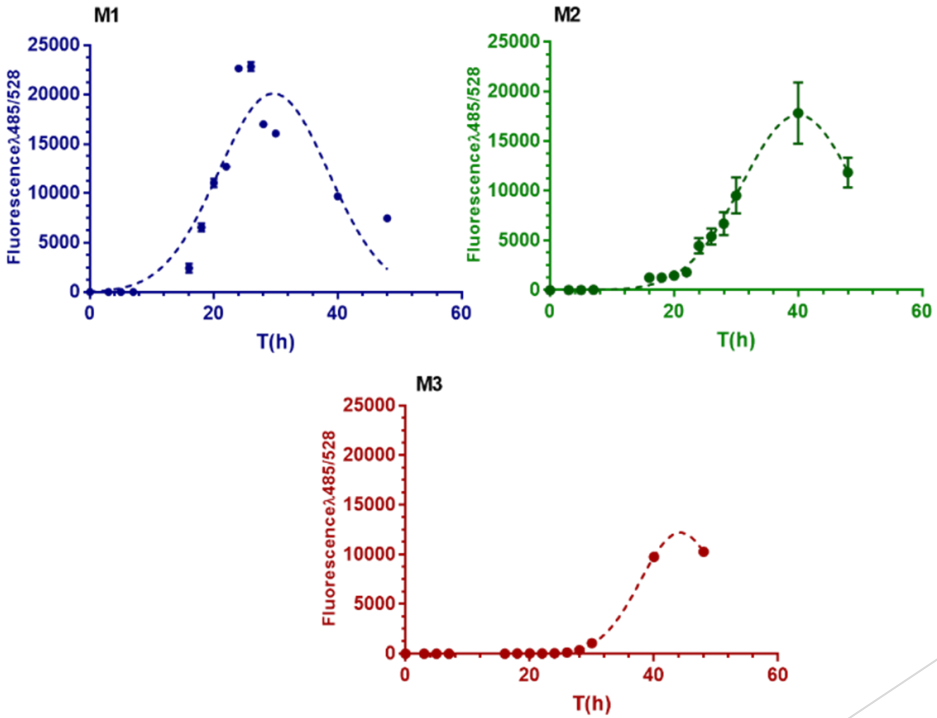
The presence of xylose (M3) inhibited the expression of *spoIIE*

Expression of *spoIIE* in *B. subtilis* P_{spoIIE}-*gfp*

- Absorbance (600 nm)



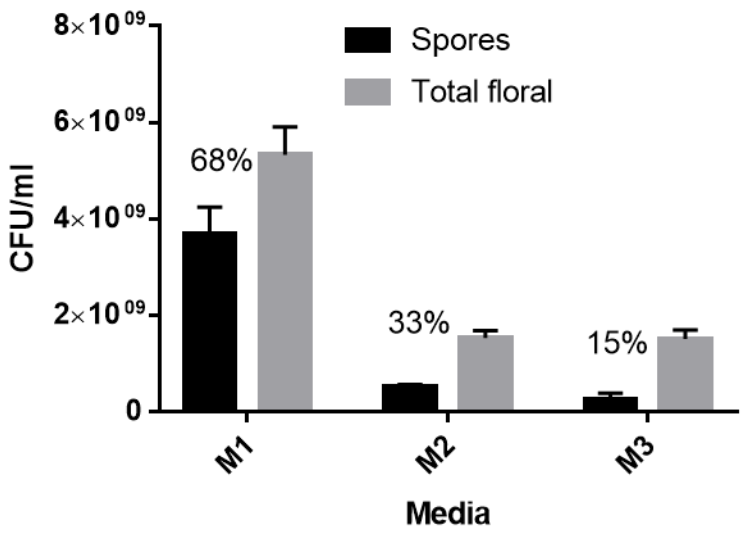
- Fluorescence (λ485/528 nm)



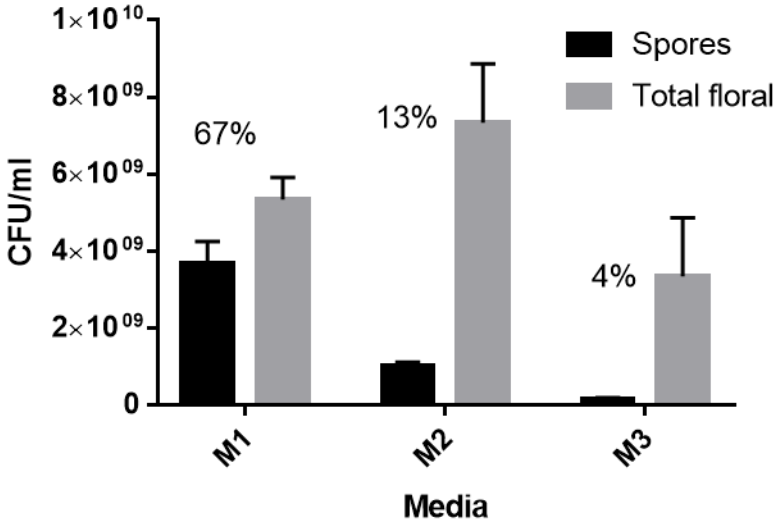
The fluorescence signal in M1 is higher than one in M2 and M3
The presence of glucose and xylose affected the expression of *spoIIE*

Spores count

- *B. licheniformis* P_{spoIIE}-*gfp*



- . *B. subtilis* P_{spoIIE}-*gfp*



The higher sporulation rate was observed in M1: 68 %

The presence of glucose and xylose inhibits the sporulation

Conclusion

- Our results show that *B. licheniformis* P_{spoIIE}-gfp and *B. subtilis* P_{spoIIE}-*gfp*, are good reporter promoter to follow the sporulation initiation in both strain.
- The expression of *spoIIE* gene was affected by the presence of xylose in the culture media.
- The presence of glucose inhibit or delays the expression of *spoIIE* gene and the sporulation of the strain.
- We have demonstrated that the sporulation rate is higher in M1 medium without added sugar.

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