



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

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## I. Introduction

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#### Historic

#### **1857-1864** Pateur identified the microorganisms responsible for fermentation

**1878:** Henry Tessier describes *Bacillus bifidus* isolated in the faces of babies Proposed oral feeding of the bacteria to prevent infant diarrhea

1900

**1953:** Werner Kollath Defined probiotics as active substances that are essential for healthy development of life **2001**: FAO and WHO defined probiotic as « Live microorganisms which when administered in adequate amount confer health benefits on the host »

2000

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#### 1850

#### 1950

**1907:** Elie Metchnikoff Nobel Prize winner Proposed beneficial effects of Probiotics microorganisms on human health.

Bulgarians were healthy and lived long beacause of the consumption of fermented milk products which consists of rod shaped bacteria named *Lactobacilus bulgalicus* 

doi: 10.1097/MCG.00000000000697

1965: Lilly et Still

The term probiotic was first used to describe substances which stimulates the growth of other microorganisms

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#### Mode of action of probiotics



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#### 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

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

### III. Results

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#### **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 spollE 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* ( $\lambda^{exc} = 485 \text{ nm}$  and  $\lambda^{emi} 528 \text{ nm}$ ).
- The number of spores and total floral were count in both species.

### Expression of *spollE* in *B. licheniformis* P<sub>spollE</sub>-gfp

Absorbance (600 nm)

Fluorescence ( $\lambda 485/528$  nm)



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The presence of xylose (M3) inhibited the expression of spollE

## Expression of *spollE* in *B. subtilis* P<sub>spollE</sub>-gfp

Absorbance (600 nm)

Fluorescence ( $\lambda$ 485/528 nm)



#### Spores count



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<sub>spollE</sub>-gfp and B. subtilis P<sub>spollE</sub>-gfp, are good reporter promoter to follow the sporulation initiation in both strain.
- The expression of *spollE* gene was affected by the presence of xylose in the culture media.
- The presence of glucose inhibit or delays the expression of spollE 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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