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

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A. Introduction

Bacillus probiotics have been extensively studied and isolated since many years from different food products. Although generally used in dairy products, they also widely used in various commercial food such as fermented meats, cereals, baby foods, fruits juices and ice creams.

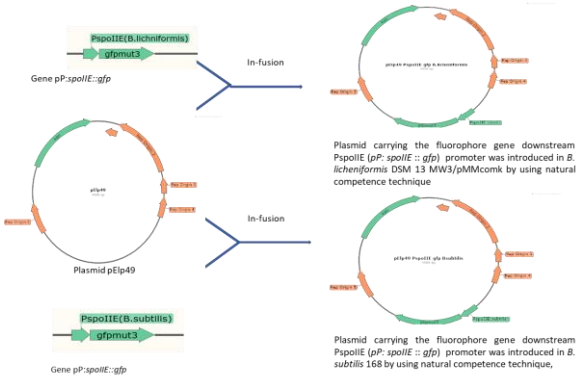
The interest to study *Bacillus sp* are related to their ability to produce spores that have several advantages compared to non-spore-forming *Lactobacillus sp.* which are also used as probiotics. *Bacillus sp* benefits are linked to spore resistance to heat, chemical agents and enzyme degradation. In the form of spore, *Bacillus sp.* can be stored at room temperature, without any deleterious effect on their viability. They can survive in acid conditions of the stomach and so grow in the intestines in which they can play their role of probiotics.

Although, a large number of studies have been carried out on the production of industrially *Bacillus* sp.'s derivative products such as lactic acid and various enzymes, a few number of studies have been carried on the spores production by *Bacillus* species.

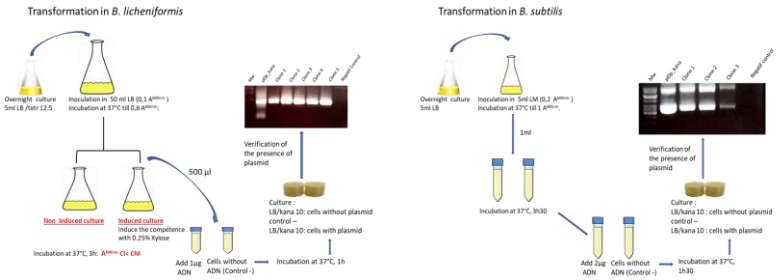
In the present study, we follow the expression of *spoIIE gene*, which plays a crucial role in *B. subtilis*, *B. licheniformis* and *B. coagulans* sporulation, by following its expression by using the gene encoding GFP under the control of *spoIIE* promoter. The plasmid carrying the fluorophore gene downstream P*spoIIE* promoter have been harboured by *B. licheniformis* and *B. subtilis*. In our studies, cultures were carried out in presence of different sugars used as carbon source.

B. Methods

Plasmid construction



Transformation by natural competence

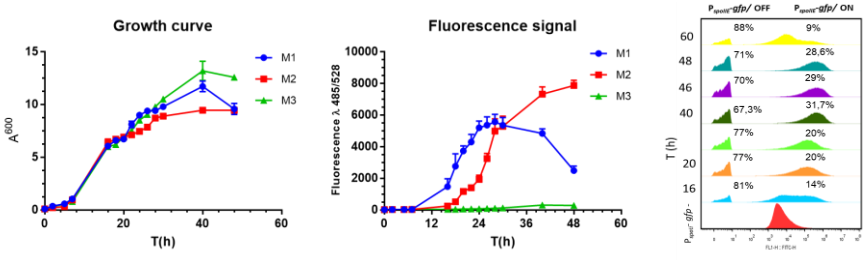


Time course of *SpoIIE* gene expression and sporulation

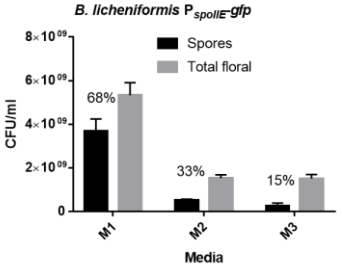
Both *B. licheniformis* and *B. subtilis*, cells were grown at 37°C in M1 medium , M2 medium Artechno production (supplemented with 0.5% of glucose) and M3 Artechno production medium (supplemented with 0.5% xylose) . After inoculation, samples were withdrawn at increasing time and their pH, absorbance and fluorescent intensity were recorded. The *spoIIE* expression of was monitored by measuring the fluorescent intensity of Gfp reporter protein (λ^{exc} = 485 nm and λ^{emi} 528 nm) by using fluorimeter and flow cytometer . The number of spores and total floral were count in both species,

C. Results

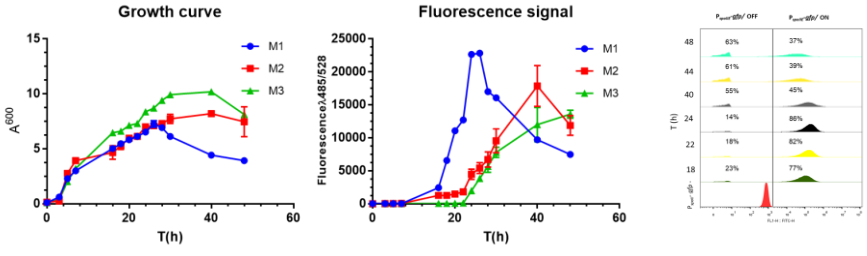
Expression of *spoIIE in B. licheniformis* DSM13/pP$_{spoIIE}$::*gfp*



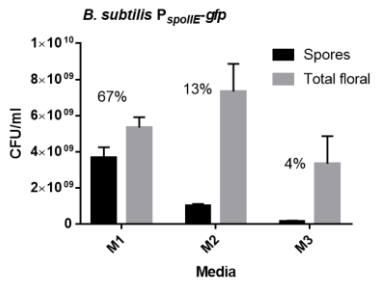
Total floral and spores counts



Expression of *spoIIE in B. subtilis* P$_{spoIIE}$-*gfp*



B. subtilis P$_{spoIIE}$-*gfp*



The fluorescence in M1 culture media is higher than those observed in M2 and M3

The presence of glucose and xylose delayed *spoIIE*expression of

The higher sporulation rate was observed in M1: for B. licheniformis 68 % and B. subtilis 67% .

The presence of glucose and xylose inhibits the sporulation

D. Conclusion

Our results shows that i) *B. licheniformis* DSM13 /pP$_{spoIIE}$::gfp and *B. subtilis* P$_{spoIIE}$-*gfp*, are good reporter promoters to follow sporulation initiation in both strains . ii) The addition of xylose in the culture media affect *spoIIE* expression in the two strains studied, and iii) the presence of glucose inhibit or delays the expression the sporulation gene. Finally, among the three artechno culture media, M1 media without added sugar, is the best media to obtain a high degree of sporulation.

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