SpoIIE promoter fused to *gfp* gene: a good reporter promoter to monitor sporulation initiation in *Bacillus sp*.

J.F Kabanyana[1], M. Dauvin[1], P. Thonart [2] and B. Joris [3]

1 University of Liège, 2 Artechno sa. rue Hermann Meganck, 3052, Gembloux , 3 InBios/CIP, Institut de Chimie B6a, University of Liège, B4000, Belgium

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.

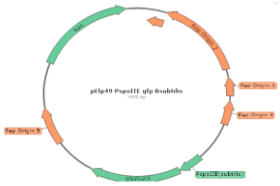
Although, a large number of studies have been carried out on the production of industrially *Bacillus* sp.'s derivative products such as lactic acid and various enzymes, a few number of studies have been carried on the spore's production by *Bacillus* species.

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

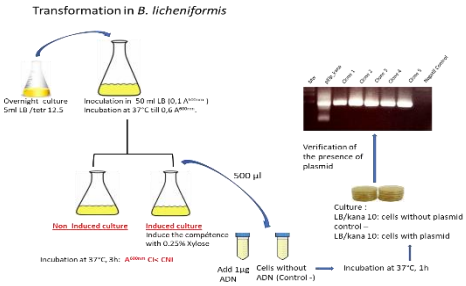
Plasmid construction

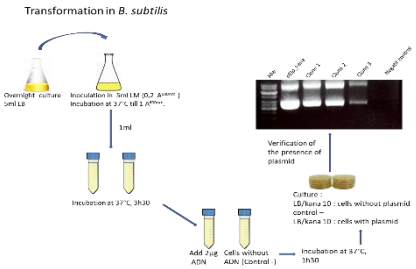
Plasmid carrying the fluorophore gene downstream PspoIIE (*pP: spoIIE :: gfp*) promoter was introduced in *B. licheniformis* DSM 13 MW3/pMMcomk by using natural competence technique



Plasmid carrying the fluorophore gene downstream PspoIIE (*pP: spoIIE :: gfp*) promoter was introduced in *B. subtilis* 168 by using natural competence technique,

Transformation by natural competence





Time course of *SpoIIE* gene expression and sporulation

Both *B. licheniformis* and *B. subtilis*, cells were cultured at 37°C in Artechno production media (M1 , M1G, M1X and M4) supplemented with 0.5% of glucose or xylose depending on the case under investigation.

After inoculation, samples were withdrawn at increasing times and their pH, absorbance at 600 nm (A^{600}) and gfp fluorescence intensity were recorded.

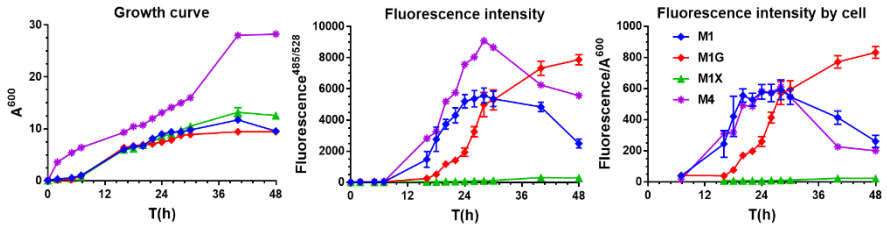
The *spoIIE* expression of was monitored by measuring the fluorescence intensity of Gfp reporter protein (λ^{exc} = 485 nm and λ^{emi} 528 nm), by using fluorimeter,

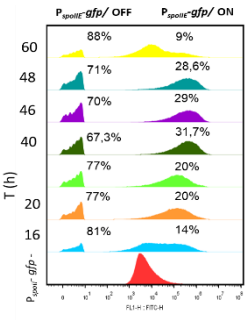
The flow cytometer was used to distinguish fluorescent and non-fluorescent cells.

The number of spores and total floral were count in both species.

C. Results

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





The fluorescence intensity in M4 culture media is higher than those observed in the media M1, M1G and M1X.

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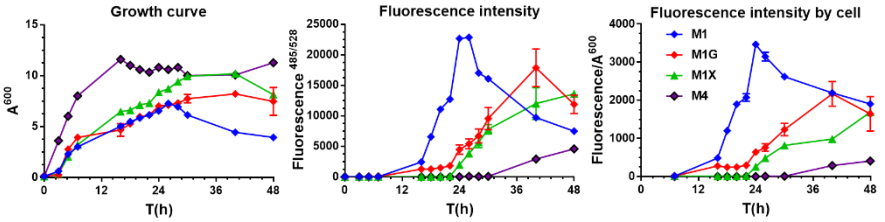
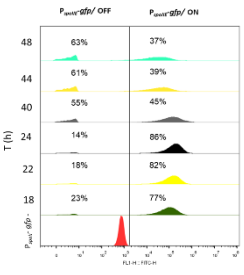
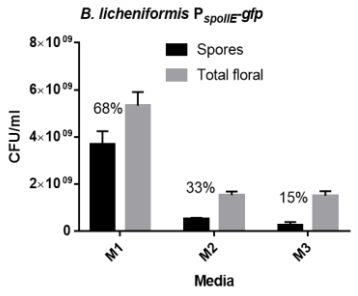
Expression of *spoIIE in B. subtilis* P$_{spoIIE}$-*gfp*

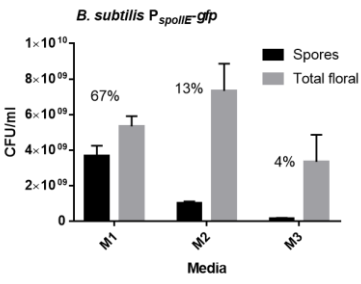




The presence of glucose and xylose delayed *spoIIE* expression in media M1G and M1X, and M4 medium were not optimised for sporulation initiation.

Total floral and spores counts





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 inhibits or delays the expression of the sporulation genes. Finally, among the three artechno culture media, M1 medium without added sugar, is the best medium to obtain a high degree of sporulation.

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