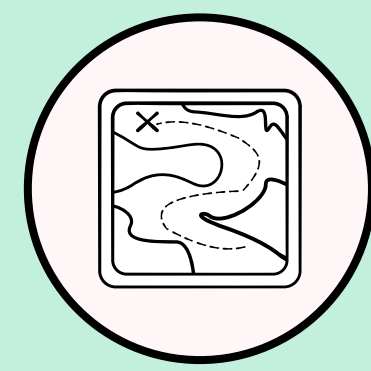


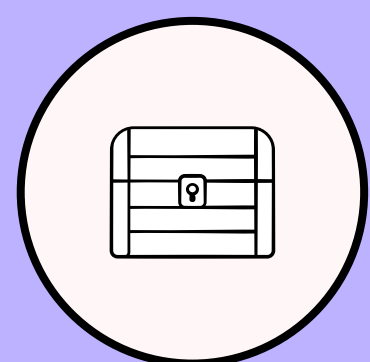
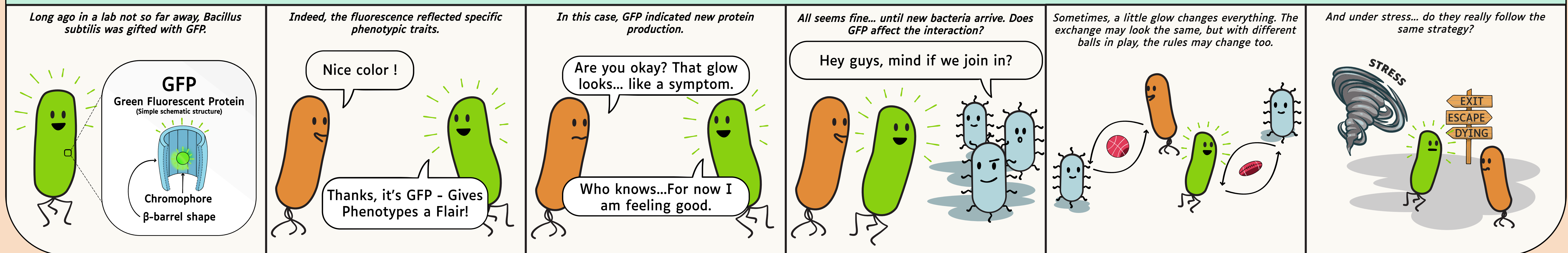


Do fluorescent proteins disrupt microbial balance in *Bacillus subtilis*?

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CONTEXT



RESULTS

A Do Fluorophores Affect Growth? Sometimes

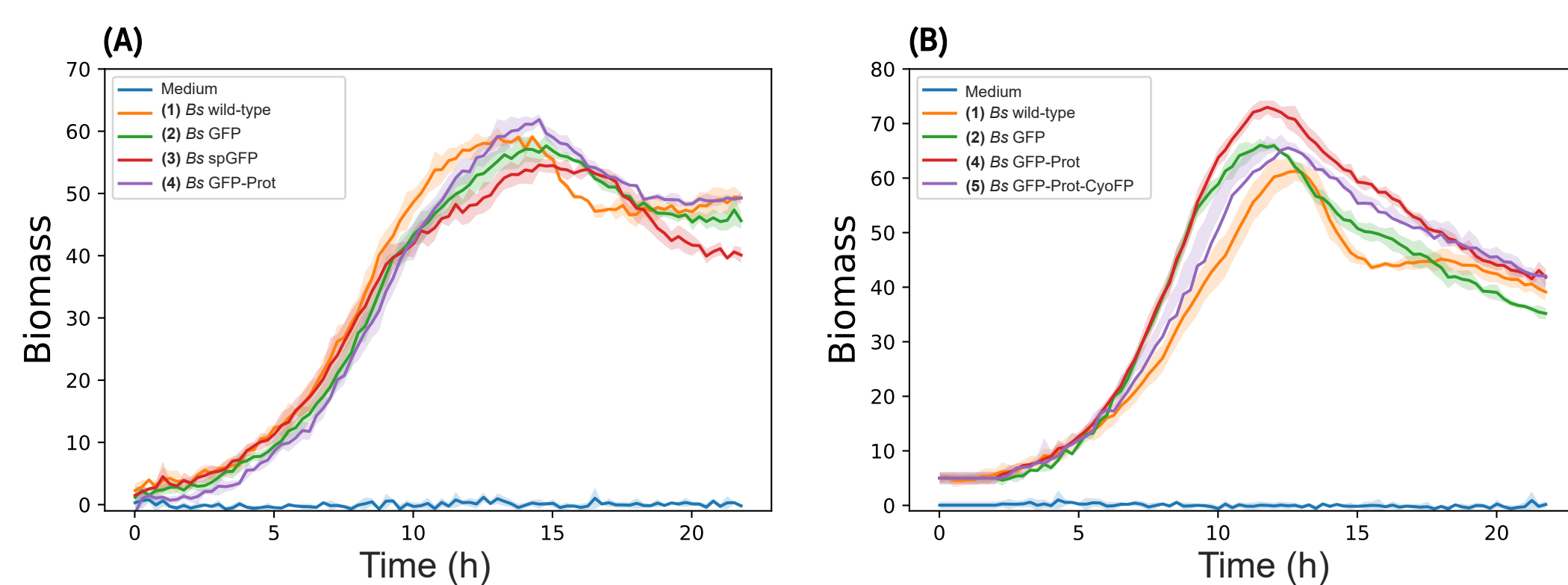
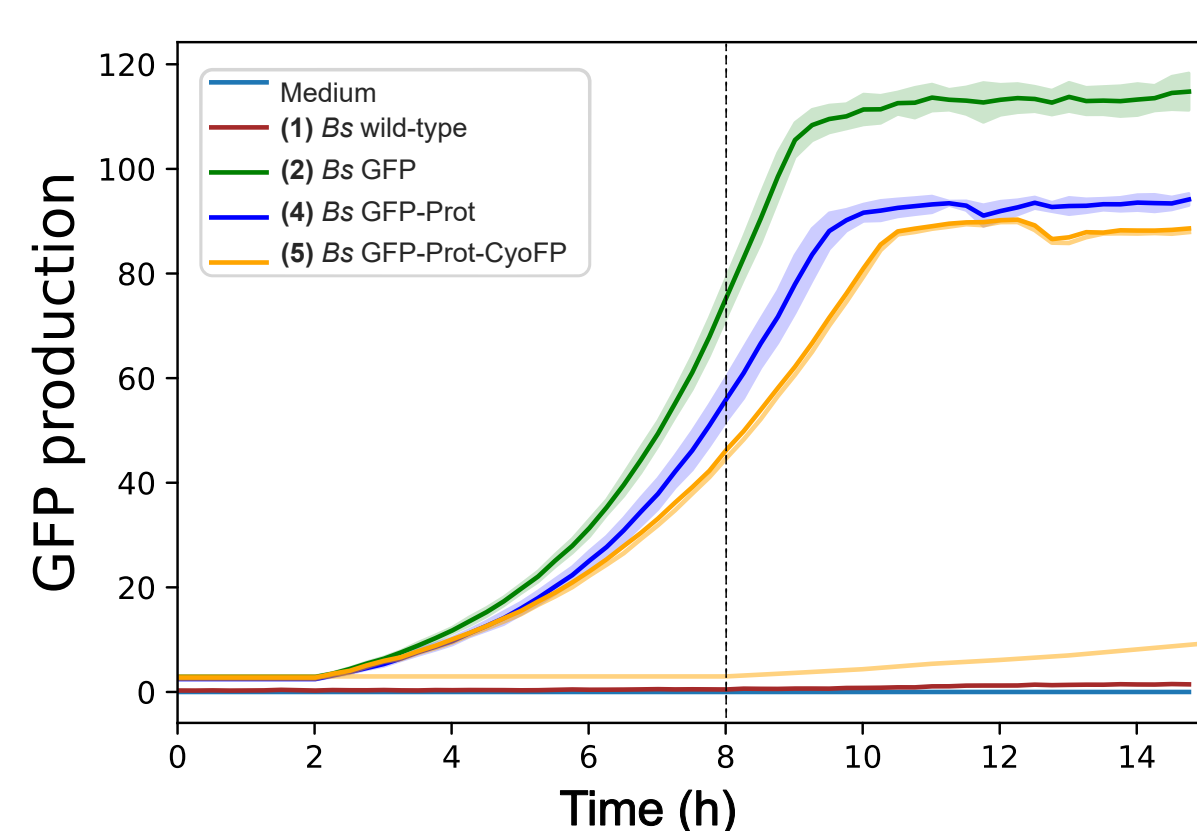


Figure 1 – Biomass of the different *B. subtilis* strains over time in the Biolector system, cultivated in M9 medium supplemented with 5 g/L glucose and IPTG at concentrations of (A) 0.5 mM and (B) 0.6 mM.

Growth rates are equivalent during the exponential phase. But at higher IPTG concentrations, there is a slight increase.

B Is Fluorophore Expression Consistent Across Different Strains? No



The GFP signal is significantly different among the strains.

More complex is the system and less GFP is produced.

C What Happens in a Continuous Culture? No impact

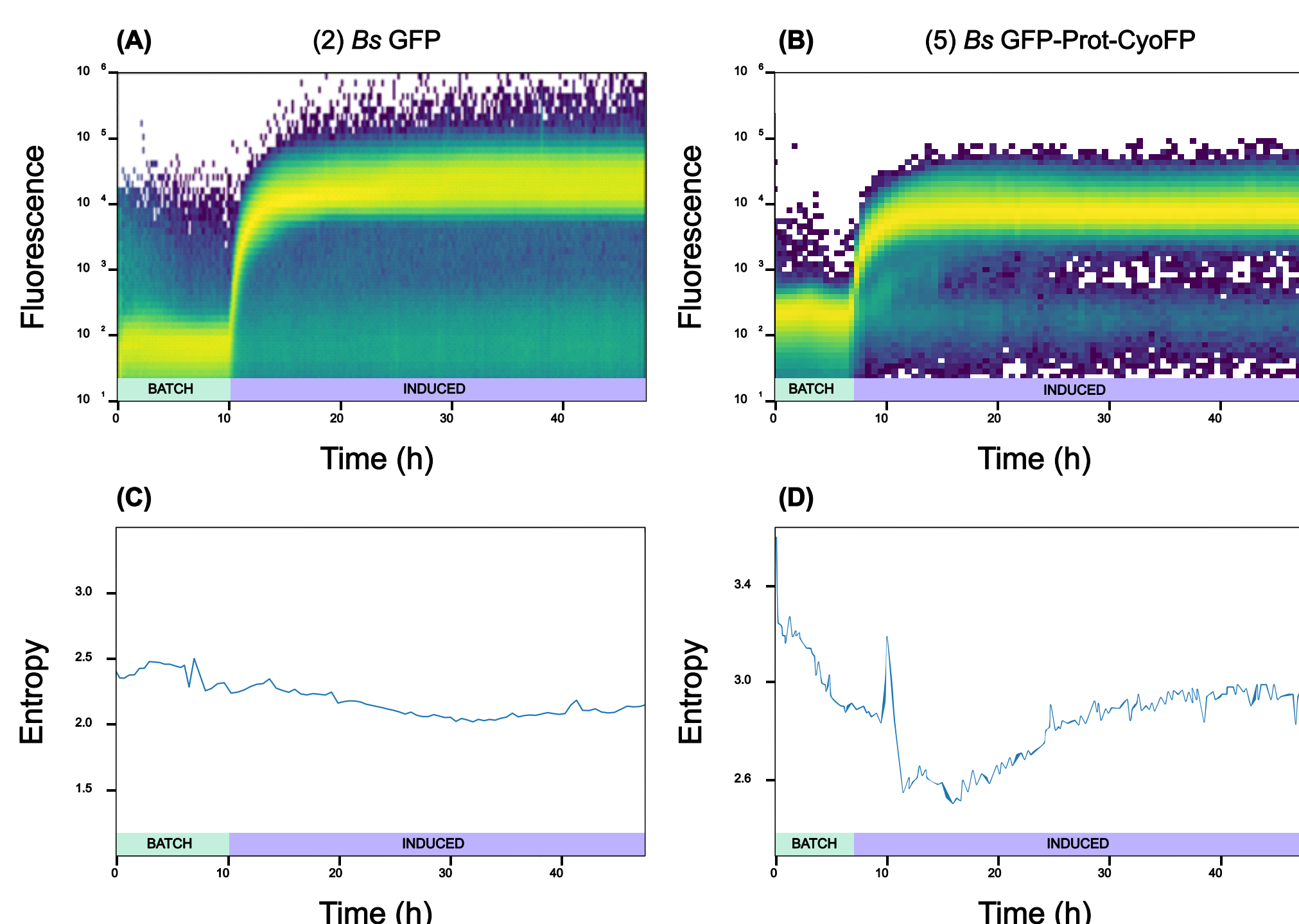
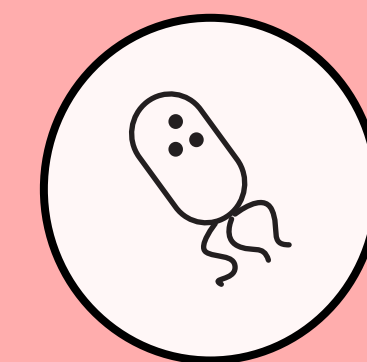


Figure 3 – GFP (fluorescence) and their entropy in continuous culture of (A & C) *Bs* GFP (1) and (B & D) *Bs* GFP-Prot-CyoFP (5) in the Dasgip system, cultivated in M9 medium supplemented with 5 g/L glucose and induced with 0.5 mM of IPTG.

Homogeneous population, all producing GFP under the induced condition.



B. subtilis STRAINS

(1) *B. subtilis* wild-type

SYSTEM INDUCIBLE WITH IPTG (Isopropyl-β-D-Thiogalactopyranoside)

(2) *B. subtilis* GFP - Producing Green Fluorescence Protein (GFP)

(3) *B. subtilis* GFP - Producing and secreting GFP

(4) *B. subtilis* GFP-Prot - Producing GFP & producing and secreting Prot

(5) *B. subtilis* GFP-Prot-CyoFP - Producing GFP & producing and secreting Prot & producing and secreting CyoFP

Induced if there is a general stress

D Can Secondary Metabolite Profiles Distinguish Between Strains? Yes

The number of VOCs decreases with the complexity of the genetic construct.

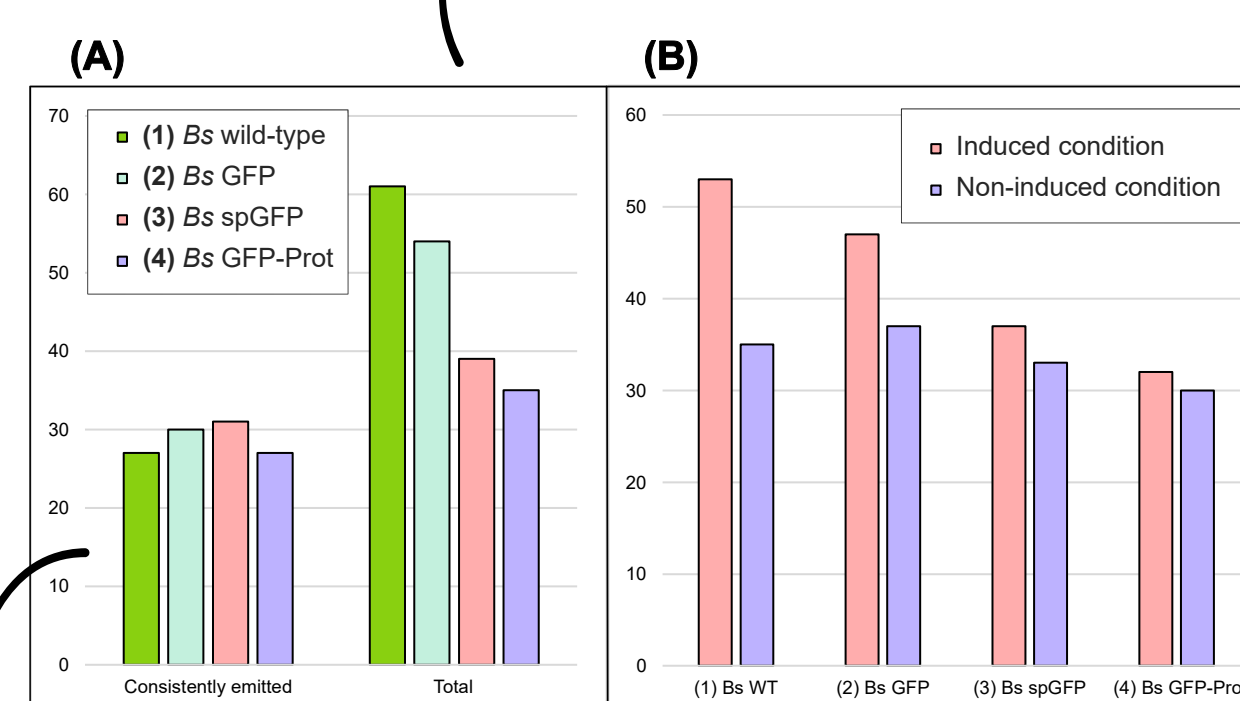


Figure 4 – Number of VOCs detected: (A) across all strains, and (B) under different conditions.

Among them, all strains emit the same 18 VOCs consistently.

VOC emission is higher under the induced condition than under the non-induced one.

In the induced condition, three VOCs are consistently detected. (RI 1016, 1227 & 1292)

Volatile Organic Compounds

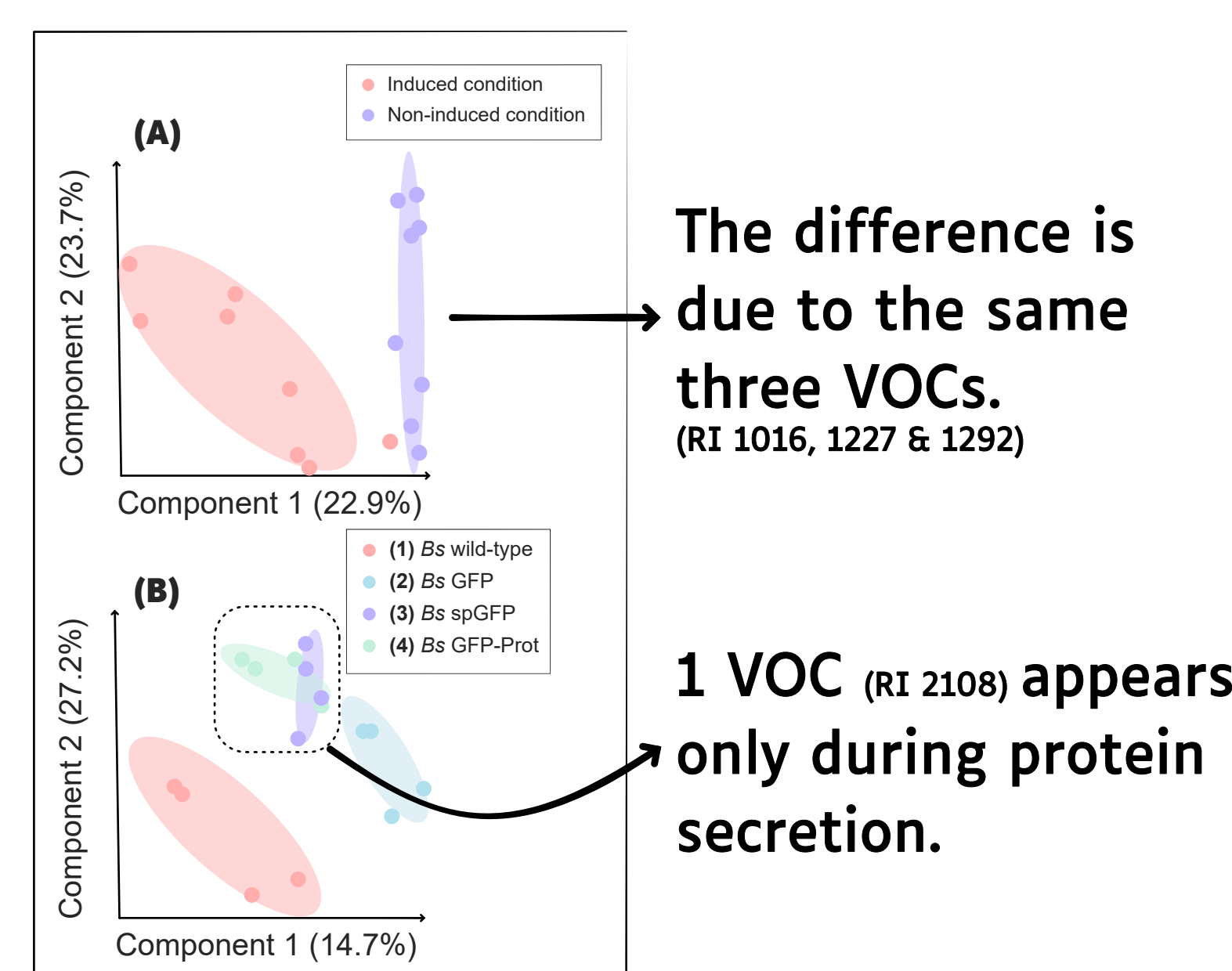


Figure 5 – PLS-DA of VOC profiles: (A) according to strain, and (B) according to condition (induced vs. non-induced).

Soluble <2000 Da

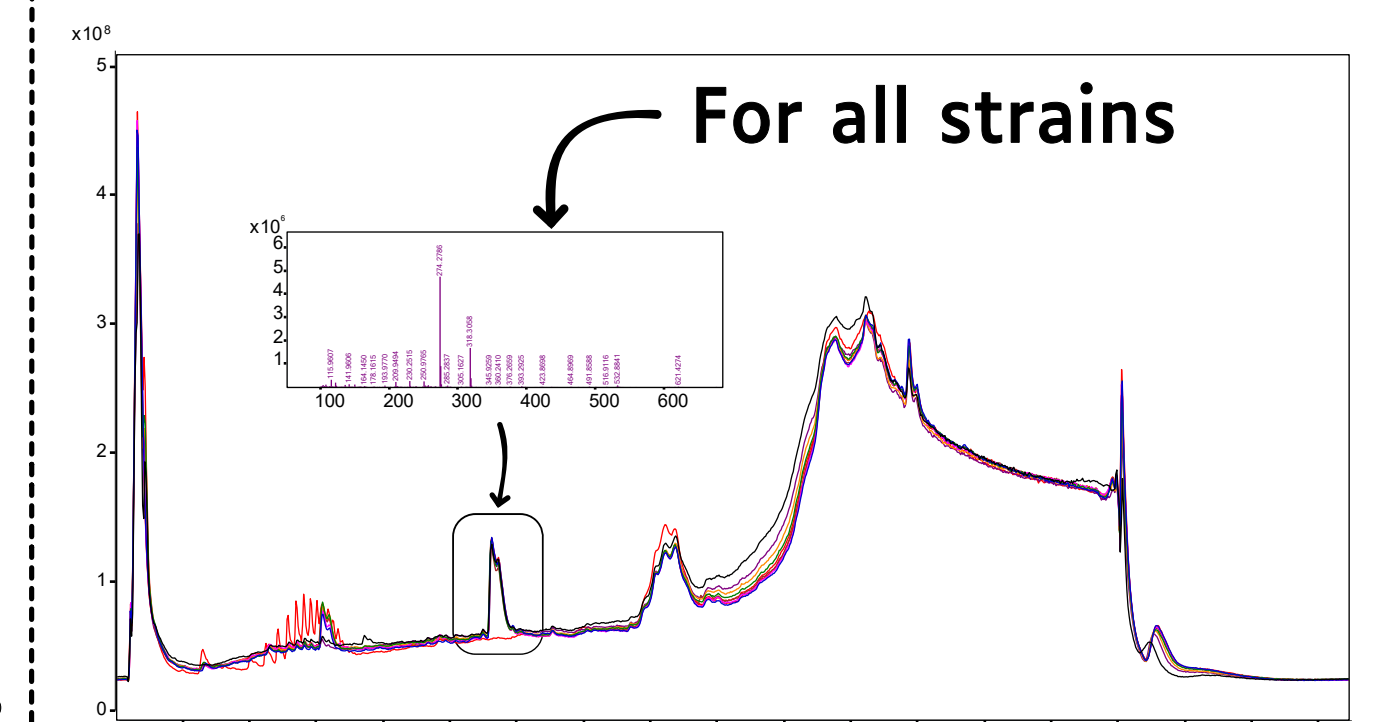


Figure 6 – Analysis of soluble secondary metabolites under 2000 Da by HPLC-QTOF from the supernatants of *B. subtilis* strains (1) to (4). Bacteria were cultivated in M9 medium supplemented with 5 g/L glucose and, in the case of induction, with 0.5 mM IPTG.

This range of secondary metabolites does not appear to differ.