Gram proportion determination in complex ecosystems using flow cytometry

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INTRODUCTION

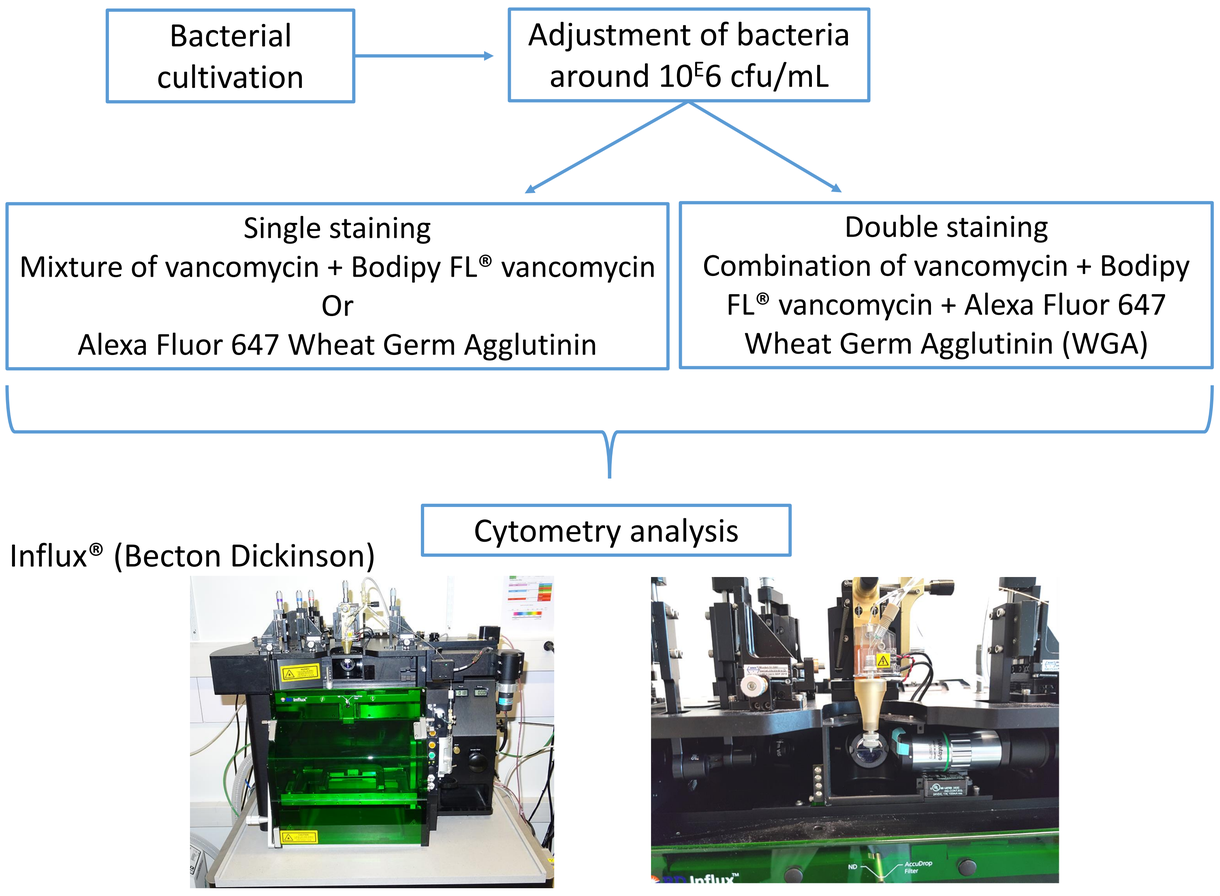
A first glimpse of the evolution of a complex ecosystem during in vitro fermentation can be assessed using flow cytometry methods. General bacterial profiles, overall viability as well as Gram status can be evaluated.

Here, we adapted a Gram-staining method using flow cytometry for in vitro ecosystems characterization. The method was developed and validated using pure cultures of aerobic bacterial species and then applied to anaerobic species and complex ecosystems.

OBJECTIVES

The objective was to develop a Gram staining method adapted for the analysis of complex ecosystems. We aimed at evaluating the most appropriate staining conditions to discriminate Gram-positive from Gram-negative bacteria. Aerobic as well as strictly anaerobic strains were tested.

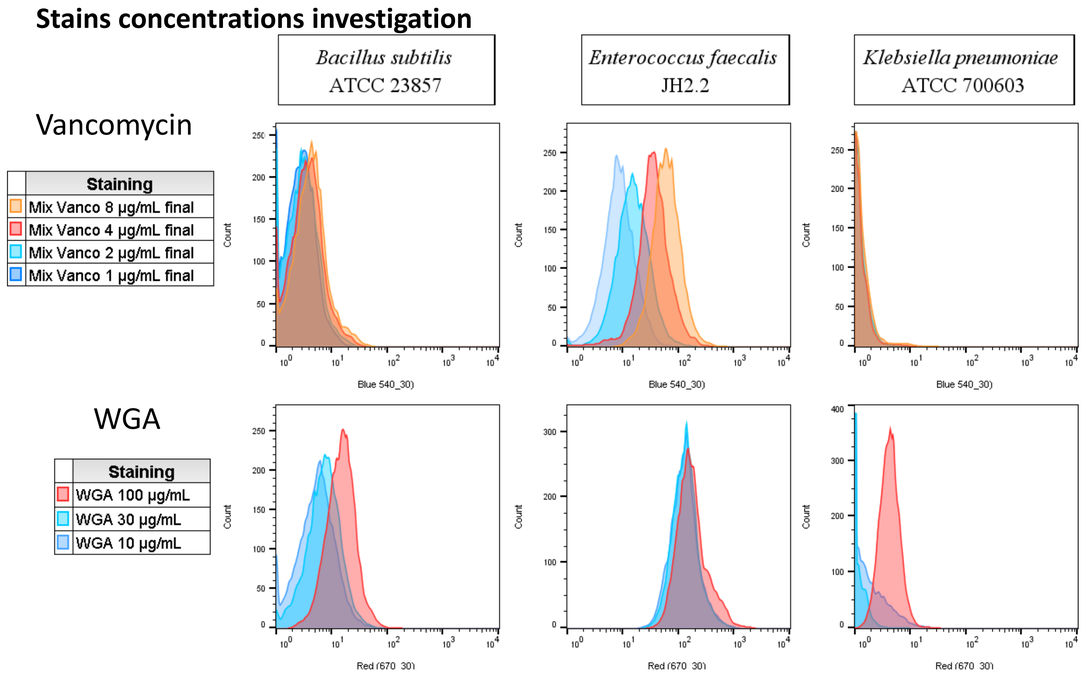
METHODS



RESULTS

Optimization of Gram-positive and Gram-negative bacteria differentiation

Stains concentrations investigation



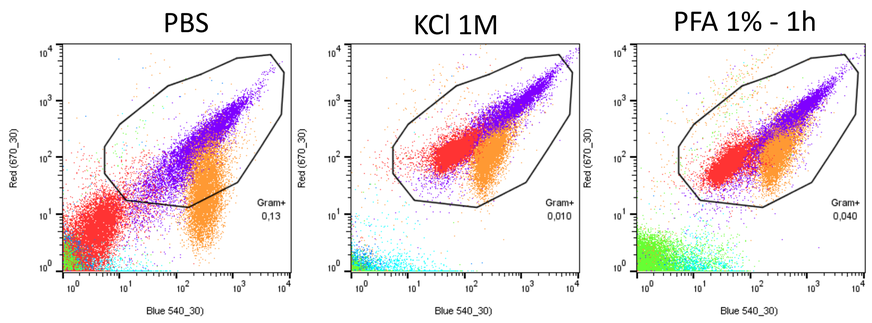
Vancomycin

- Gram-positive bacteria: higher concentrations **increased** the staining
- Gram-negative bacteria: **no staining**

WGA

- **Better staining** of Gram-positive bacteria with increased concentrations
- Higher concentrations: unwanted Gram-negative bacteria staining

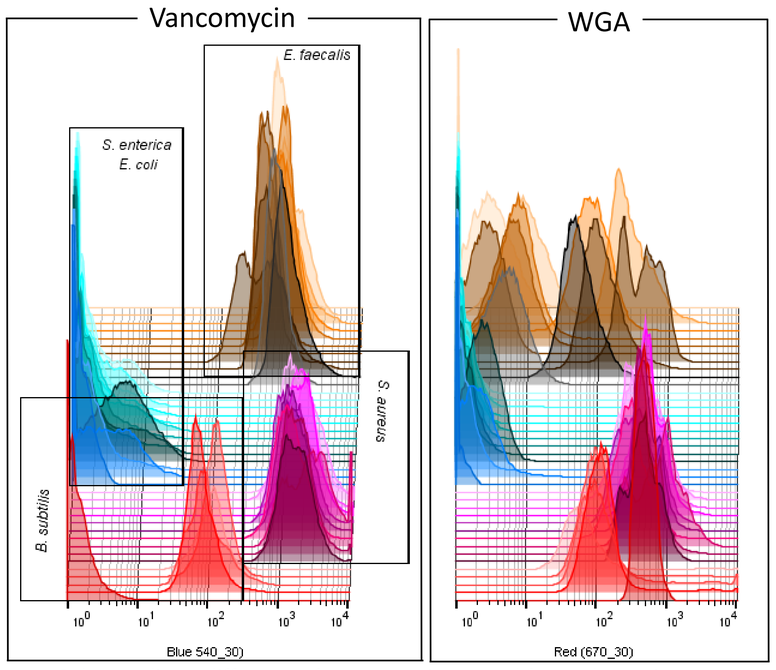
Stains combination and fixation condition



green: *K. pneumoniae* ATCC 700603, light blue: *Salmonella enterica* ATCC 13311, dark blue: *Escherichia coli* ATCC 35218, orange: *E. faecalis* ATCC 29212, purple: *Lactococcus lactis* ATCC 11454, red: *B. subtilis* ATCC 23857

KCl treatment improves Gram-positive bacteria staining leading to a better discrimination from Gram-negative bacteria especially for *B. subtilis*.
In addition, the PFA fixation method after KCl treatment does not impact the staining.

Screening of different strains of *E. faecalis*, *B. subtilis*, *S. aureus*, *L. lactis* *E. coli* and *S. enterica*



Color gradient: different reference strains tested for each species

Vancomycin

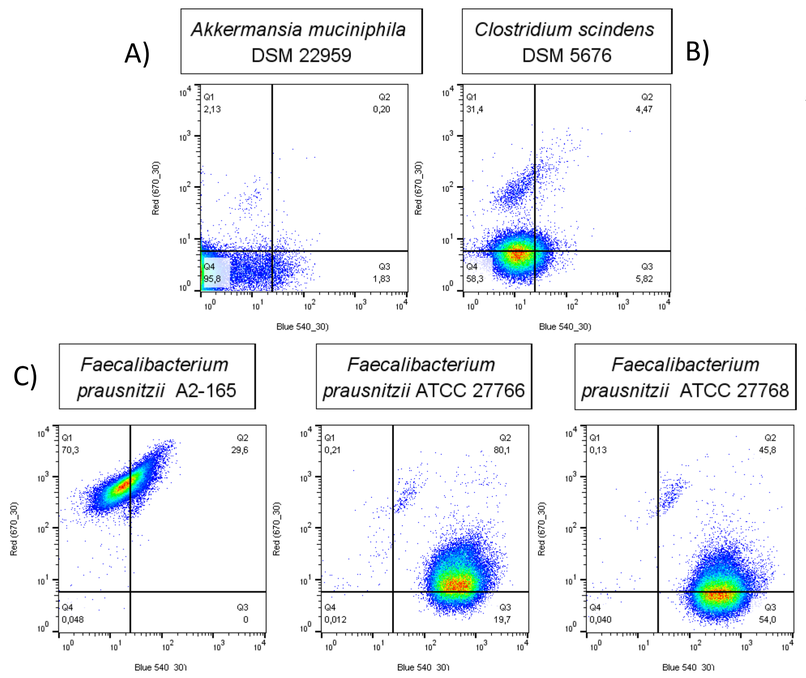
- **Gram -: not stained**
- Gram +: almost always stained (one strain of *B. subtilis* not stained

WGA

- **Gram -: not stained**
- Gram+: heterogeneous staining depending on the tested strains

➢ The **combination of both stains** represents an interesting option for Gram-positive bacteria that might not be stained by one of the selected stains

Screening of different anaerobic strains



A) *A. muciniphila* is stained as a Gram-negative bacteria as expected
B) *C. scindens* appears to be stained as a Gram-positive bacterium
C) The three *F. prausnitzii* strains are stained as Gram-positive bacteria. A significant difference is observed between the A2-165 strain and the two ATCC strains that might be due to differences on cell walls composition.

CONCLUSIONS

- Differentiation of Gram-positive bacteria and Gram-negative bacteria based on their cell wall structures
- Under the conditions and on the strains tested, gram differentiation was possible whatever bacterial growth phases (data not shown)
- Observation of staining variability for different strains belonging to a same species (likely due to differences in proteins expressed at the surface of bacteria)
- Gram-positive bacteria could always be differentiated from Gram-negative bacteria
- Gram-staining method that may be used in the future to predict the presence of specific bacterial groups in *in vitro* cultures as well as in complex ecosystems