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CO17 >> OPTIMIZATION OF FLUORESCENCE IN SITU HYBRIDIZATION TECHNIQUE FOR ANALYZING MICROORGANISMS INVOLVED IN BIOPROCESSES

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The use of the powerful technique Fluorescence *In Situ* Hybridization (FISH) has been exponentially increased in all fields of microbiology. Its analytical potential has thereby been exploited for analyzing microorganisms involved in several bioprocesses.

FISH is based on the use of fluorescent labeled oligonucleotide probes which bind specifically to the target sequence in cell, that in turn become fluorescent maintaining its integrity and allowing its detection, identification and quantification.
A typical FISH protocol includes four steps: i) fixation and permeabilization of the cells that allows FISH probes penetration and protects the RNA from degradation by endogenous RNAses; ii) hybridization of the FISH probe to the complementary sequence in the target cells; iii) washing; and iv) analysis of stained cells by microscopy or flow cytometry.

It is important to optimize each step, particularly fixation and hybridization, for increasing its potentialities and becoming the technique also faster and cheaper.

Thus, this work is focused on FISH technique optimization for yeast and bacteria analysis, by enhancing the FISH signals by increasing the number of fluorescent cells and their fluorescence intensities. With this aim different fixation procedures and hybridization conditions were tested. The modifications introduced in the FISH procedure represent an important contribution for analyzing microorganisms involved in bioprocesses.