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New water-soluble amine-reactive reagents for labeling microbial cells: application to mural paintings

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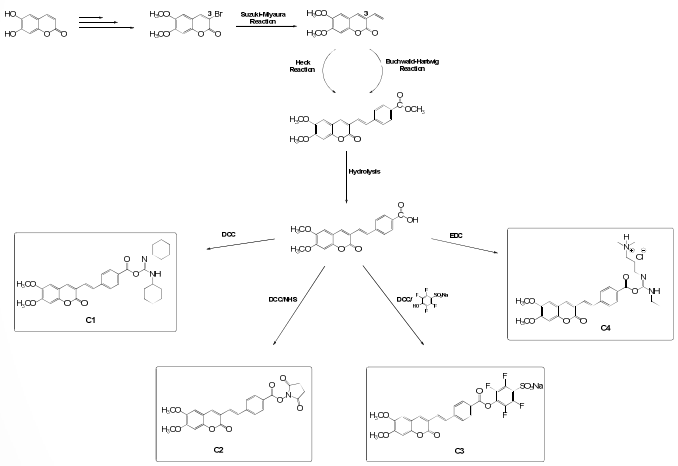
INTRODUCTION

Mural paintings are continuously exposed to physical, chemical and biological degradation, however, among the biological agents that cause deterioration, microorganisms are of critical importance, due to their metabolic versatility and flexibility, and their ability to tolerate unfavourable conditions. Thereby, microorganisms can cause damages in mural paintings surfaces through a variety of mechanisms, including biofilm formation, chemical reactions, physical penetration into the substrate and production of pigments [1-3].

It is imperative the development of new strategies that detect microbial contamination in order to plan effective remediation strategies. This research focuses on the application of fluorescent compounds. In this context we developed new water-soluble coumarin derivatives (4-Sulfotetrafluorophenyl Esters) as long-wavelength fluorescent labels for biomolecules[4]. These esters are easily synthesized, and react readily with primary amines to give acylation products.

METHODOLOGY

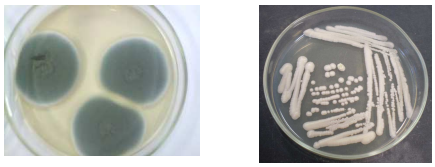
A – Synthesis of Coumarin Derivatives as Potential Fluorescent Labels (C1 to C4)



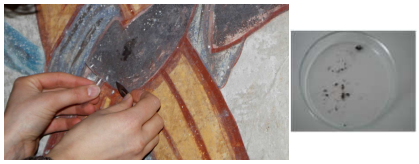
B – Fluorescent Labeling Optimization using Bovine Serum Protein (BSA)

Temperature, pH, solvent, stoichiometry, concentration and reaction duration

C – Fluorescent Labeling of Microbial Cells (Bacteria and Fungi) with C3



D – Fluorescent Labeling of Contaminated Mortars with C3



RESULTS

In a first attempt we successfully label a protein (BSA) with C3. The optimized protocol was used for labeling bacterial and yeast culture cells and contaminated mortar samples (Fig 1).

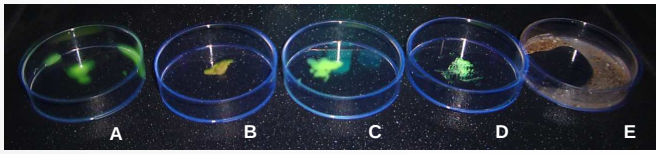


Fig 1- Labeled bacterial and yeast culture cells (A and B) and contaminated mortar samples (C and D) observed at 254 nm. E- Contaminated mortar without fluorescent label.

CONCLUSIONS

Coumarin derivative C3 (4-Sulfotetrafluorophenyl Ester) is a highly potential fluorescent label for biomolecules.

Fluorescent labeling protocol, was developed to detect microbial cells in real mortar micro samples.

This methodology is a culture-independent technique that gives a direct indication about the microbial contamination and shows clearly proliferation within mortars.

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