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ANTIBODY-BASED FLOW CYTOMETRY METHOD FOR THE DETECTION OF *A. MUCINIPHILA* IN COMPLEX CELL SUSPENSIONS

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Akkermansia muciniphila is a mucin-degrading bacterium abundantly present in the human gut. Since its discovery, *A. muciniphila* has been recognize as a beneficial intestinal symbiont and is considered a promising next-generation probiotic. However, an efficient strategy for isolation of new strains is needed. In this study an antibody-based flow cytometry (FCM) method, for the specific detection of *A. muciniphila* in complex cell suspensions, was developed.

Rabbit polyclonal antibodies were produced against whole cells of *A. muciniphila* type strain Muc^T and used, after their purification, as primary antibodies. Optimal concentration of the primary antibodies was defined by titration experiments. Alexa-488 goat anti-rabbit IgG was used as secondary antibody. Briefly, *A. muciniphila* cells, in pure culture or in mixed cell suspensions, were incubated for 30 min at room temperature in Phosphate Buffer Saline (PBS, pH 7.4), 5% (v/v) goat serum for blocking potential nonspecific antibody-binding sites. Anti-*A. muciniphila* whole cells purified IgGs (1 µg/ml) were added to samples, then incubated for 1h at room temperature. Cells were washed once and resuspended in PBS, 3% (v/v) bovine serum albumin (BSA) and the fluorochrome-labeled secondary antibody (1 µg/ml) was added. Samples were incubated for 1 h at room temperature in the dark. Finally, samples were diluted in PBS and analyzed by FCM (C6 plus BD Accuri) (excitation: 488 nm; emission: 530 nm).

Our assays confirmed the antibodies specificity at species level. The antibodies obtained from the strain Muc^T were able to detect five newly isolated *A. muciniphila* strains. Conversely, no signals were detected with other intestinal species. The FCM method was applied to quantify *A. muciniphila* in pure culture and in mixed populations, containing the target bacteria and *E. coli* or *Lactobacillus acidophilus* at different ratio. The accuracy of the absolute quantification made by antibodies labelling was compared to SYBR Green I staining and resulted variable depending on cell density. *A. muciniphila* was specifically detected in all mixed populations, even at the ratio 1:1000 and a cell density of 10^5 FU/ml. The developed method was also able to detect *A. muciniphila* in enrichment cultures obtained by inoculating fecal samples in minimal medium supplemented with mucin.

Our method could be applied for the detection and quantification of *A. muciniphila* in multi-species samples. Moreover, considering that antibody-labelled cells showed to retain their viability, our protocol will be further combined with fluorescence-activated cell sorting (FACS) for isolation of new *A. muciniphila* strains from fecal samples of healthy donor. Improving the isolation process (rate and selectivity) could greatly enhance availability of new strains and, consequently, our knowledge of this bacterial species and its diversity.