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LABELING OF *BIFIDOBACTERIUM LONGUM* CELLS WITH 13C-SUBSTITUTED LEUCINE FOR QUANTITATIVE PROTEOMIC ANALYSES

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Bifidobacteria are anaerobic bifid or multiply branching gram-positive rods that constitute one of the most numerous populations in the gastrointestinal tract of humans. Particularly in breast-feed infants, they can represent more than 90% of the total gut microbiota, and *Bifidobacterium longum* is one of the most representative species. The genome sequencing of several *B. longum* strains has recently prompted some investigations at the proteomic level.

Stable isotope labeling of amino acids in cell cultures (SILAC) is a simple and accurate procedure that can be used as a quantitative proteomic approach with many growing eukaryotic cell types. It is based on a comparison of the protein levels in cells grown in two formulations of the same medium that differ only by the fact that one formulation contains a nonradioactive, isotopically labeled form of an amino acid. By measuring the ratio of light peptides to heavy peptides, the relative abundance of proteins from cultures treated under different conditions can be determined.

In the present work we have adapted a SILAC procedure to the quantitative analysis of *B. longum* proteins. A medium that allows growth of *B. longum* NCIMB8809 with high level, stable incorporation of [13C6]leucine was developed. Incorporation of [13C6]leucine (containing six 13C atoms) into a protein or peptide leads to a 6-Da shift in the molecular mass due to the labeled leucine compared to the protein or peptide that contains natural leucine. Using this strategy, proteins having variations of at least 50% in their expression rates can be quantified with great confidence.