World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:10, No:07, 2016

Fluorescence in situ Hybridization (FISH) Detection of Bacteria and Archaea in Fecal Samples

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Abstract : The fluorescence in situ hybridization (FISH) is a staining technique that allows the identification, detection and quantification of microorganisms without prior cultivation by means of epifluorescence and confocal laser scanning microscopy (CLSM). Oligonucleotide probes have been used to detect bacteria and archaea that colonize the cattle and swine digestive systems. These bacterial strains have been obtained from fecal samples issued from cattle manure and swine slurry. The collection of these samples has been done at 3 different pit's levels A, B and C with same height. Two collection depth levels have been taken in consideration, one collection level just under the pit's surface and the second one at the bottom of the pit. Cells were fixed and FISH was performed using oligonucleotides of 15 to 25 nucleotides of length associated with a fluorescent molecule Cy3 or Cy5. The double hybridization using Cy3 probe targeting bacteria (Cy3-EUB338-I) along with a Cy5 probe targeting Archaea (Gy5-ARCH915) gave a better signal. The CLSM images show that there are more bacteria than archaea in swine slurry. However, the choice of fluorescent probes is critical for getting the double hybridization and a unique signature for each microorganism. FISH technique is an easy way to detect pathogens like E. coli O157, Listeria, Salmonella that easily contaminate water streams, agricultural soils and, consequently, food products and endanger human health.

Keywords : archaea, bacteria, detection, FISH, fluorescence
Conference Title : ICBB 2016 : International Conference on Biotechnology and Bioengineering
Conference Location : Stockholm, Sweden
Conference Dates : July 11-12, 2016