In recent times, there has been increasing interest on developing probiotics of food and feed value. To develop a probiotic for a particular organism, it is needful to know the micro-flora of its alimentary tract. Although, Pasteur’s observation suggested that the host and its intestinal micro-flora were interdependent, the intellectual origins of probiosis started with the work of Metchnikoff in 1907 who thought that on balance the intestinal micro-flora was detrimental to the host because of the absorption of toxic bacterial metabolites. A study was designed to isolate and identify the microbial strains suitable for probiotic application in chicken. The aim was to provide beneficial micro-organisms that were not present in the alimentary tract of the chicken, or those who could exert beneficial effect. The selection of the strain was in agreement with bio-safety aspects, viability during storage, tolerance for low pH/gastric juice bile, resistance for antibiotics and anti-microbial activity. The following sources were used for strain isolation: crops, proventriculus, gizzard, ileum and caeca of chicken. Diluted samples were mixed with MRS medium (Merck) and incubated at 37°C for 24 h in anaerobiosis. Identification of the strains was done as per the Bergey’s Manual of Determinative Bacteriology. Finally, one strain was selected for probiotic application in chicken and it was identified as *Lactobacillus fermentum* (designated as *L. fermentum* LPB).

**Key Words:** chickens, *Lactobacillus*, isolation.