The intestinal microbiota may affect gut motility by altering host metabolism and production of metabolites, including gases, short chain fatty acids and bile acids, and therefore is considered to contribute to chronic constipation (CC). The aim of this study was to characterize mucosal microbiota and contractility of colonic muscle in CC patients. Colonic tissue samples were obtained from patients undergoing colectomy for CC and contractile activity was analyzed. Outcome was compared with the intestinal muscle contractions of patients undergoing colorectal surgery for gut diseases not associated with disorder of motor function. In CC patients, the spontaneous contractions were higher in both longitudinal (12.3±4.4 vs 1.9±0.9 g/s) and circular (13.47±3.3 vs 5.8±2.2 g/s) smooth muscle strips compared to controls. Moreover, the carbachol-induced response was also increased in CC in both longitudinal (EC50 0.50±0.05 vs 0.65±0.14 µM) and circular (EC50 0.76±0.06 vs 2.01±1.05 µM) muscle layers compared with those of the control group. The juxta-mucosal microbiota was studied with culture-based and 16S rRNA pyrosequencing techniques. The microbiota in constipated patients was dominated by bacteria belonging to the phyla Bacteroidetes (34-43%) and Firmicutes (31-52%), followed by Proteobacteria (4-26%) and Actinobacteria (1-4%). No definitive association between constipation and the abundance or lack of certain prokaryotic taxa in the gut microbiome was observed. Yet, we identified some microbes which may affect motility via production of methane, hydrogen sulfide, butyrate, acetate, and propionate. Lactobacillus species were isolated from colonic tissue samples from CC patients and their probiotic properties were compared with those of lactobacilli isolates from feces of healthy subjects, but no significant differences were detected. Our findings suggest a role for gut microbiota in constipation and promote new therapeutic approaches for treatment of patients with CC.