Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. Recent reports describe the role of gut microbiota in health and disease emphasized on the potential of probiotics as a biotherapeutic with benefits against in CRC. Probiotics can be used to balance dysbiotic gut microbial community toward a symbiotic state that will provide means of defense against disease initiation healthy individuals at risk or recurrence in CRC patients. There is still, however, a need for more studies that allow the proper identification of potent probiotic strains with potential in reducing CRC risk. The main goal of this study is to identify potent probiotic strains and formulate a novel active probiotic Lactobacillus biotherapeutic for CRC. Several Lactobacillus strains (L. reuteri and L. fermentum) were screened in vitro for their anti-proliferative activity and fatty acid production. Later, the most effective bacterial formulation was characterized in a genetically induced animal CRC model. The findings demonstrated that the probiotic anti-cancer activity is strain-dependent and is mainly associated to the production of short chain fatty acids (SCFAs). Interestingly, when probiotic extracts inhibited CRC cell growth, they still promoted healthy growth of non-neoplastic cells. Later, probiotic co-culture (La-Lf) of potent strain with synergistic effect was characterized for antioxidant and anti-cancer activity. The oral administration of this Lactobacillus formulation reduced tumor growth and cellular proliferation in the intestines of Apc Min/+ mice. These observations support the use of a novel Lactobacillus probiotic treatment for use in CRC prevention and bring more inquiries for further investigations.