Escherichia coli can commonly be found, either as a commensal, probiotic or a pathogen, in the human gastrointestinal (GI) tract. Biofilm formation and its regulation is surprisingly variable, although distinct regulatory patterns of red, dry and rough (rdar) biofilm formation arise in certain pathovars and even clones. In the GI tract, environmental conditions, signals from the host and from commensal bacteria contribute to shape E. coli biofilm formation within the multi-faceted multicellular communities in a complex and integrated fashion. Although some major regulatory networks, adhesion factors and extracellular matrix components constituting E. coli biofilms have been recognized, these processes have mainly been characterized in vitro and in the context of interaction of E. coli strains with intestinal epithelial cells. However, direct observation of E. coli cells in situ, and the vast number of genes encoding surface appendages on the core or accessory genome of E. coli suggests the complexity of the biofilm process to be far from being fully understood. In this review, we summarize biofilm formation mechanisms of commensal, probiotic and pathogenic E. coli in the context of the gastrointestinal tract.