

# In vitro evaluation of single- and multi-strain probiotics: Inter-species inhibition between probiotic strains, and inhibition of pathogens

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## Abstract

Many studies comparing the effects of single- and multi-strain probiotics on pathogen inhibition compare treatments with different concentrations. They also do not examine the possibility of inhibition between probiotic strains with a mixture. We tested the ability of 14 single-species probiotics to inhibit each other using a cross-streak assay, and agar spot test. We then tested the ability of 15 single-species probiotics and 5 probiotic mixtures to inhibit *Clostridium difficile*, *Escherichia coli* and *S. typhimurium*, using the agar spot test. Testing was done with mixtures created in two ways: one group contained component species incubated together, the other group of mixtures was made using component species which had been incubated separately, equalised to equal optical density, and then mixed in equal volumes. Inhibition was observed for all combinations of probiotics, suggesting that when used as such there may be inhibition between probiotics, potentially reducing efficacy of the mixture. Significant inter-species variation was seen against each pathogen. When single species were tested against mixtures, the multi-species preparations displayed significantly ( $p < 0.05$  or less) greater inhibition of pathogens in 12 out of 24 cases. Despite evidence that probiotic species will inhibit each other when incubated together in vitro, in many cases a probiotic mixture was more effective at inhibiting pathogens than its component species when tested at approximately equal concentrations of biomass. This suggests that using a probiotic mixture might be more effective at reducing gastrointestinal infections, and that creating a mixture using species with different effects against different pathogens may have a broader spectrum of action than a single provided by a single strain.