Motility Buffer (MB) Hinders the Motility of Fluorescent *E. coli* (AD62)

Very likely, the clusters are induced by PVA, instead of MB. This issue should be considered as resolved. I will modify the protocol, so we don't add PVA to MB in the future.

I. AD62 with and without MB

Two videos from the same day shows that AD62 slows significantly and forms clusters in the presence of MB or BMB. When MB is replaced with water, AD62 swims faster and less clusters are observed.

video 1: AD62 slow with MB \ / video 2: AD62 fast withtout MB

II. Is the MB too old?

The MB in the previous experiment was prepared 2 weeks before the experiment. While this is not typically considered "too old", I did a test with freshly prepared MB. *Freshly prepared: made on 11/29 and used on 12/01.*

video 3: clusters in fresh MB BF \ / video 4: clusters in fresh MB FL

III. MB has stronger effect on AD62 (than JEK *from Chile*)

MB hindering motility is strain specific. Two bacterial strains are used in my experiment: AD62 and JEK. AD62 as the fluorescent bacteria and JEK as the wild type. MB significantly hinders the motility of AD62. However, its effect on JEK is not very pronounced. *It could be the case that clusters are more obvious in fluorescent images. JEK can only be imaged in bright field, so I didn't see obvious clusters. But based on the motility of JEK, there were less clusters than in AD62.*

video 5: JEK in MB BF