

S3. Plantain NSP inhibits adhesion to, and invasion of Caco2-cl1 cells by adherent, invasive *E. coli* (AIEC).

Three adherent, invasive Crohn's disease *E. coli* isolates (HM605, HM615 and LF82) and the non-pathogenic reference strain *E. coli* K12 were selected to examine the effect of plantain NSP on the ability of *E. coli* to adhere to, and invade the Caco2-cl1 cell-line which was to be used to generate M-cells. Briefly, Caco2-cl1 cells were seeded (5×10^4 cells per 1 ml well) to 24-well tissue culture plates (Corning-Costar; High Wycombe, UK) and maintained in culture. Following pre-treatment of confluent cells for 30 min, with or without plantain NSP (0 to 50 mg/mL, in DMEM without antibiotics), each monolayer was infected for 4 h with approximately 1×10^6 bacteria per well (MOI 10:1). Adherence and invasion was assessed using a gentamicin protection assay as previously described [Martin HM *et al. Gastroenterology* 2004;127:80-93.]

Caco2-cl1 epithelial cell invasion by all three colonic and ileal AIEC isolates investigated was inhibited by the presence of plantain NSP both at 5 and 50mg/mL. For *E. coli* K12, significant inhibition of invasion was only observed at plantain NSP levels of 50 mg/mL. Similarly, bacterial adhesion to Caco2-cl1 cells by all *E. coli* isolates assessed was also significantly blocked by the presence of plantain NSP at either 5 or 50 mg/mL. *, $P < .05$; **, $P < .01$; and ***, $P < .001$; ANOVA (n=3).

<i>E. coli</i> isolate	Plantain NSP (mg/mL)					
	0.5		5		50	
	Bacterial Invasion (% inhibition)			Bacterial adhesion (% inhibition)		
K12	0.88 ± 12.74	25.44 ± 4.88	49.12 ± 15.37 *	13.13 ± 22.92	46.20 ± 6.80 *	47.75 ± 20.25
LF82	14.17 ± 5.37	68.46 ± 11.76 *	66.67 ± 2.69 **	-2.01 ± 10.70	40.97 ± 2.18 **	43.56 ± 13.12
HM580	26.52 ± 6.00	67.53 ± 1.59 **	77.08 ± 5.41 **	2.49 ± 7.32	45.13 ± 17.58	68.56 ± 11.86 *
HM605	6.57 ± 6.89	86.42 ± 1.51 ***	90.60 ± 1.13 ***	12.22 ± 8.17	42.53 ± 6.47	70.89 ± 7.18 *
HM615	19.59 ± 14.22	79.95 ± 5.22 ***	81.80 ± 3.00 ***	9.56 ± 10.61	59.52 ± 9.38 *	65.43 ± 6.03 *