

Supplemental Material

Table S1. Diarrhea and fecal shedding of bacteria after challenge of *A. nancymae* with H10407, H10407-P or HS ^{a,b}

Treatment (CFU)	No. of animals	Diarrhea			Fecal shedding of H10407		
		No. with diarrhea/N (%)	Median no. days to onset (IQR)	Median no. days of duration (IQR)	% Incidence	Median no. of days to onset (IQR)	Median no. of days of duration (IQR)
H10407 (8.0x10 ⁹)	5 ^c	2/5 (40)	6.5 (4-9)	4.5 (2-7)	100	1 (-)	10 (4-10)
H10407 (5.1x10 ¹⁰)	5	3/5 (60)	1 (1-5)	3 (2-3)	100	1 (1-1.5)	4 (1-10)
H10407 (5.8x10 ¹¹)	5	4/5 (80)	1 (-)	8.5 (5.5-10)	100	1 (-)	9 (5.5-10)
H10407 (7.5x10 ¹¹)	10	8/10 (80)	1 (1-5.75)	7.5 (2.25-10)	100	1 (-)	7 (5-8)
H10407-P (6.4x10 ¹¹) ^d	5	1/5 (20)	1(-)	10 (-)	-	-	-
HS (7.9x10 ¹¹) ^d	5	2/5 (40)	3.5 (2-5)	6 (-)	-	-	-

^a A diarrheal episode was defined as 2 or more consecutive days of grade 3 or higher stool consistency that begins as the first day of at least 2 consecutive days of diarrhea starting as early as day one after challenge, and ends when 2 or more days pass without diarrhea.

^b Fecal shedding defined as isolation of H10407 from stool collected after challenge that begins (onset) as early as day one after challenge and ends (duration) on last day, up to day 10, that H10407 are shed in the stool.

^c Two animals were excluded from the fecal shedding analysis only, due to missing data on study days 1 and 2; (n = 3).

^d Fecal shedding was not assessed in control groups due to limitations in the ability to differentiate from other commensal *E. coli* colonies.

Table S2. Diarrhea and fecal shedding of bacteria after IN vaccination with dscCfaE and challenge with H10407 (~5x10¹¹) ^{a,b}

Expt	Vaccine (no. of doses) ^c	No. of animals	Diarrhea			Fecal shedding of H10407		
			No. with diarrhea/N (%)	Median no. days to onset (IQR)	Median no. days duration (IQR)	% Incidence	Median no. days to onset (IQR)	Median no. days of duration (IQR)
1	dscCfaE (4)	10	3/10 (30)	1 (1-3)	2 (2-3)	100	1 (-)	4.5 (3-7.25)
	dscCfaE+CTB (4)	10	2/10 (20)	1 (-)	4 (-)	100	1 (-)	4.5 (2.5-7)
	PBS (4)	10	9/10 (90)	1 (-)	4 (2.5-6)	100	1 (-)	4.5 (3.75-7)
2	dscCfaE+LTB (4)	8	1/8 (12.5)	6 (-)	2 (-)	100	1 (-)	3.5 (3-5.5)
	dscCfaE+LTB (3)	8	3/8 (37.5)	1 (1-5)	7 (3-10)	100	1 (-)	5 (3-6)
	PBS (4)	8	6/8 (75)	1 (-)	4.5 (3.75-6.25)	100	1 (-)	5 (4-5.75)
3	dscCfaE+LTB (4)	10	4/10 (40)	1 (1-1.75)	2.5 (2-8.25)	100	1 (-)	4 (3.75-5.25)
	PBS (4)	10	7/10 (70)	2 (1-5)	3 (2-5)	100	1 (-)	6 (5-8.25)

^a A diarrheal episode was defined as 2 or more consecutive days of grade 3 or higher stool consistency that begins as the first day of at least 2 consecutive days of diarrhea starting as early as day one after challenge, and ends when 2 or more days pass without diarrhea.

^b Fecal shedding defined as isolation of H10407 from stool collected after challenge that begins (onset) as early as day one after challenge and ends (duration) on last day, up to day 10, that H10407 are shed in the stool.

^c All vaccine antigens were administered intranasally; for all vaccinations, doses of 0.2 mg dscCfaE and 0.29 mg adjuvant were given; vaccinations were performed on study days 0, 14, 28, 84 (4) or study days 56, 70, 84 (3).

23 Table S3. Serum antibody titers in *A. nancymaae* vaccinated with dscCfaE and challenged with H10407 (~5x10¹¹)

24

Expt	Vaccine (no. of doses) ^a	No. of animals	Anti-dscCfaE				Anti-toxoid	
			Mean IgG titers (SD)		Mean IgA titers (SD)		Mean IgG titers (SD)	
			Day 42	Day 98	Day 42	Day 98	Day 42	Day 98
1	dscCfaE (4)	10	2.19 (1.0)	3.14 (0.64)	0.93 (0.65)	1.70 (0.51)	0.43 (0.07)	0.42 (0.03)
	dscCfaE+CTB (4)	10	3.77 (0.30)	3.89 (0.35)	1.98 (0.29)	2.11 (0.26)	3.80 (0.32)	3.74 (0.20)
	PBS (4)	10	0.57 (0.54)	0.57 (0.52)	0.42 (0.08)	0.45 (0.09)	0.4(0)	0.4 (0)
2	dscCfaE+LTB (4)	8	3.12 (0.41)	3.70 (0.47)	1.24 (0.53)	1.84 (0.45)	2.19 (0.70)	2.85 (0.57)
	dscCfaE+LTB (3) ^b	8	-	2.77 (0.58)	-	1.07 (0.59)	-	2.63 (0.78)
	PBS (4)	8	0.77 (0.58)	0.75 (0.51)	0.44 (0.12)	0.47 (0.18)	0.4 (0)	0.4 (0)
3	dscCfaE LTB (4)	10	1.54 (1.10)	2.52 (0.79)	0.75 (0.51)	1.35 (0.77)	2.20 (0.56)	2.35 (0.46)
	PBS (4)	10	0.4 (0)	0.4 (0)	0.4 (0)	0.4 (0)	0.54 (0.23)	0.48 (0.16)

25 ^a All vaccine antigens were administered intranasally; for all vaccinations, doses of 0.2 mg dscCfaE and 0.29 mg adjuvant were given; vaccinations were performed on
 26 study days 0, 14, 28, 84 (4) or study days 56, 70, 84 (3)

27 ^b Data was not collected on day 42 for the three dose group