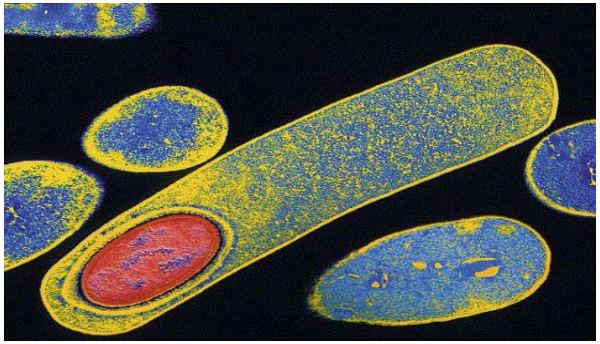
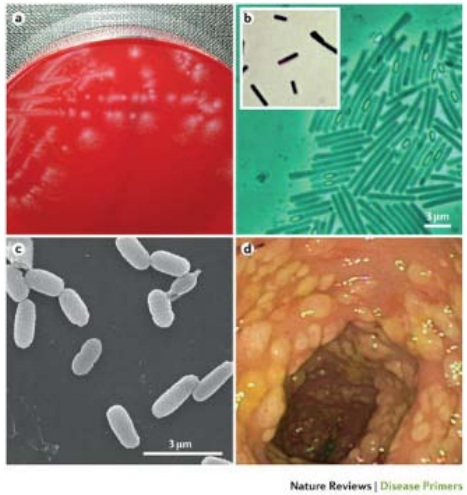
Identification of host factors involved in the *Clostridium difficile* toxins-induced cytotoxicity



Yung-Fu Chang, Professor
Cornell University

Clostridium difficile infection (CDI)

- *C. difficile* : G+, spore-forming, obligate anaerobic bacterium, colonized in intestine.
- The leading cause of antibiotic-associated intestinal disease by disrupting normal gut microbiota
- The clinical outcomes can range from asymptomatic carrier to diarrhea and pseudomembranous colitis
- The currently available antibiotics for treatment of CDI are metronidazole, vancomycin and fidaxomicin, but high recurrence.
- *Clostridium difficile* infection (CDI) caused 453,000 cases occurred in the United States in 2011 resulting in 29,000 deaths.



Morphology and colon pathologic change caused by *C. difficile*

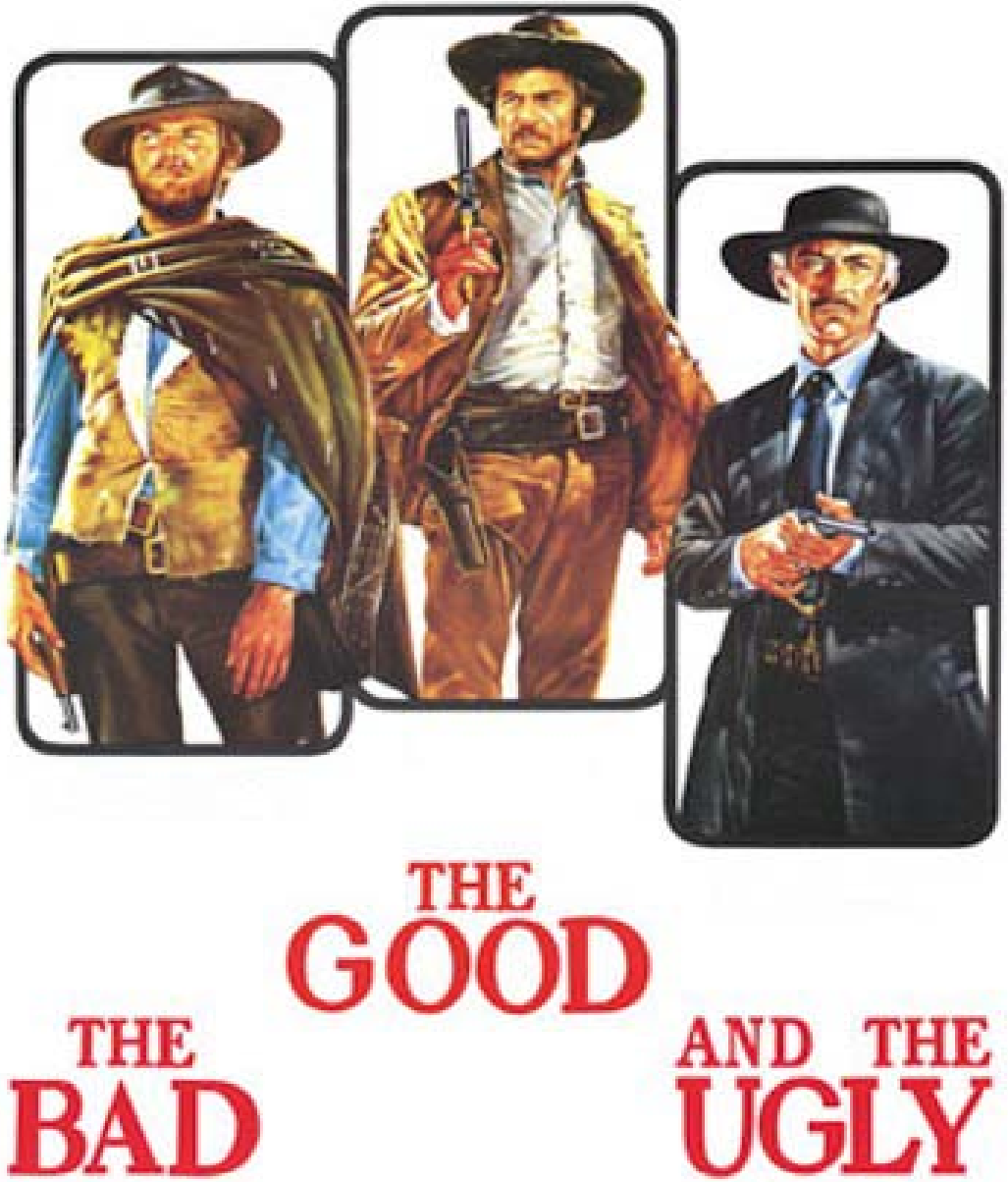
(from Nature Reviews, 2016)

http://tombanwell.blogspot.com/2010_12_01_archive.html

Clostridium difficile infection is a modern day plague

Starr & Campbell; *Clin Microbiol Infect* 2001

C. difficile = Difficult to deal with

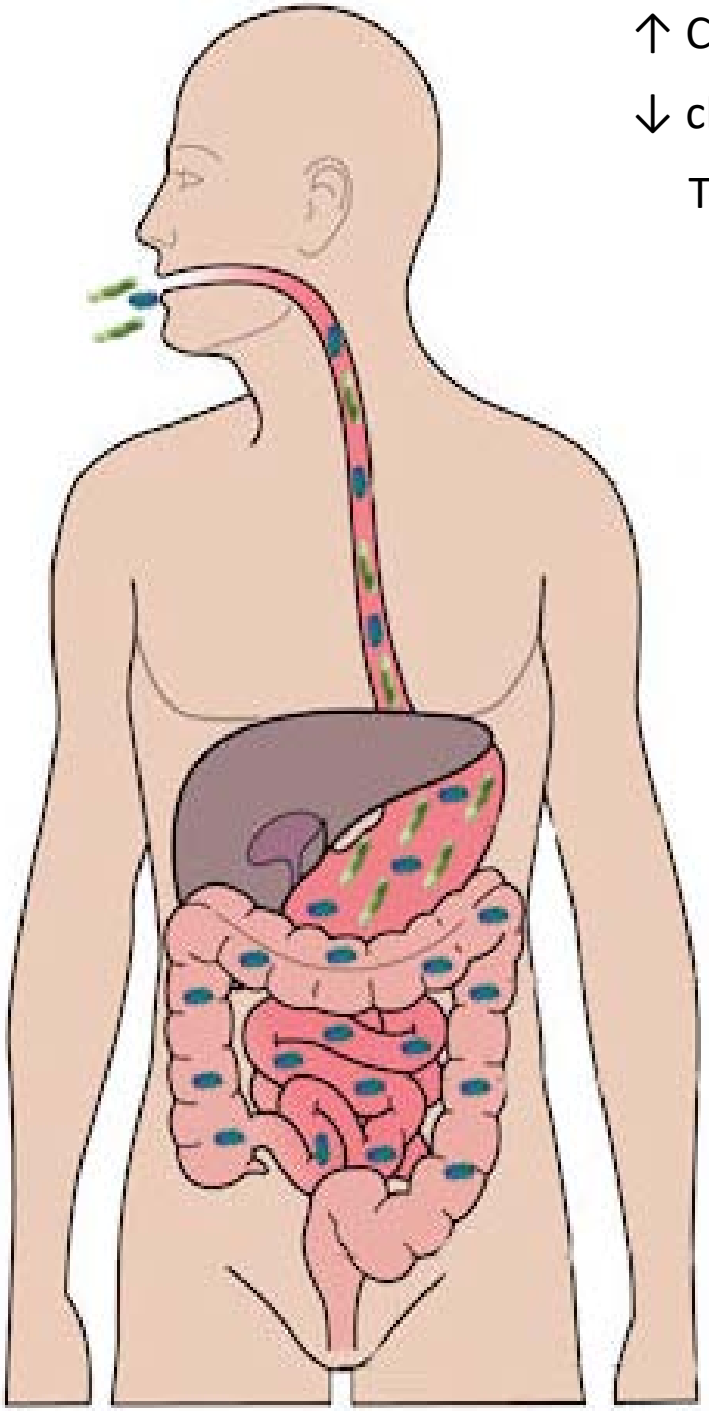


The good
C. acetobutylicum

The bad
C. perfringens

The ugly
C. difficile

Image credit: imagesci.com



↑ Cholate--taurocholate, glycocholate, deoxycholate

↓ chenodeoxycholate

TcdA & TcdB

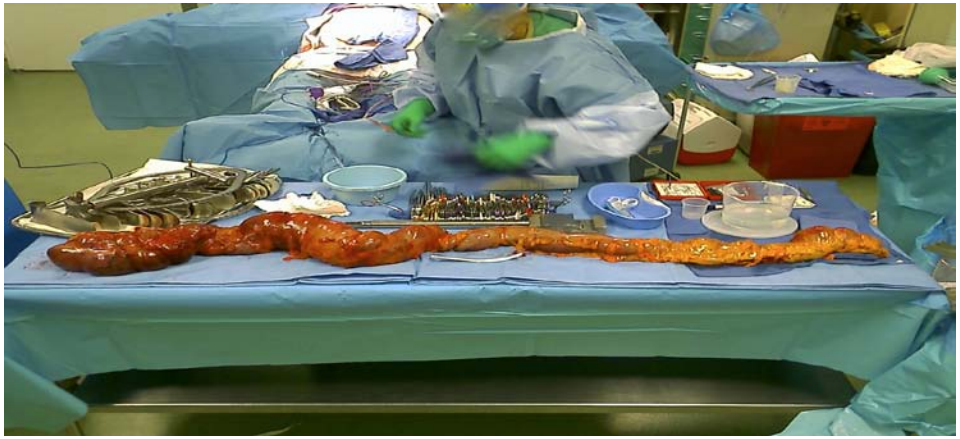
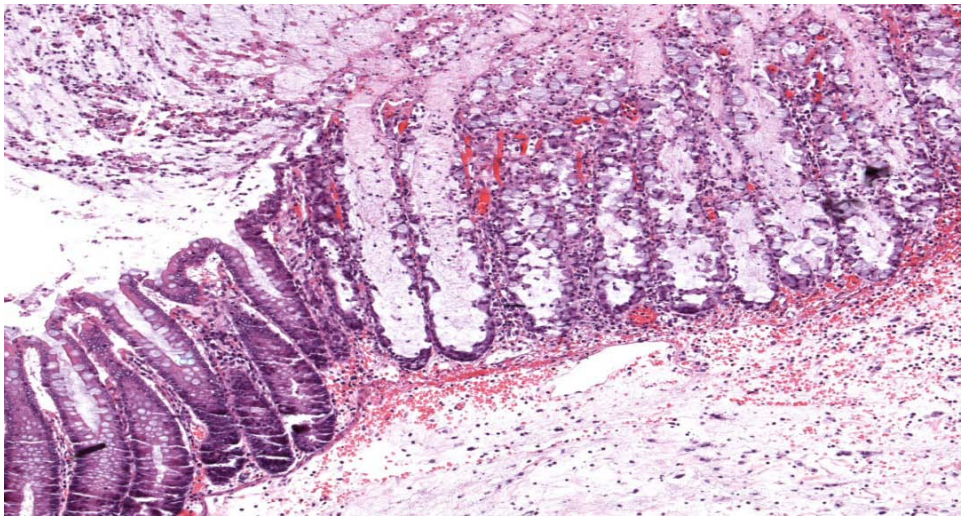
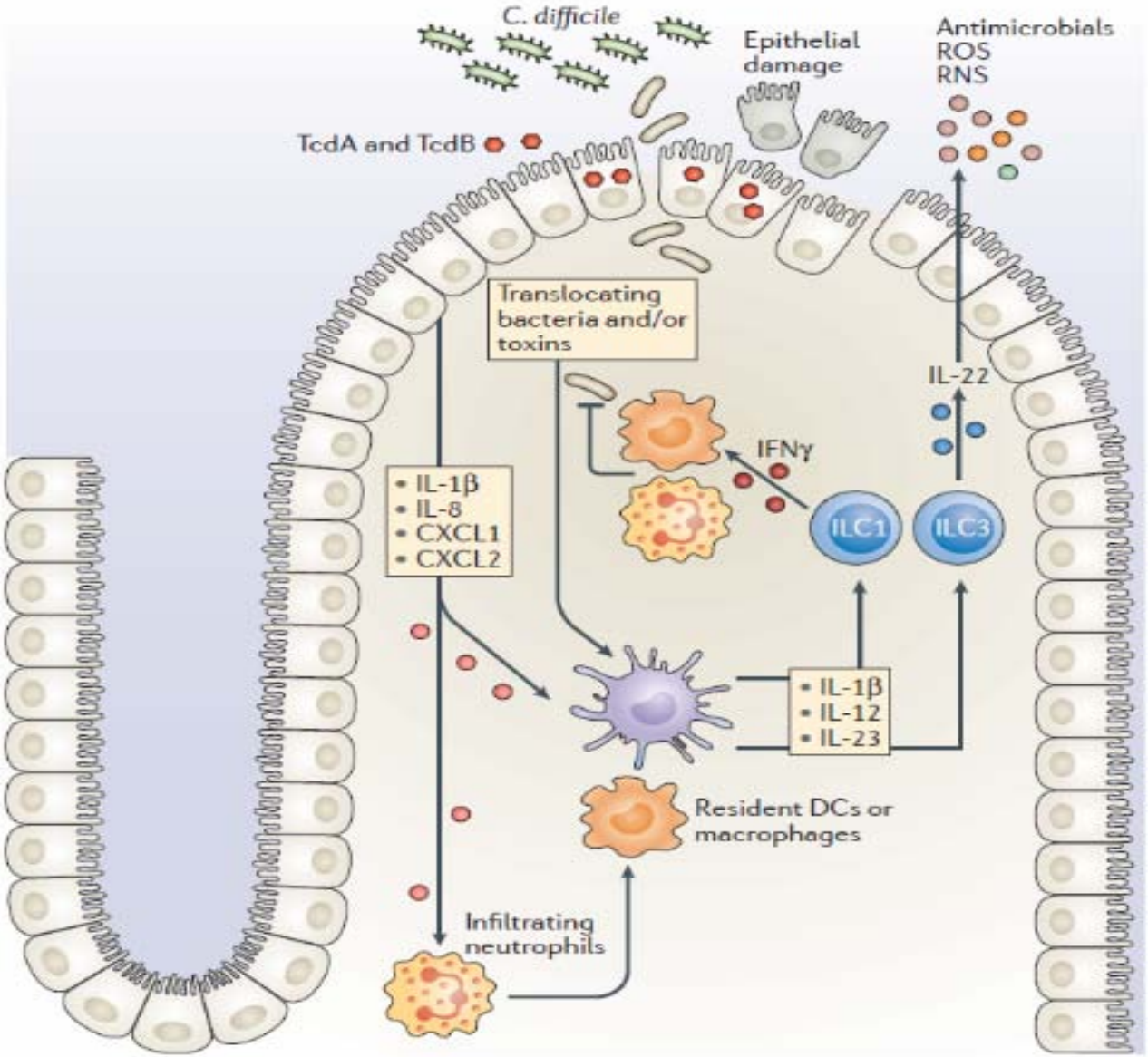


Image credits: Nature Reviews Microbiology, Dr. Nir Hus



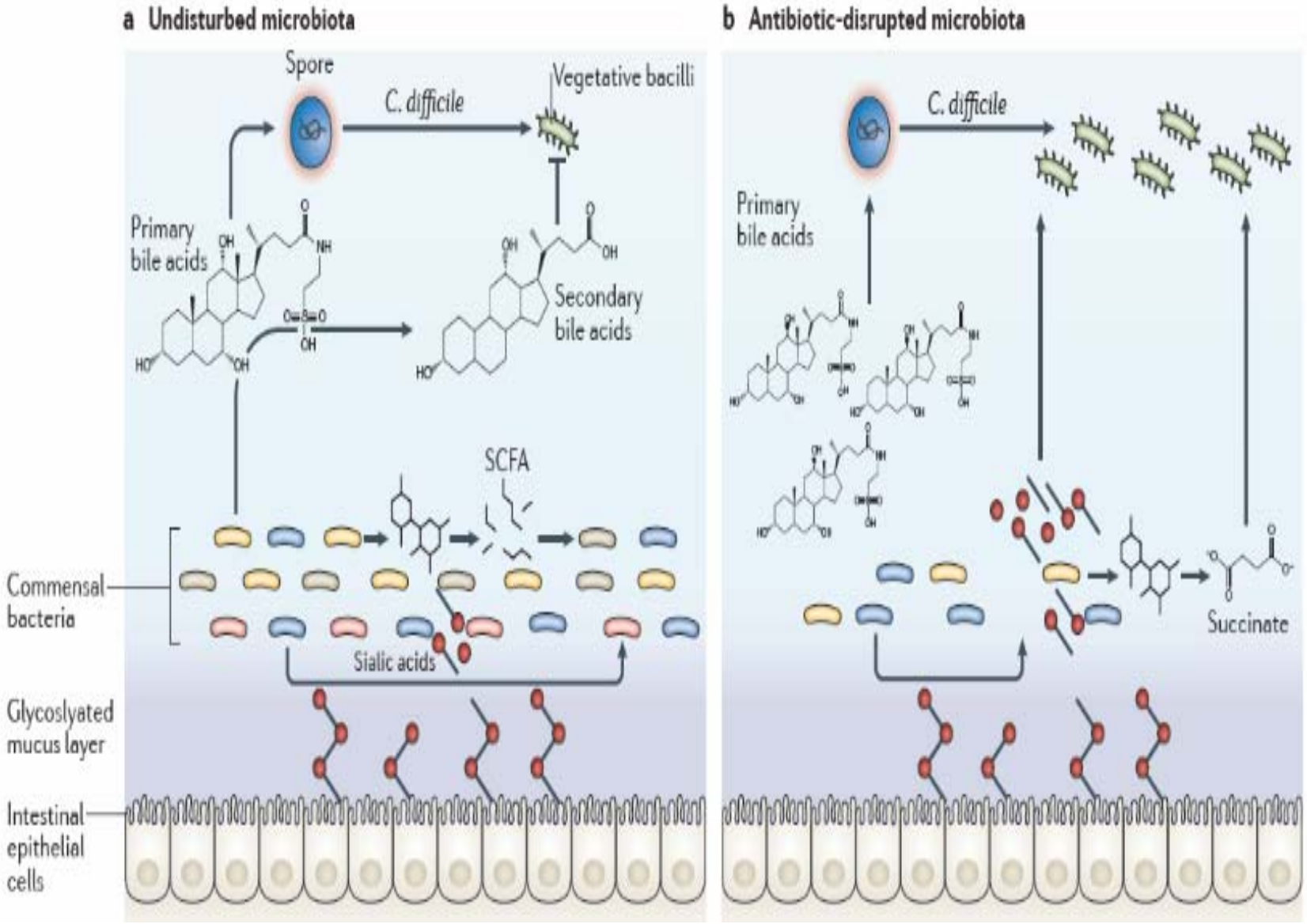
Courtesy Dr. Jeanette Guarner, Infectious Disease Pathology Activity, DVRD, CDC, Sunenshine RH and McDonald LC. *Clev Clinic J Med.* 2006;73: 1987-97.



Limit bacterial dissemination, attenuate toxin activity and repair epithelial damage

Innate immune-mediated defences against *C. difficile*

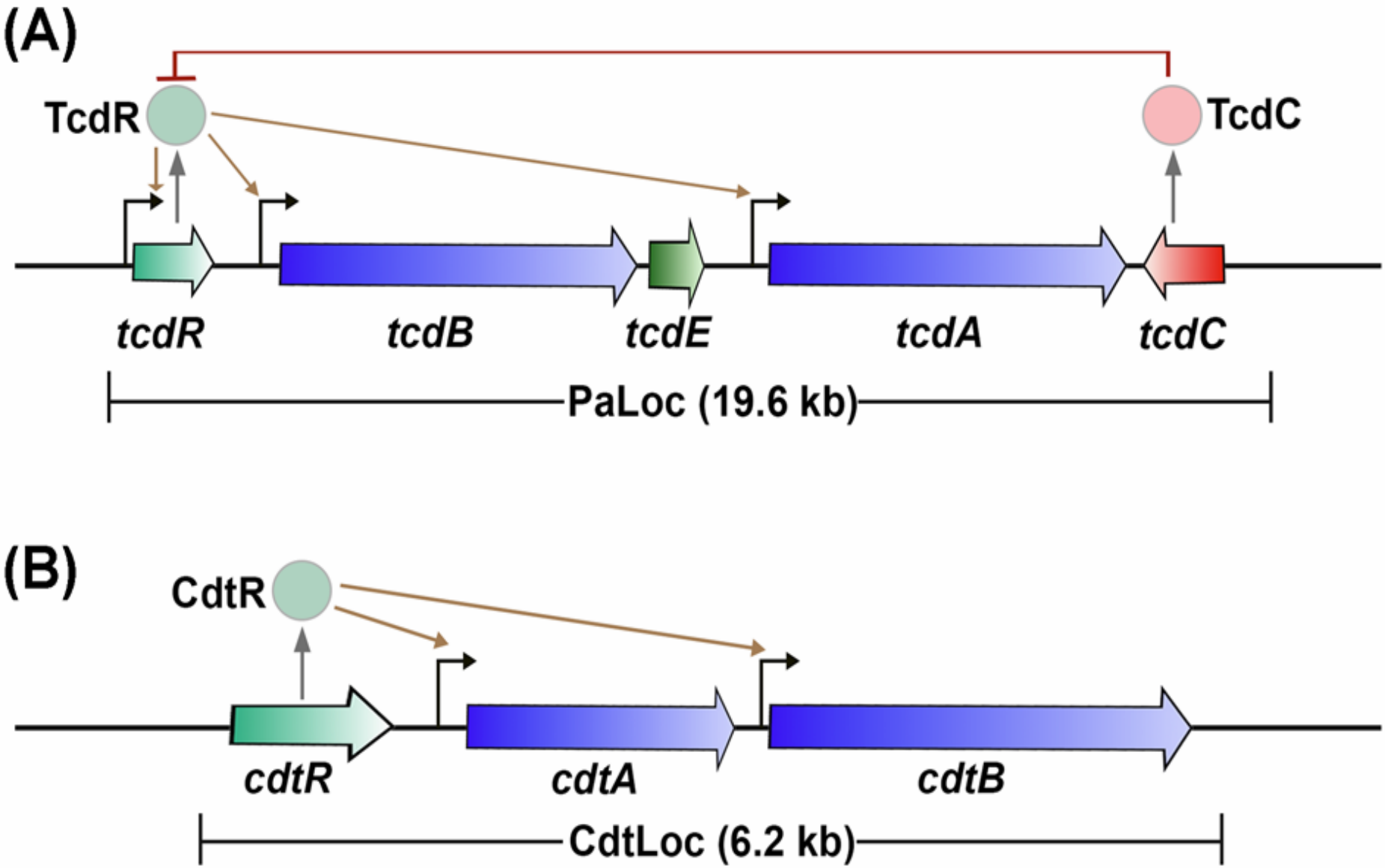
Nat. Rev. Microbiol. 14:616, 2016



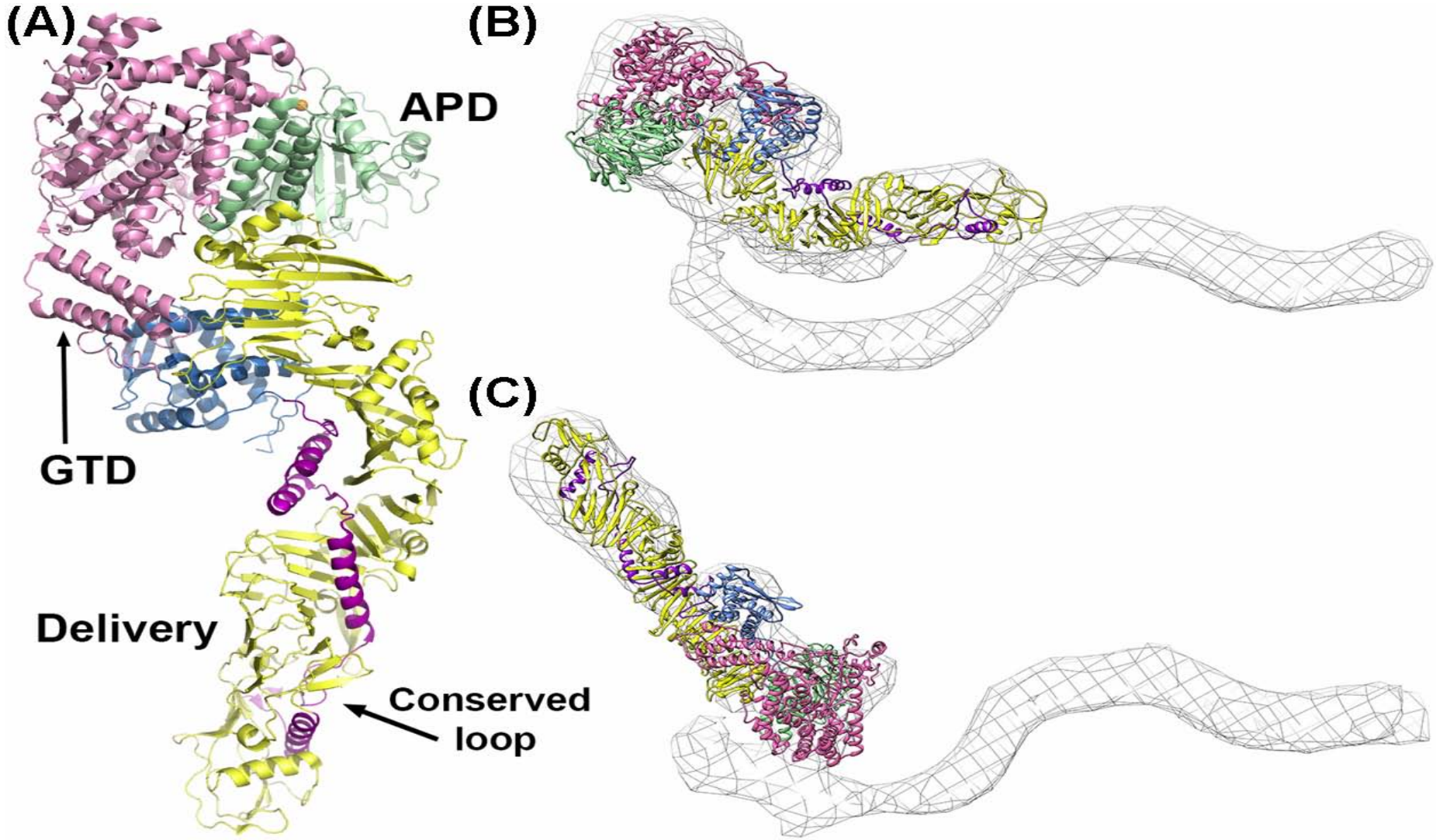
Microbiota-mediated defences against *C. difficile*

Nat. Rev. Microbiol. 14:609, 2016

Organization of toxin genes

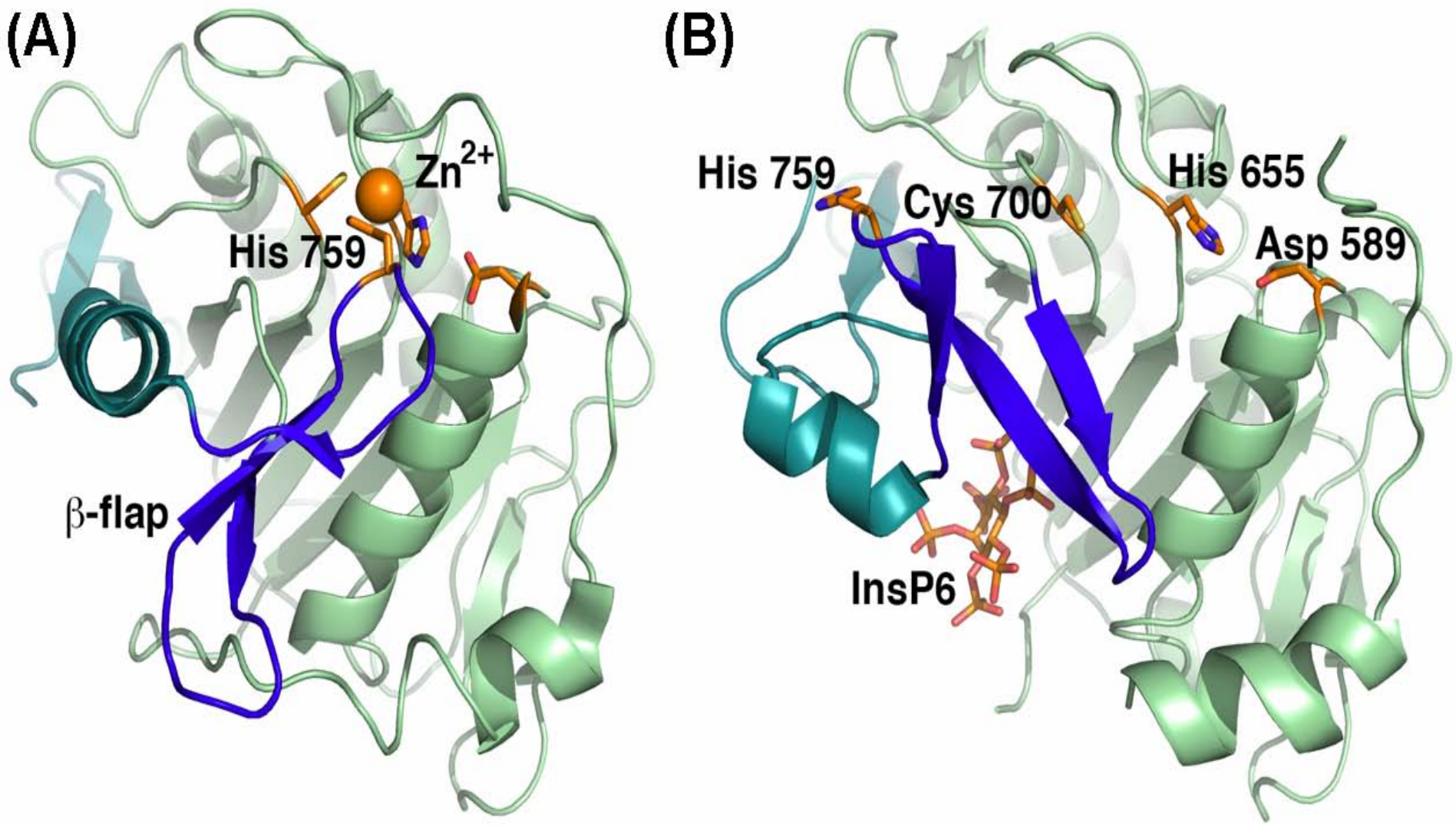


FEMS Microbiol. Rev. 41:723.



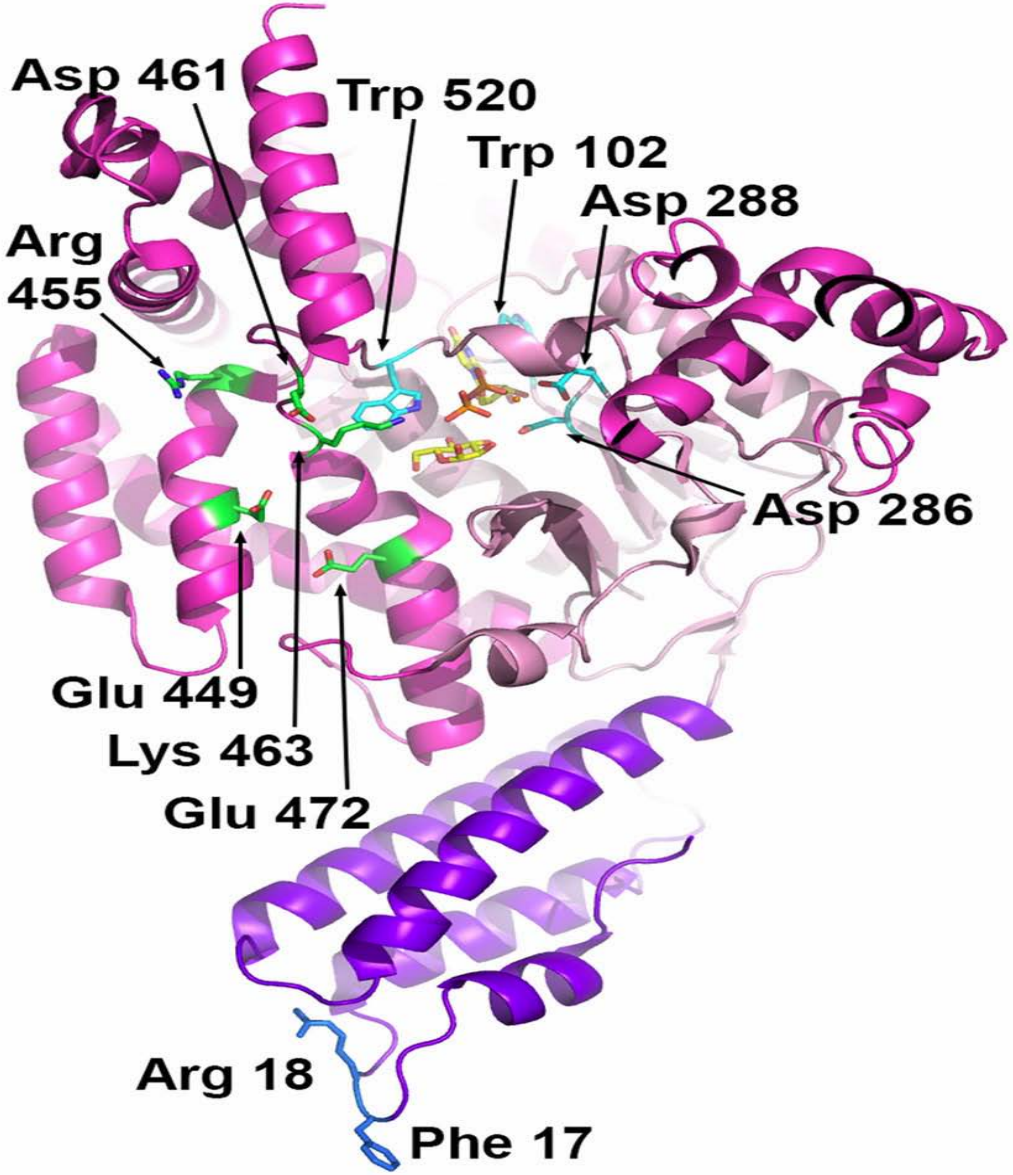
TcdA structure

FEMS Microbiol. Rev. 41:723



The autoprocessing domain (APD) undergoes a significant conformational change upon binding to InsP6

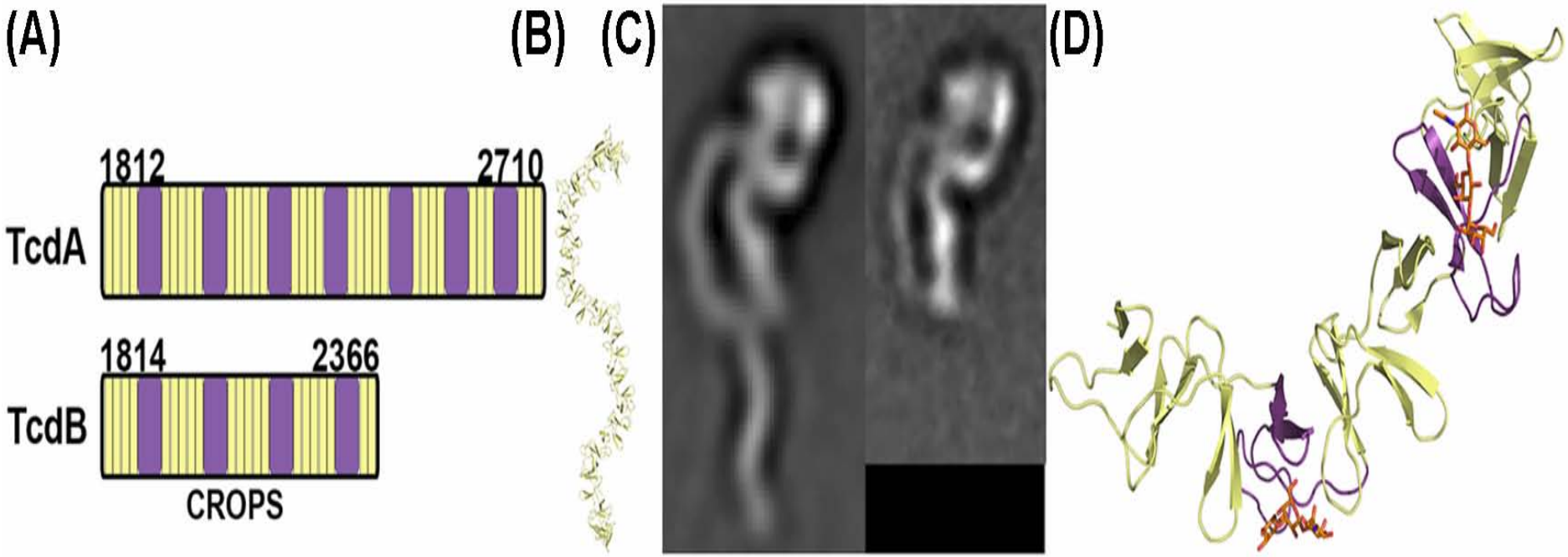
FEMS Microbiol. Rev. 41:723



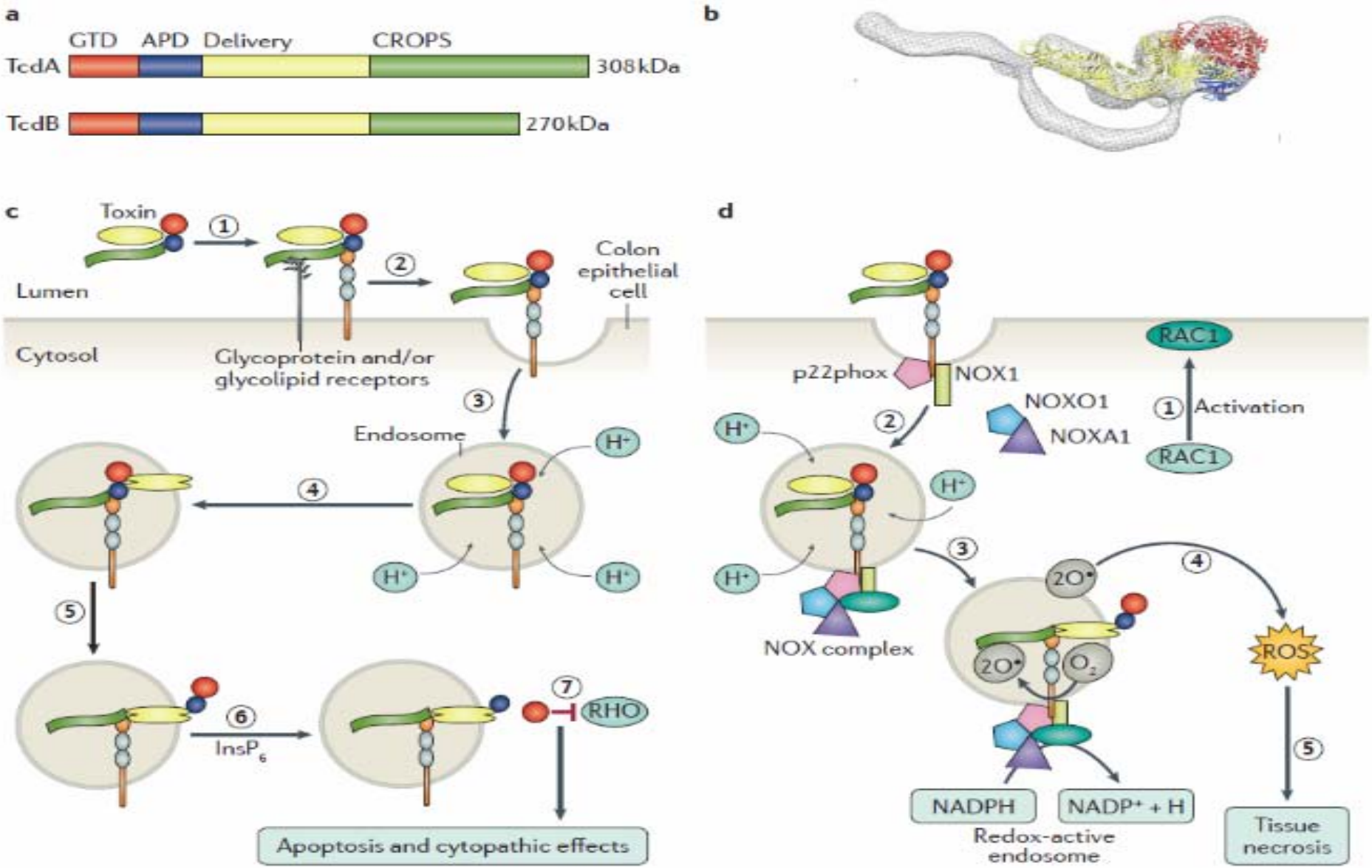
The glucosyltransferase domain

FEMS Microbiol. Rev. 41:723

Structure of the CROPS domain



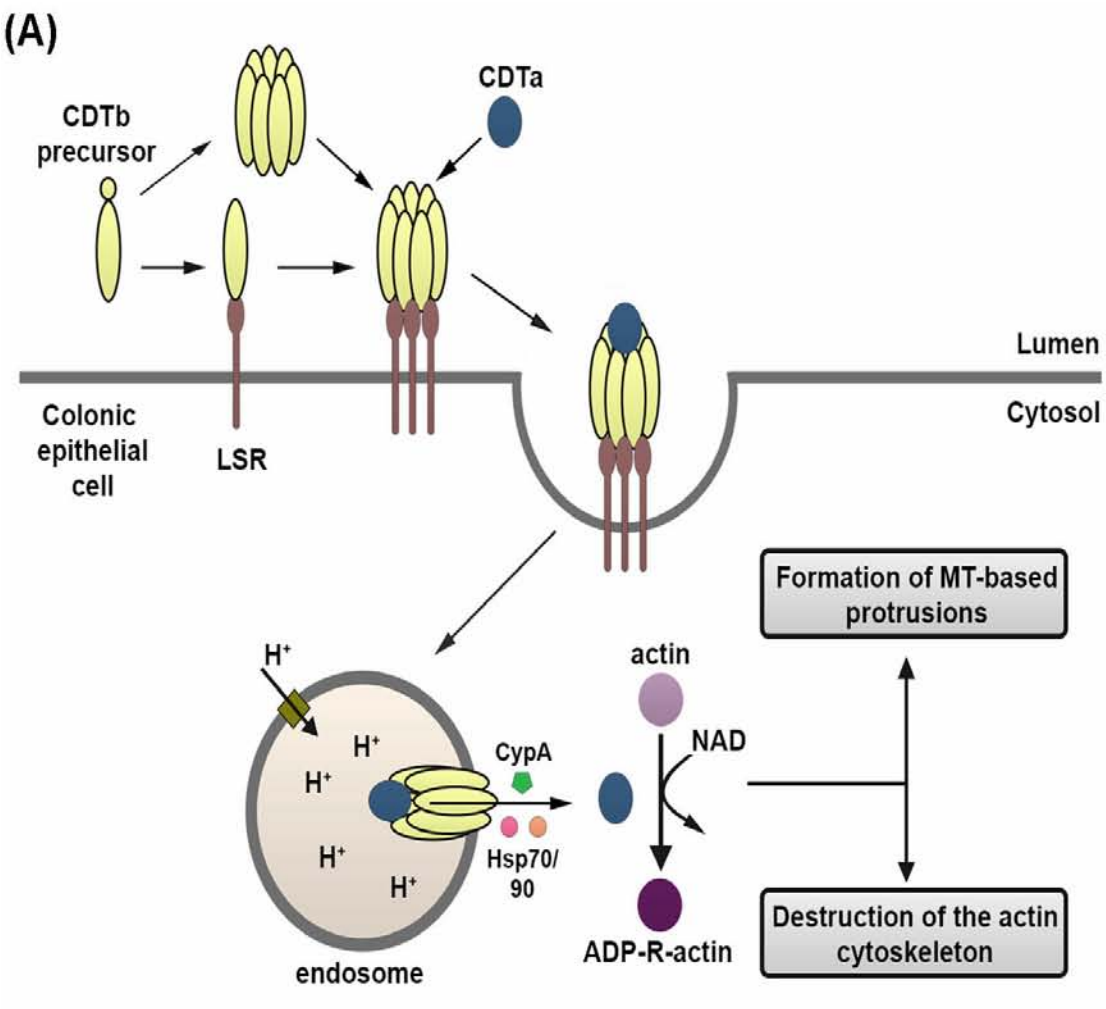
FEMS Microbiol. Rev. 41:723

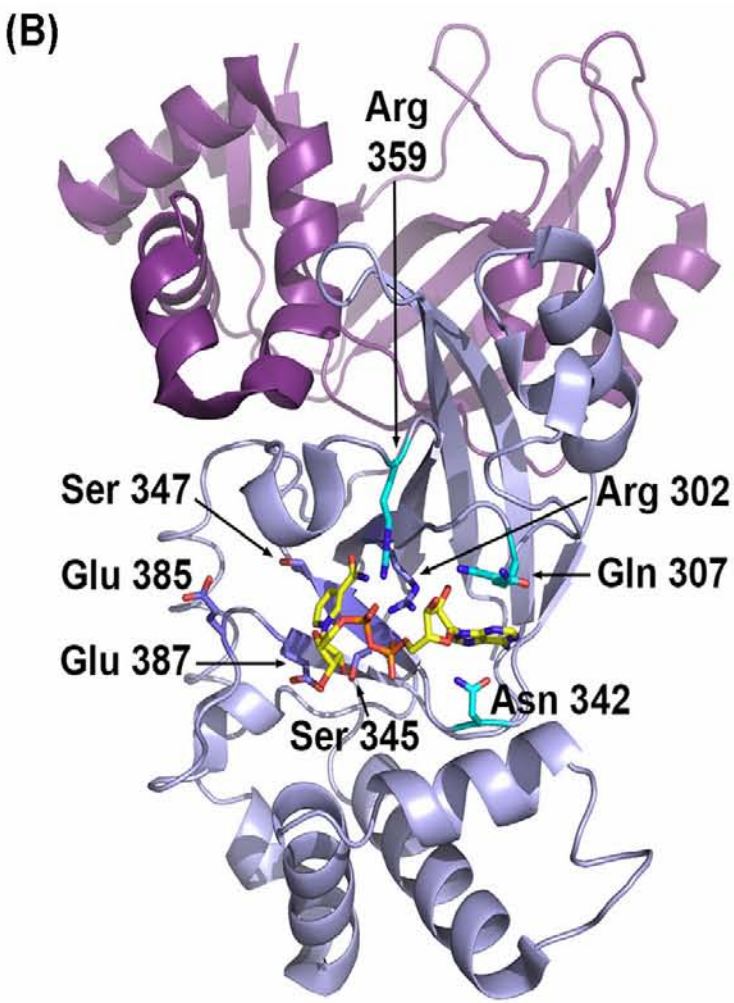


Inositol hexakisphosphate

Structure and function of the large clostridial toxins

Nat. Rev. Dis. Pri. 2:1, 2016



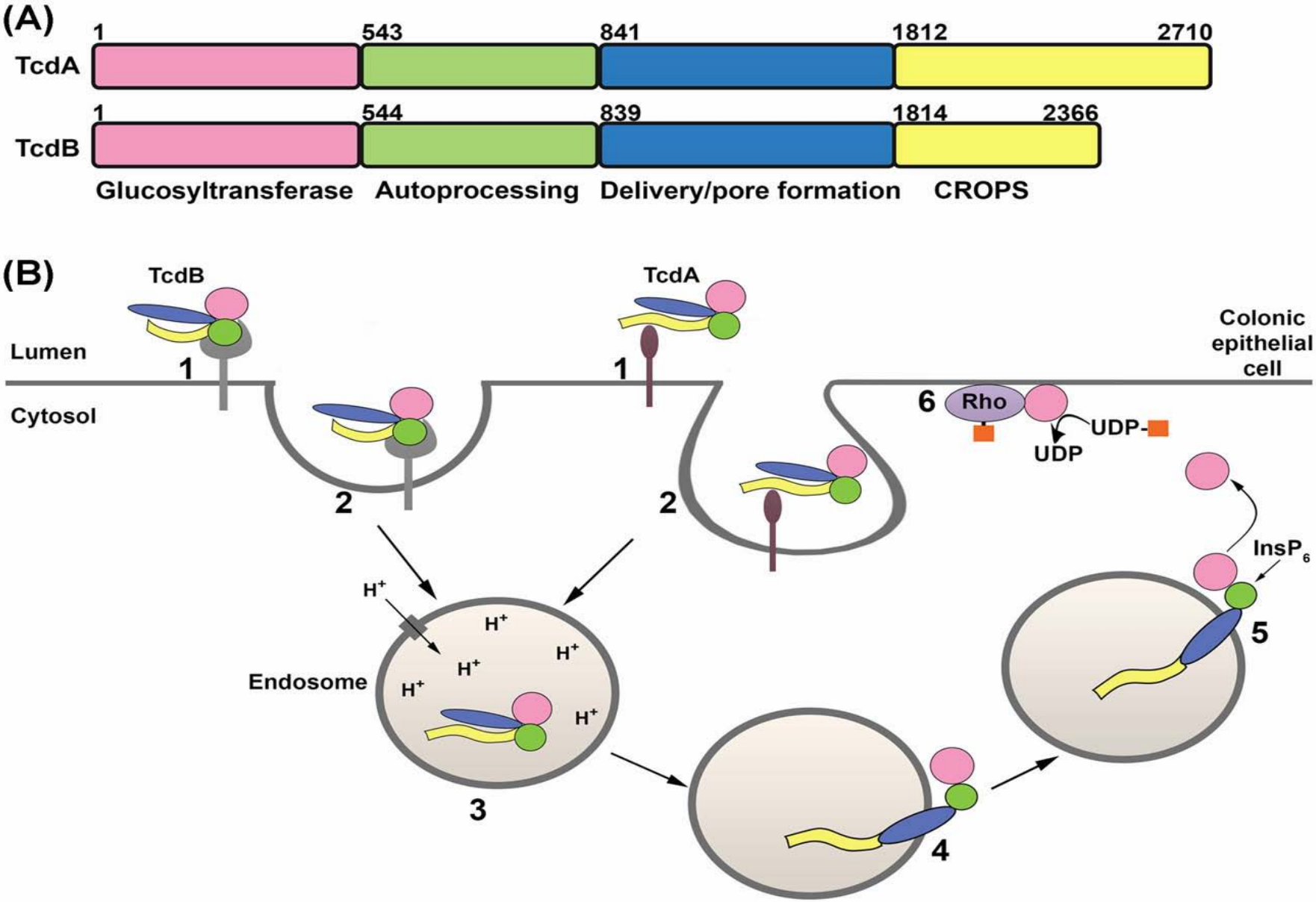


lipolysis-stimulated
lipoprotein receptor

The CDT binary toxin

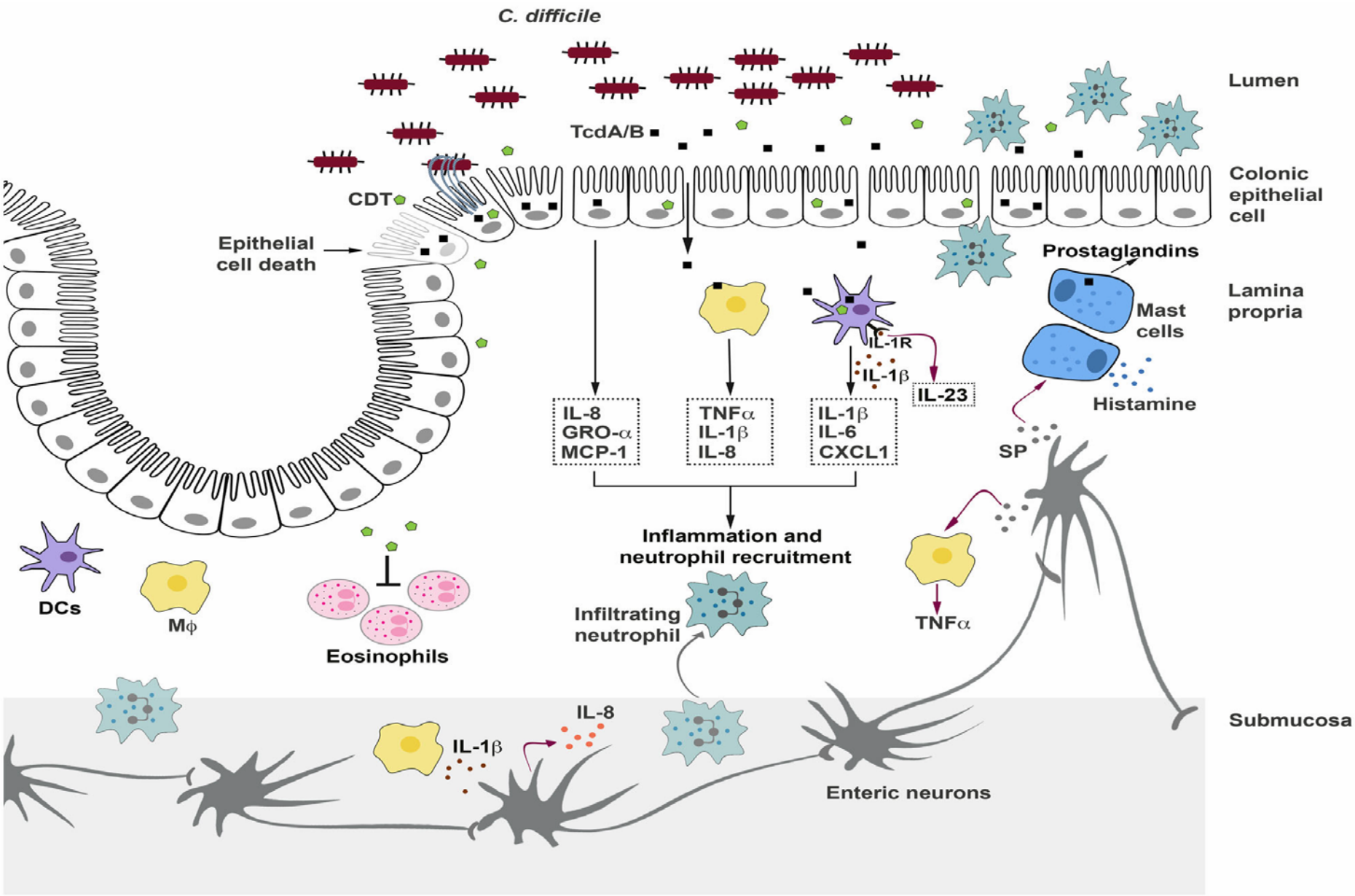
cyclophilin A

FEMS Microbiol. Rev. 41:723



TcdA and TcdB primary structure and mechanism of action

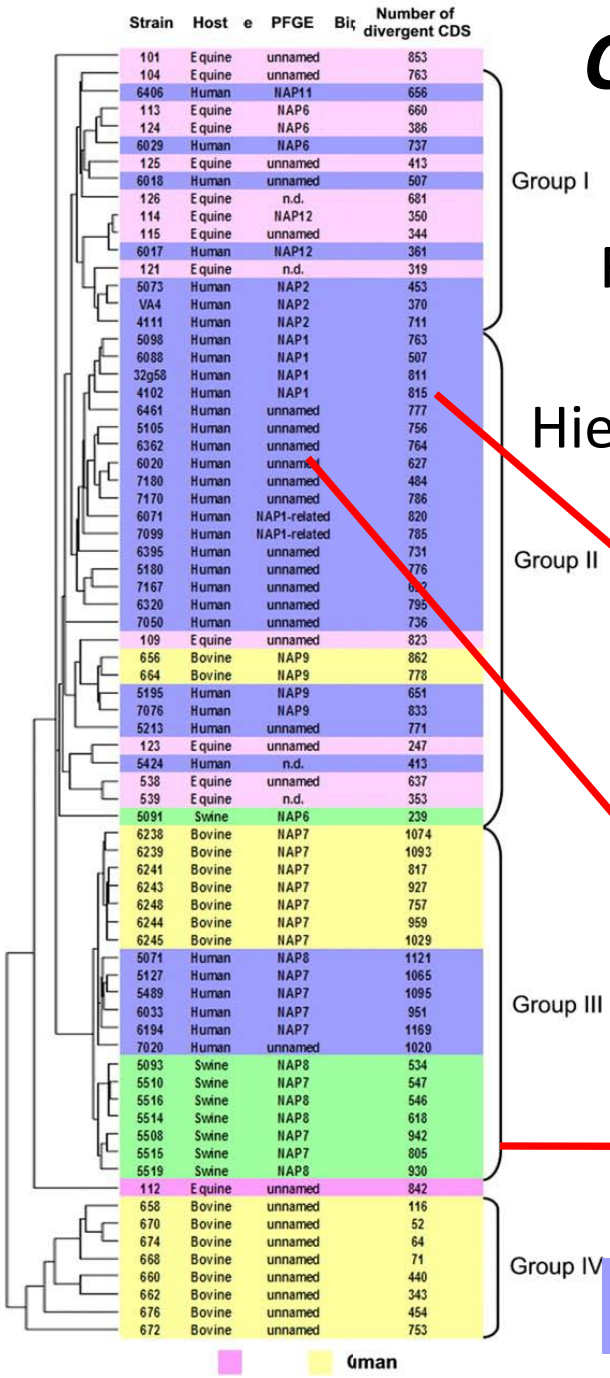
FEMS Microbiol. Rev. 41:723



Cellular effects of *C. difficile* toxins

FEMS Microbiol. Rev. 41:723

C. difficile genome is highly diverse



Reference strain – CD630 (1982, Switzerland)

Hierarchical clustering (73) - US, Canada, UK, France

Each strain contained hundreds of divergent genes

Some strains could not be typed by PFGE

Possibility of zoonotic transmission

Human **Swine** **Bovine** **Equine**

Janvilisri, J. bacteriol. 2009

Core genome of *C. diff* is extremely low

	QCD_76w55	630	6503	QCD_23m63	QCD_97b34	6407	CIP_107932	6466	QCD_37x79	ATCC_43255	QCD_63q42	QCD_32g58	R20291	CD196
6534	7924	7627	7766	7479	7616	6461	7538	7913	7965	7816	8046	7722	7900	7642
	5668	5840	5505	5264	5648	3114	5511	5170	5931	5783	6080	5492	5432	5513
	2256	1787	2261	2215	1968	3347	2027	2743	2034	2033	1966	2230	2468	2129
	0	82	22	0	2	58	4	72	52	6	36	30	48	13
QCD_76w55		7765	7904	7617	7754	6599	7676	8051	8103	7954	8184	7860	8038	7780
		6624	6741	6238	7337	3482	7031	6116	7399	6832	7037	6766	6818	7032
		1141	1163	1379	417	3117	645	1935	704	1122	1147	1094	1220	748
		1	4	4	0	2	0	4	2	0	6	0	2	0
630			7607	7320	7457	6302	7379	7754	7806	7657	7887	7563	7741	7483
			6482	6249	6603	3459	6546	5986	6734	6837	6882	6464	6279	6413
			1125	1071	854	2843	833	1768	1072	820	1005	1099	1462	1070
			5	19	0	1	0	5	0	6	8	0	0	1
6503				7459	7596	6441	7518	7893	7945	7796	8026	7702	7880	7622
				6080	6728	3434	6546	5975	6799	6649	6736	6390	6287	6513
				1379	868	3007	972	1918	1146	1147	1290	1312	1593	1109
				10	2	12	2	14	0	6	22	10	10	0
QCD_23m63					7309	6154	7231	7606	7658	7509	7739	7415	7593	7335
					6224	3240	6200	6121	6309	6315	6377	6102	5908	6062
					1085	2914	1031	1485	1349	1194	1362	1313	1685	1273
					0	2	6	232	2	4	12	4	6	2
QCD_97b34						6291	7368	7743	7795	7646	7876	7552	7730	7472
						3468	7015	6091	7385	6814	7008	6762	6825	7054
						2823	353	1652	410	832	868	790	905	418
						0	0	2	0	0	2	0	0	0
6407							6213	6588	6640	6491	6721	6397	6575	6317
							3416	3232	3523	3574	3576	3368	3350	3457
							2797	3356	3117	2917	3145	3029	3225	2860
							0	68	0	54	4	38	20	10
CIP_107932								7665	7717	7568	7798	7474	7652	7394
								6021	7074	6792	6769	6664	6632	6853
								1644	643	776	1029	810	1020	541
								2	4	10	0	6	2	4
6466									8092	7943	8173	7849	8027	7769
									6210	6090	6213	5972	5883	5997
									1882	1853	1960	1877	2144	1772
									2	4	4	42	66	18
QCD_37x79										7995	8225	7901	8079	7821
										6892	7311	6834	6924	7097
										1103	914	1067	1155	724
										2	160	4	10	6
ATCC_43255											8076	7752	7930	7672
											7049	6554	6426	6659
											1027	1198	1504	1013
											30	0	4	0
QCD_63q42												7982	8160	7902
												6648	6561	6730
												1334	1599	1172
												2	4	1
QCD-32g58													7836	7578
													6438	6565
													1398	1013
													48	2
R20291														7756
														6706
														1050
														20

Total
Shared
Difference
PairUnique

Strain 6534 vs. 630	
Total	7627
Shared	5840
Different	1787
Pair Unique	82

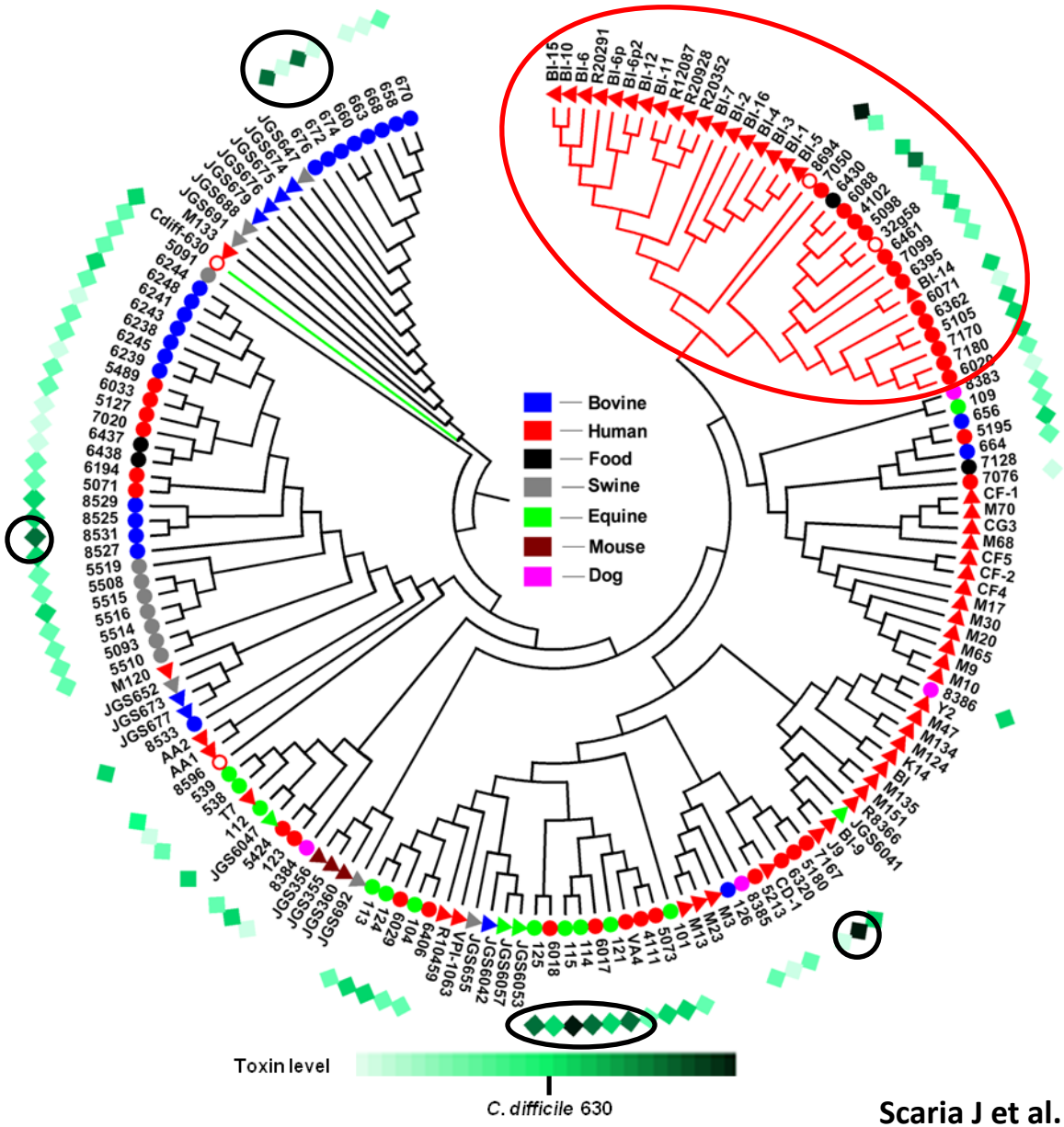
Core genome is about 25% of the pangenome

***E. coli* core genome is about 75% of the pangenome**

Pairwise genome sequence comparisons

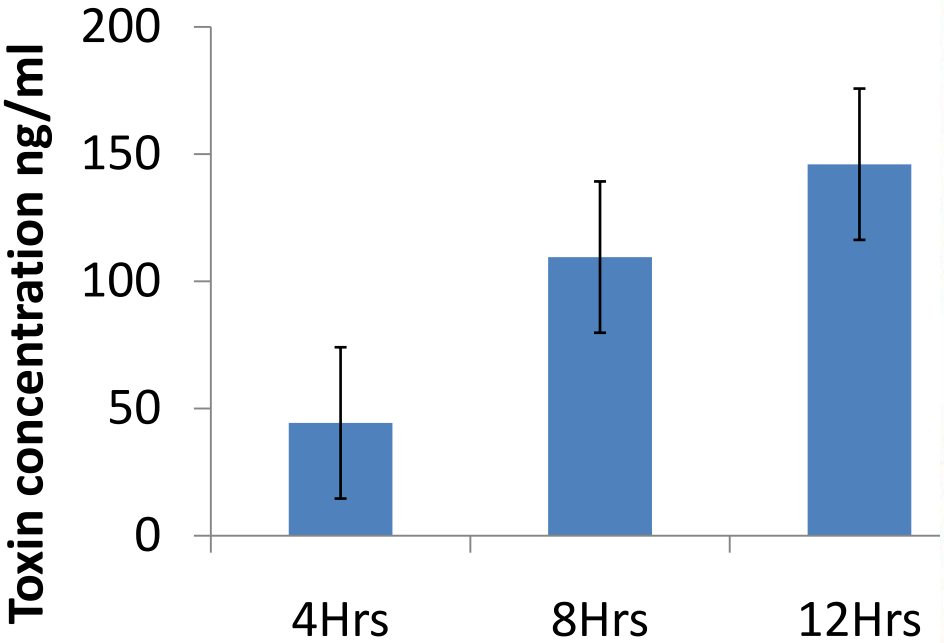
Scaria J *et al*. PLOS one : e15147, 2010

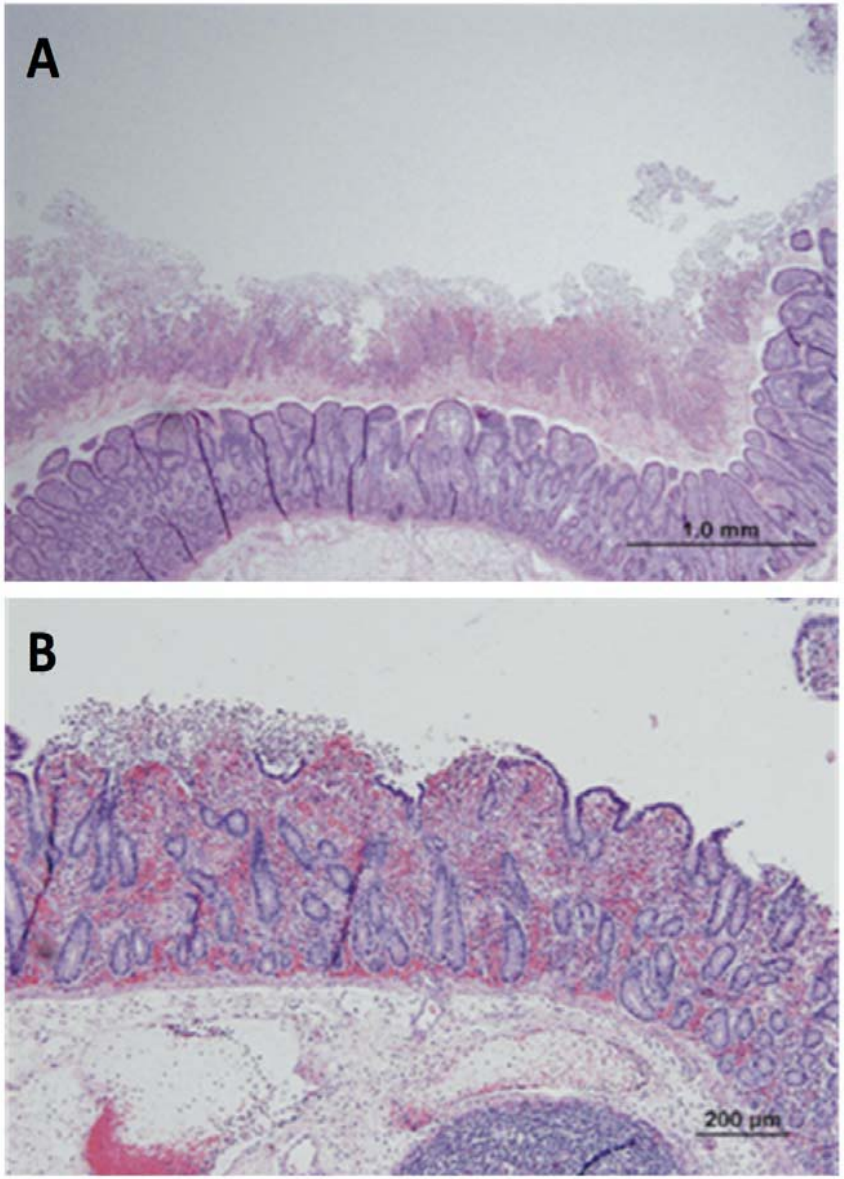
Strain (167) typing does not correlate with hypervirulence



Scaria J et al. PLOS one : e15147, 2010

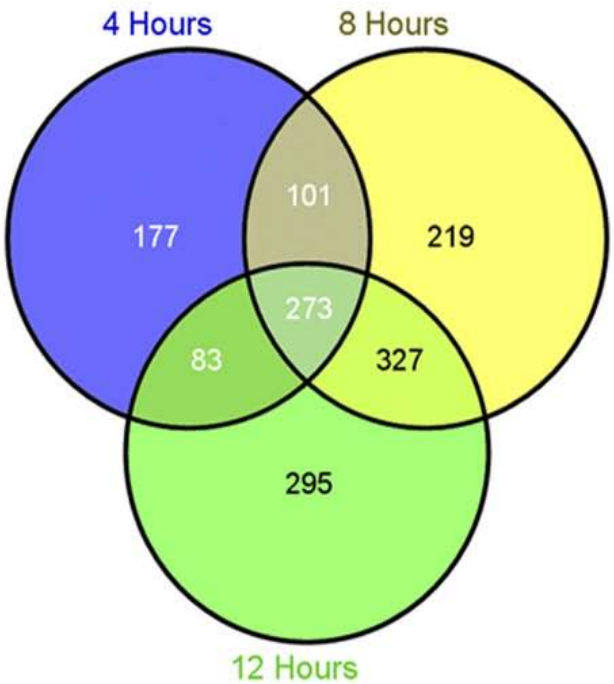
CDI symptoms



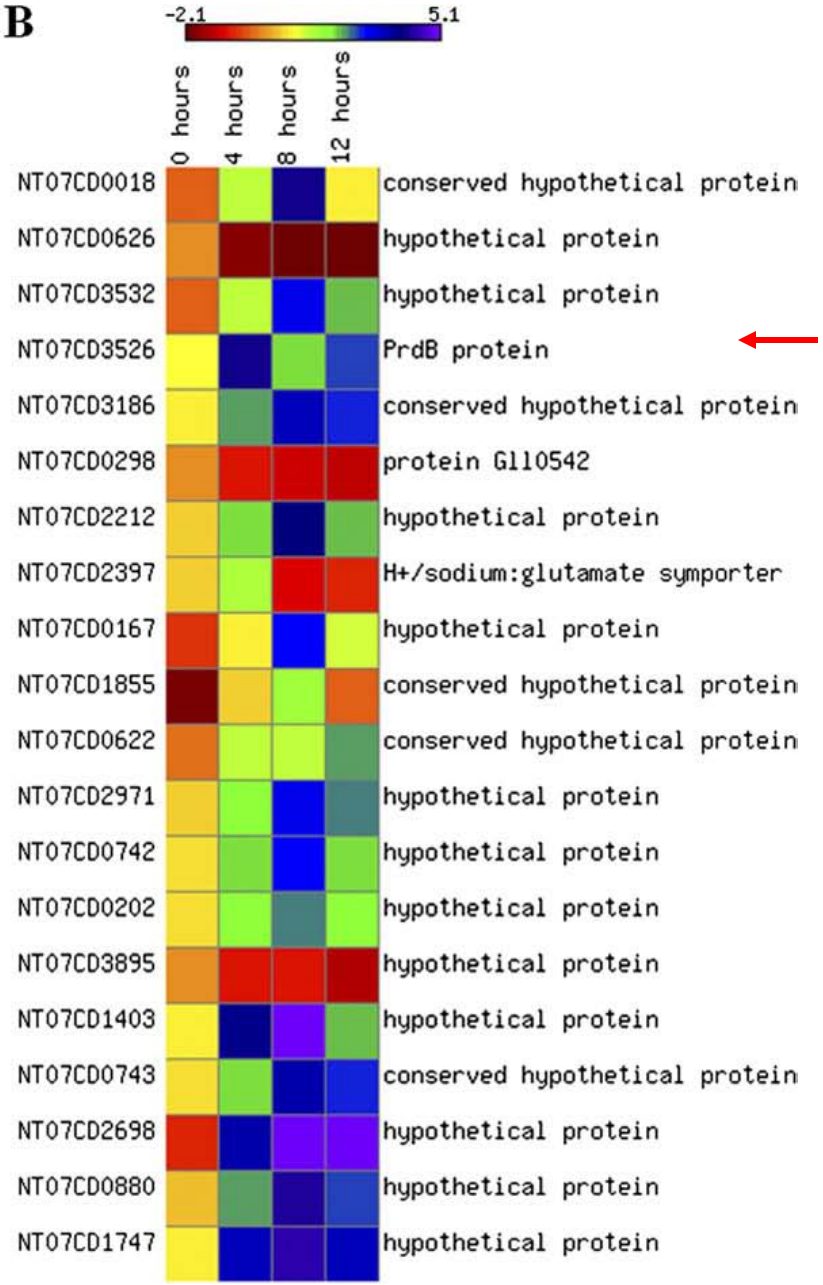


Janvilisri et al, PLoS One, e45608, 2012

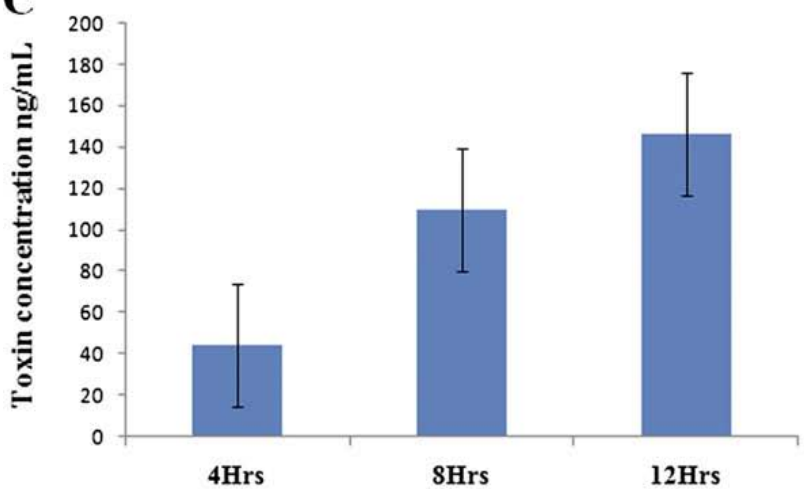
A



B



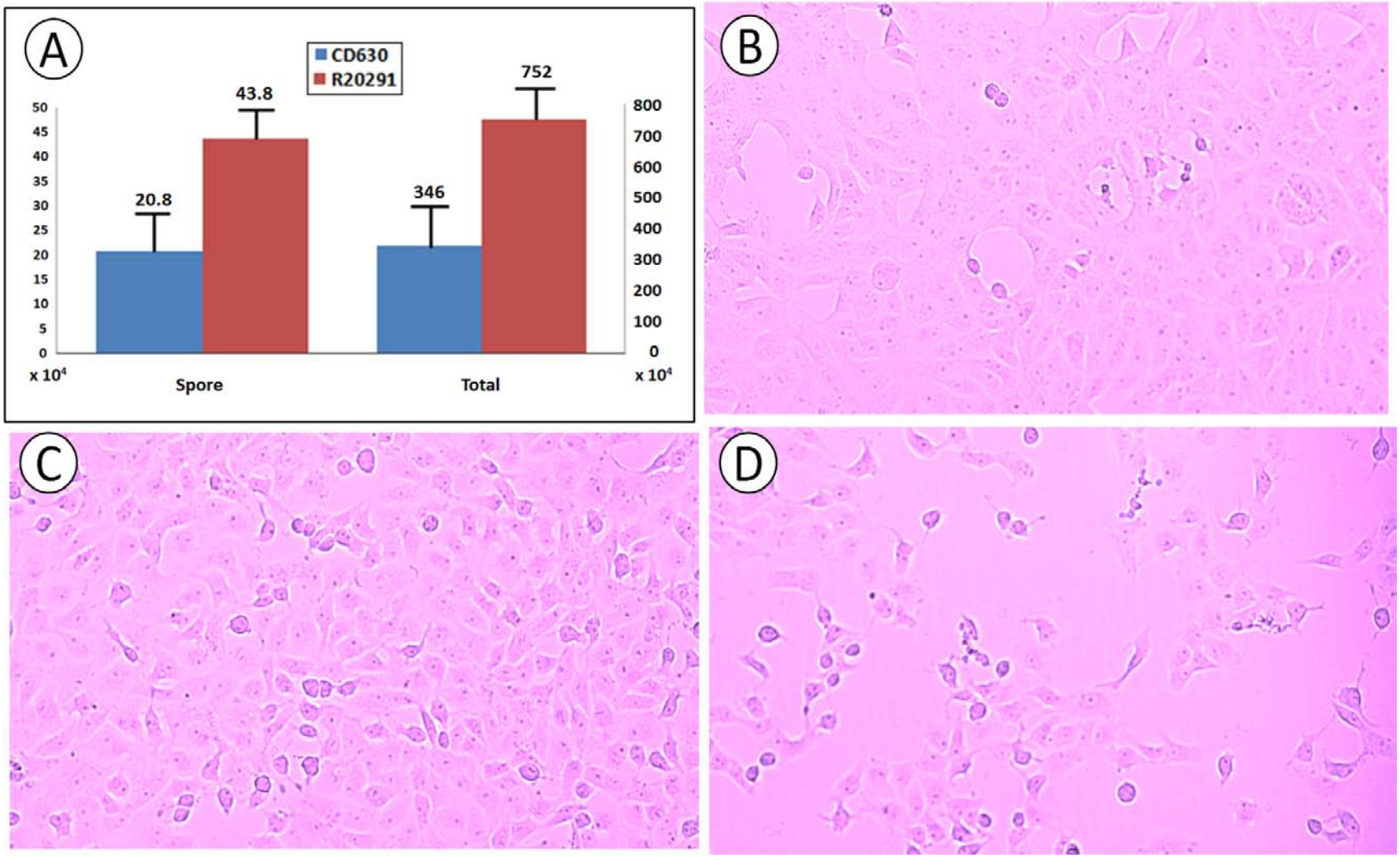
C



Proteomics

Scaria J *et al*. JID, 203, 1613 2011

Catabolic expansion leads to increased rate of colonization and virulence



Scaria J *et al*.