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BAB I
PENDAHULUAN

Abses cerebri dapat terjadi pada anak usia berapapun namun yang paling lazim antara usia 4 sampai 8 tahun. Penyebab abses cerebri adalah embolisasi karena penyakit jantung kongenital dengan *shunt* dari kanan ke kiri (terutama tetralogi Fallot), meningitis, otitis media kronis dan mastoiditis, infeksi jaringan lunak dari muka atau kulit kepala, selulitis orbita, infeksi gigi, luka tembus kepala, status imunodefisiensi, dan infeksi *shunt* ventrikulo-peritoneum.[1]

Abses serebri adalah infeksi intraserebral fokal yang muncul sebagai area serebritis lokal dan berkembang menjadi kumpulan pus yang dikelilingi oleh kapsul yang bervaskularisasi baik. Abses serebri sering terjadi berkaitan dengan malformasi jantung kongenital dan dapat muncul nyata pada saat lahir atau bermanifestasi pada usia dewasa. Dua komplikasi pada susunan saraf pusat yang paling serius yang berkaitan dengan penyakit jantung bawaan (PJB) adalah trombosis serebral dan abses serebri.[2]

Abses serebri dapat berasal dari: (1) penyebaran langsung dari infeksi jaringan non-neural di sekitarnya seperti sinusitis paranasal, otitis media, mastoiditis atau infeksi gigi; (2) penyebaran hematogen dari tempat infeksi yang jauh seperti endokarditis, infeksi paru, infeksi gastrointestinal; (3) akibat trauma kepala atau tindakan pembedahan yang menyebabkan infeksi langsung pada otak.[2]

Pasien dengan PJB sianotik (dengan right-to-left shunt) memiliki resiko yang lebih tinggi untuk menderita abses serebri dimana PJB sianotik merupakan faktor resiko pada 12,2-69,4% dari seluruh kasus serebri. Studi dari Menon et al melaporkan bahwa dari 75 pasien abses serebri, enam diantaranya (8%) memiliki PJB sianotik dan keseluruhannya merupakan penderita Tetralogi of Fallot.[2]

IMAGES IN NEUROLOGY

Cranial Nerve VII Palsy as the First Sign of Cephalic Tetanus After an Earthquake

A 29-YEAR-OLD MAN sustained right-sided periorbital and frontal scalp abrasions caused by falling cement during the January 12, 2010, earthquake in Haiti. Twelve days later, he developed weakness of the right side of his face. He received local wound care and intravenous cefazolin sodium. On physical examination, he had a subconjunctival hemorrhage and an isolated right-sided cranial nerve VII palsy. He reported pain in his jaw; however, he had a normal range of motion. Computed tomography of the head revealed no skull or jaw fractures. One day after discharge from the hospital, he noted difficulty in opening his mouth and new numbness on the right side of his face. His symptoms worsened over 7 days. He was evaluated at another hospital, where a gastric feeding tube was placed because of his inability to open his mouth. On readmission to the hospital, a new cranial nerve V palsy, a new subtle cranial nerve VI palsy, and a persistent cranial nerve VII palsy were noted (all right-sided) in addition to trismus and neck rigidity. His voice was soft; he experienced frequent left-sided facial spasms (**Figure 1**). Tetanus toxoid, tetanus immune globulin (3000 U intramuscularly), and intravenous metronidazole phosphate were given. A second computed tomograph of the head showed no abnormalities. Results of cerebrospinal fluid testing were normal. The patient underwent surgical exploration and debridement of his wounds that revealed no persistent infection. He never required intubation. Over 16 days, his trismus, neck rigidity, and left-sided facial spasms resolved, and he was able to drink a liquid diet through a straw. A residual cranial nerve VII palsy persisted 21 days after hospital discharge (**Figure 2**).

COMMENT

Tetanus is increasingly uncommon in developed countries owing to immunization programs. In developing countries, however, tetanus remains a significant health problem. Earthquakes can create soil-contaminated wounds that in an underimmunized population can lead to numerous cases of tetanus. Physicians working in such regions should be aware of all clinical presentations of tetanus. Cephalic tetanus is a form of localized tetanus characterized by trismus plus paralysis of 1 or more cranial nerves, with cranial nerve VII most frequently involved. The pathophysiology of cranial nerve paralysis in cephalic tetanus is still unclear.[1] Likewise, why paresis may persist for weeks after resolution of spasms is also unknown.[1,2] To our knowledge, a complete seventh cranial nerve palsy inaugural in the evolution of cephalic tetanus has been reported in only 4 other cases.[2-4]

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Table 1. Selected Demographic Characteristics of Pregnant Women Who Received Tdap During Pregnancy by Vaccination Status Recorded in the Vaccine Safety Datalink Sites From January 1, 2007, Through November 15, 2013

	No. (%)			
	Time Since Prior Tetanus-Containing Vaccination, y			
Variable	<2 (n = 4812)	2-5 (n = 9999)	>5 (Control) (n = 14 344)	P Value
Maternal age, mean (range), y	30.5 (15-49)	30.7 (14-49)	28.8 (14-48)	<.001
Enrollment prior to pregnancy, mean (range), mo	49.8 (6.0-99.9)	62.4 (6.0-100.5)	63.9 (6.0-99.8)	<.001
Gestational age at Tdap, mean (range), wk	30 (1-39)	30 (1-41)	27 (1-40)	<.001
Adequate prenatal care[a]	3629 (75)	7324 (73)	10 542 (73)	.01
Other vaccines in pregnancy	3012 (63)	6179 (62)	8996 (63)	.33
Maternal comorbidity[b]	1383 (29)	2956 (30)	4394 (31)	.03
Pregnancy complication[c]	2514 (52)	5230 (52)	7565 (53)	.74
Prior tetanus vaccine Tdap[d]	4542 (94)	8511 (85)	2477 (17)	<.001

Abbreviation: Tdap, tetanus, diphtheria, and acellular pertussis.

[a] Adequate or adequate plus prenatal care based on Kotelchuck Adequacy of Prenatal Care Utilization Index.

[b] Presence of hypertension in pregnancy, diabetes, cardiovascular disease, or asthma.

[c] Includes any of the following diagnoses: fetal abnormality affecting maternal management, fetal or placental problems affecting maternal management, polyhydramnios, oligohydramnios, premature rupture of membranes, amnionitis, antepartum hemorrhage, placental abruption, placenta previa, or antepartum complications.

[d] Compared with non-Tdap tetanus vaccines (ie, tetanus diphtheria; tetanus toxoid; diphtheria and tetanus toxoids and acellular pertussis, etc).

Table 1. Continued

	HD Cohort, No. (%)	SD Cohort, No. (%)	Risk Ratio (95% CI)	P Value	HD (≥65 yrs), No. (%)	SD (≥65 yrs), No. (%)	Risk Ratio (95% CI)	P Value
2015–16 Season	n = 176976	n = 1597003			n = 164111	n = 854757		
Lab-confirmed influenza	168 (0.095)	1901 (0.11)	0.84 (0.72–0.99)	0.03155	[illegible] (0.094)	806 (0.095)	1.0 (0.84–1.19)	1.0
Influenza hospitalization	124 (0.07)	937 (0.059)	1.19 (0.99–1.44)	0.06114	(0.069)	568 (0.066)	1.05 (0.85–1.29)	0.68

Disclosures. **All authors:** No reported disclosures.

1467. Effectiveness of a Web-Based Intervention to Increase Uptake of Maternal Vaccines

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Session: 162. Maternal/Infant Immunization
Friday, October 6, 2017: 12:30 PM

Background. Tetanus-diphtheria-acellular pertussis (Tdap) and influenza (flu) vaccines are recommended for all pregnant women in each pregnancy. However, vaccination uptake is suboptimal. Our objective was to test of the efficacy of an online vaccine and social media resource in increasing uptake of Tdap and flu vaccines.

Methods. The RCT was conducted in an integrated health care system in Colorado from September 2013 to July 2016. Participants were pregnant women in the third trimester of pregnancy. Participants were randomly assigned to a website with vaccine information and interactive social media components (VSM), a website with vaccine information only (VI), or usual care (UC). To facilitate interaction on the VSM site, women were randomized 3:2:1 across the VSM:VI:UC arms. The interventions were designed and pilot tested using focus groups, individual interviews, surveys, and usability testing with vaccine-hesitant parents and pregnant women and included content on maternal and infant vaccination. Participants in the VSM and VI arms had access to the same base vaccine content. The VSM site also included a blog, discussion forum, chat room, and "Ask a Question" portal. After randomization, women in the VSM and VI arms were sent a website link. While they were encouraged to use the vaccine website, it was not required. Tdap and flu vaccination outcomes were analyzed separately. Women were included in each analysis if they had no record of vaccination for the relevant vaccine at enrollment and were >2 weeks from delivery.

Results. For Tdap (n = 172), there were no significant differences in uptake between study arms (VSM: 71%, VI: 69%, UC: 68%, P = .95). For flu (n = 284), women in both the VSM and VI arms had higher rates of uptake compared with UC, although the intervention arms were not significantly different from each other (VSM: 57%, VI: 55%, UC: 38%, P = .09). Women receiving any intervention (VSM or VI) had significantly higher uptake of flu vaccine compared with UC (VSM/VI: 56%, UC: 38%, P = .03).

Conclusion. Web-based vaccination information which is sent to pregnant women, with or without social media components, can positively influence maternal flu vaccine uptake. Because of the potential for scalability, the impact of robust vaccination information websites should be studied in other settings and with women in earlier stages of pregnancy.

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1468. Provider Attitudes and Practices Regarding Maternal Vaccination Among Obstetrician-Gynecologists: A National Survey

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Background. Obstetrician-gynecologists (ob-gyns) play a crucial role as vaccinators of pregnant women, yet little is known about their attitudes and practices in this role. Our objectives were to describe, among a nationally representative sample of ob-gyns: 1) practices and attitudes regarding vaccination of pregnant women; and 2) barriers to the use of standing orders.

Methods. An e-mail and mail survey among ob-gyns conducted March-June 2016.

Results. The response rate was 69% (331/477). Overall, 90% reported administering ≥1 vaccines to pregnant women. Almost all (97% and 95%, respectively), strongly recommend influenza (flu) and tetanus-diphtheria-acellular pertussis (Tdap) vaccines; 60% use standing orders for flu vaccination and 56% for Tdap vaccination. More (68%) always recommend Tdap vaccines to household contacts of pregnant women than flu vaccines (53%). Physician attitudes are shown in the figure.

The most significant barriers to the use of standing orders included provider concern that patients prefer to speak to them first (12% major barrier, 25% somewhat), provider belief that they should be the one to recommend vaccines (11% major, 12% somewhat), and staff discomfort because of having to answer vaccine-related questions (7% major, 17% somewhat).

Conclusion. Ob-gyn attitudinal barriers to maternal vaccination are rare, whereas barriers to use of standing orders, a highly effective strategy for increasing vaccination uptake, are common, and less than 2/3 of providers currently use them.

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1469. Durability and Kinetics of Maternal Pertussis Antibodies in Infants of Mothers Immunized with Tdap During Pregnancy

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Background. Infant protection against severe pertussis requires sufficient maternal pertussis antibodies until infant immunization begins. The kinetics of maternally-derived Tdap-induced antibodies in infants is poorly understood.

Methods. 34 healthy mother-infant pairs were followed prospectively from maternal Tdap immunization to infant age 6 weeks. Blood was collected from women pre-Tdap, 4 weeks post Tdap and at delivery, and from infants at birth, and age 3 and 6 weeks. IgG to pertussis toxin (PT), filamentous hemagglutinin (FHA), fimbrial proteins (FIM) and pertactin (PRN) was quantified by luminex assay (IU/mL). Geometric mean concentrations (GMCs) with 95% confidence intervals (C.I.) for pertussis-specific IgG and half-life of IgG to PT were calculated.

Results. Mean maternal age was 31.1 years (range 22.7–39.7); 47% were white, 32% Hispanic and 21% Black. Tdap was administered at a mean gestation of 30.7 weeks (28–32.7). Infants had a mean gestation of 39.1 weeks (36–41.1) and birthweight of 3379g (2580–4584). GMCs (95%C.I.) for maternal pertussis-specific IgG increased significantly 4 weeks post-Tdap (4-fold higher in 59%, 41%, 29% and 44% for PT, FHA, FIM and PRN, respectively) and waned before delivery. Placental transfer was 135% for PT, 141% for FHA, 131% for FIM and 136% for PRN. Maternal antibodies in infants decayed quickly, but at age 6 weeks GMC of infant PT-specific IgG was 21.1IU/mL (14.7–30.2) and 91% had PT ≥ 10 IU/mL. Estimated half-life of PT-specific IgG in infants was 30.9 days.

Time	PT (IU/mL)	FHA (IU/mL)	FIM (IU/mL)	PRN (IU/mL)
Pre-Tdap	9.85 (6.71–14.45)	32.81 (21.79–49.42)	131.55 (81.98–211.15)	55.67 (35.75–86.68)
Post-Tdap	46.8 (34.4–63.68)	116.82 (88.9–153.5)	440.35 (327.57–591.97)	233.02 (179.14–303.04)
Maternal Delivery	40.78 (29.4–56.53)	104.81 (78.27–140.35)	384.5 (287.41–514.29)	204.41 (155.42–268.84)
Infant Cord	55.12 (38.65–78.6)	147.81 (113.47–192.49)	505.36 (366.44–696.95)	278.55 (216.57–358.26)
Infant 3w	30.73 (21.57–43.79)	82.49 (63.65–106.93)	292.87 (221.11–388.06)	167.72 (129.57–217.07)
Infant 6w	21.1 (14.72–30.23)	54.98 (41.97–72.03)	210.52 (153.76–288.27)	113.03 (88.94–146.99)

Conclusion. Although the half-life of maternal PT- specific antibodies induced by Tdap immunization during the third trimester of pregnancy is shorter than previously thought, this strategy results in levels likely sufficient to protect infants through the start of the immunization series.

Disclosures. **All authors:** No reported disclosures.

1470. Tdap and Influenza Vaccination Among Women with a Live Birth, Internet Panel Survey, United States, 2015–2016

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Availabe: jsessionid=5669A4B18FD265C14C1B2459FCF491B5?sequence=1 [accessed 18 April 2020].Page 2Skilled Birth AttendanceDTP3 CoverageÃ ÂRural-UrbanMaternal EducationWealth QuintileRegional DifferenceMedianRural- UrbanMaternal EducationWealth QuintileRegional DifferenceMedianGrand MedianBenin (MICS erbop od sitniuq e sacir siam serehlum san sa§Ãnairc uo serehlum me arutreboc(azeuqir ed litniuQ ;)o£Ã§Ãacude mes sair¡Ãdnuces se£Ãm sartuo ed anretam o£Ã§Ãacude moc se£Ãm me arutreboc(anretam o£Ã§ÃacudE ;)onabru larur on arutreboc(onabru-larur :somertxe siod ertne a§Ãnerefid a atneserper arugif A HCAEA)8(54.9259.9)4(9.91)5(9.6)6(31)7(3,464)5(93)21(7,86)ht11(6,14)31(4.05)41/3102 SHD(OGOT)ºÂ1(51 .2154.7)dn2(1.31)4(5.6)5(4.8)ºÂ3(8.0'1.61)ºÂ2(12)ºÂ1(4.71)ºÂ2(8.41)ºÂ2(2.11)7102 SCIM(enoeL arreiS)9(53.2350.7)11(6.83)dn2(9,1)4(9.7)8(2,62.54)8(65)9(85)6(3.03)8(4,43)7102 SHD(lageneS)41(359.25)31(7.25)41(1.35)41(9.46)41(5.521.85)ht41(7.76)ht41(1.27)21(5.84)7(2.43)71/6102 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Among them are global regulators, such as two-component systems (TCs) and Cody, to detect the relevant environmental signals [17,18]. The TCSs are ubiquitous among bacteria. They consist of a chinidine kinase (SHK) sensor connected to the membrane or the cytosolic sensor (SHK) that detects a stimulus and its response regulator to the cytoplasmic cognate (RR) that measures the cellular response. After a specific stimulation, the autophosphophollyl SHK in a retained histidine residue. The phosphoryl group is then transferred from histidine to a retained aspartate residue in the RR, which after phosphorylation is able to control the expression of its target genes [18]. Depending on the availability of nutrients, bacteria need to adjust their gene expression. The global regulator that Cody has shown to be an important regulatory link between metabolism and virulence factor synthesis in many bacteria Gram-positives for G + C drops. Cody shows the affinity enhanced by its DNA target when linked to GTP and/or branched chain amino acids [19]. In C. botulinum ATCC 3502, Cody positively demonstrated Bont's expression and connected to the promoter of GTP-dependent operan ntnH-Bonta [20]. In addition, it was found that the SPO0A spore regulator positively regulates the synthesis of BONT in type E C. botulinum, which is part of group II of C. botulinum and lacks BOTR. The SPO0A binds to the promoter of Operon Bont/E and enhances its transcript [21]. The SPO0A is expressed in the exponential growth phase of C. botulinum and begins the cascade of alternative sigma factors involved in the spulation [22]. However, SPO0A targets several hundred other genes and has pleiotropic effects [23]. In addition, the mutation frequency decline gene (MFD), which encodes a repair factor coupled to transcription, alsoPleepic effects: Increases the expression of toxin A and toxin toxin Genes in C. difficile [24]. These Cody, Spo0A and MFD genes are stored in the C. tetani genome. Genomic analysis of the strain C. tetani E88 reveals the presence of numerous slutty regulating genes, some of them are homologous to regulatory genes in other chlorstridia or bacteria. The aim of this study was to determine the role of the 12 slutty regulatory systems in the synthesis of tents, nine two-component systems and global regulators Cody, Spo0a and Mfd.Genome Sequencing of the E88 strain showed that the large plasmid containing tents also Ports of regulatory elements: TETR Located immediately upstream of the tent, a two-component system (TCS) (CTC_RS13805/CTC_RS13810, previously called CTP21/CTP22) homologous in systems in other Gram-positive bacteria, including C. botulina and bacilue sp. additional slutty sigma factors [9]. In addition, refined genomic analysis showed that the E88 strain chromosome has 30 TCSs based on the preserved histidine kinase motifs as part of the SHK and a Rec of the receptor domain as part of the adjacent RR. Most of the 30 TCSs belong to the OMPR family (External Membrane Protein Regulation) (19 TCSs), two to (lithic gene regulator/alginate biosynthesis regulator) Lytr/Algr, two a (reductor nitrate) NARL, two for (factor for inversion stimulation) FIS, one for (L-arabinose gene regulator) ARAC, one for (catabolic purine gene regulator) PUCR, one for Lysr (lysine synthesis regulator) and one for XRE (((((Profage Bacillus subtilis) PBSX repressor) Families (Table 1). All 30 TCSs, except for a homology with TCSs whores in other species of Clostridia. Among them, 19 TCSs are homologous to related genes in the C. botulinum strain hall (identity level ¥ 45%) (Table 1). It has been demonstrated previously that five TCSs in the tension hall positively regulate the synthesis of botulinum neurotoxin (BONT)Two of them are homologous for C. tetani TCSS, i.e. CLC0661/CLC0663 of C. botulinum withThe protein identity for CTC_RS02080/CTC_RS02085 of C. tetani and CLC0410/CLC0411 shows 68% protein identity for CTC_RS10030/CTC_RS10035 (Table 1). Another study identified a TCS (CB00786/CB00787) in the strain of C. botulinum ATCC3502, which negatively regulates the expression of the BONT gene [26]. This TCS shares a significant identity with a TCS of C. tetani (CTC_RS07310/CTC_RS07315). The C. tetani CN655 C. tetani genome [10] shares the same TCs genes as the E88 strain. As we have already characterized tetr in the CN655 strain, a strain that is more easily transformable compared to other strains of C. tetani under the applied conditions, the CN655 strain was selected for additional genetic investigations, including the analysis of nine TCs, MFD, SPO0A and Cody genes. Res, breathing; BAC, bacitracin; Vir, virulence; Spa, subtiline gene; Van, vancomycin; GTC, gramicidine transcript; Arc, aerobic breathing control; Pho, phosphate; FEU, fertile pickup; DCU, dicarboxylate pickup; Sin, inhibition of sporesporulation; EU, use of ethanolamine; ATO, acetoacetyl-coenzyme to transferase. Plasmids who were able to generate anti-sensus mRNA from nine TCs genes, as well as from MFD, Spo0A and Cody, were built in analogy for the construction of PMRP306, which was previously used in research in the research previously tetr investigation into C. tetani and regulatory genes in C. botulinum [13,14]. The DNA segments for anti-sense mRNA production were designed, located in the RR gene of three TCSs (CTC_RS04785, CTC_RS10155 and CTC_RS07315) and in the shk gene in six other TCSS (CTC_RS13810, CTC_R Mesa 2). Although the growth kinetic of the CN655 that houses the empty vector PAT18 (CN655/PAT18) was slightly smaller than that of the WT CN655 during the exponential growth phase and the start of the stationary phase, the total tent production was slightly higher (additional material figure S1). marartsom marartsom TW 556NC otnauq 81TAP/556NC Growth and production of toxins from 72 to 144 h (extra material figure s1.) no difference was observed in bacterial size and microscopic morphology between anti-sensus strains CN655 and CN655/PAT18. directing mfd, p1472 directing spo0a and P1418 directed to cody,) showed similar growth kinetic compared to the CN655/PAT18 control strain (figure 1.) however, the growth of CN655/P1307 strains directing CTC_RS13810, CN655/P1309 directing CTC_RS04705, singatinatimatinatimatinatimatinatimatinating C5/PENTAT C50/P155 c50/C555/P155 Strain/C555 Finict/C555/P155 Strain/C555 Finict/C50/P155 c55555, C555/P135 ctc (C555/P135 C50/C50/P135 c555, cn655/p1312 target CTC_RS (12-48 h,) but the five anti-sensus strains reached a biomass similar to that monitored by OD600nm than that of other anti-sensus strains at 72-144 h (Figure 1.) the reticulated immunosorbent test (elisa) in the culture overnadante was reduced in three anti-sensus strains CN655 (CN655/P130 on the other hand, the CN655/P1310, which targets CTC_RS04785, showed a moderate decrease (about 25%) in the secreted tent. the cody anti-sensus strain (CN655/P1418) showed a significantly lower barge level (50% to 65%) in the culture supernatant in the first 56 h of culture and a less pronounced effect (20% to 25%) at the end of the growth phase (Figure 2a.) the anti-sensus strains CN655/P1308, CN655/P13, CN655/P13,and CN655/p1472 had no significant difference in the production of extracellular TeNT compared to CN655/pAT18 (S1). The total amounts of TeNT produced (i.e., TeNT in the supernatant of culture and intracellular TeNT at the end of culture growth) were significantly decreased in CN655/p1307, CN655/p1314 and CN655/p1418, while no significant difference was observed in CN655/p1310 (Figure 3A). In contrast, extracellular and total TNT concentrations were significantly increased in CN655/p1419 aiming at CTC_RS07315 (Figure 2B and Figure 3B). The anti-Sense strain CN655/p1311 aimed at the CTC_RS05750 presented an increase in the extracellular TeNT within the first 48 h of culture, but the total amount of TeNT at the end of the culture was not significantly different from the control voltage (Figure 2, Supplementary Material Figure S2). The total production of TeNT was not significantly different from that of the CN655/pAT18 control line in CN655/p1308, CN655/p1309, CN655/p1312, CN655/p13131313, CN655/p1480 and CN655/p1472 (Susplementary Materials Figure S3). The transcriptional levels of tent and tetR were monitored by qRT-PCR in 8, 24, 32 and 48 h of culture, which corresponded to the exponential growth phase (8 h) and the initial stage of stationary growth (12–48 h) (Figure 1). The limit of these analyses is based on the consideration that copy numbers of recombinant plasmids are similar in recombinant strains. Among the TCS investigated by the anti-sented RNA, CN655/p1307 and CN655/p1314 presented a significant reduction in the transcript of the tent and TTR during the growth phase of 24 to 48 h and only a moderate effect in the exponential growth phase of 8 h (Figure 4). A marked reduced tetR transcription was observed in CN655/p1310 at the early stationary growth stage of 8 h and 24-32 h, while the transcript of the tent was not mu mu uortsom 1131p/556NC esnes-itna epritse a ,etsartnoc mE .)4 arugiF(adizuder in the transcript of the tent and tetrament in the exponential growth phase of 8 h and in lesser extent in the stationary growth phase of 24 48 h (Figure 4). The anti-sensus strain CN655/P1419 also exhibited an increase in the transcription level of the barracks in the exponential growth phase of 8 hours and 48 h of culture, but a decreased transcription of tetr within the 24-32 h of early stationary growth phase, although for a lesser extent than in CN655/P1307 and CN655/P1314 (Figure 4). This suggests that CTC_ RS07315 (directed in CN655/P1419) controls the transcript of the tent regardless of tetr. The transcriptional levels of tent and tetr were not significantly different in CN655/p1308 and Suplemer CN655/P1309 compared to the CN65555555 (Supplyyy (CN655/P1309 Figure S4) control lineage. Despite a slightly reduced transcript of TETR in CN655/P1312 and CN655/P1313 during the early stationary growth phase of 24 - 32 h (Figure 4), no significant alteration of the transcript of the tent was observed in these strains (additional materials Figure S3). The anti-sensus tension in which Cody was directed (CN655/P1418) showed a decrease in the transcription of the barrack in the culture of 8 48 hours and a moderate decrease in the transcript of TETR (Figure 4). To identify the mode of action of the positive and negative regulators of the transcript of the tent gene, we investigate its possible direct interaction with tent and/or tetr genes, testing the connection of regulators to tent and tetr promoters. As shown in Figure 5, the CTC_ RS07315 (directed in CN655/P1419) RR specifically connected to the tent and tetr promoters (ptent, ptetr). The RR CTC_RS04785 (directed in CN655/P1310) caused a change in PTENT mobility, but not in PTETR, despite the significant decrease in TETR transcription. On the other hand, no specific connection to PTENT or PTETR was observed with theof CTC_RS10155, CTC_RS13805 and CTC_RS05745, although they have influenced the synthesis and transcription of the tent. How to How toelectrophoretic change was not observed with SHK, such as CTC_RS04710. Also, CodY connected to Ptent, but not to PtetR. As a positive control, TetR, which was found to positively regulate the tent, was used [13]. Since two TCSs (CTC_RS05745/CTC_RS05750 and CTC_RS10030/CTC_RS10035) encoded in the genome C. tetani are wholly engaged in control of phosphate absorption and/or metabolism, as judged of their homologies with previously characterized TCSs, we have verified the effect of inorganic phosphate (PNTi) in culture. Therefore, TGY (trpticase/glucose/east extract) culture medium containing less than 2 mM Pi was complemented with 10 to 60 mM Pi. C. tetani growth was similar in TGY complemented with 10 and 20 mM Pi than in the TGY control medium, but was slightly decreased when supplemented with 40 and 60 mM Pi (Figure 6). The extracellular and total TeNT production was increased in TGY containing 40 or 60 mM Pi, while no significant difference in TeNT production was observed in TGY complemented with 10 or 20 mM Pi. The highest level of TeNT production occurred when C. tetani was cultivated in TGY with 40 mM Pi (at least twice as extracellular TeNT compared to the TGY control medium) (Figure 6). The expression of the tent was increased in the TGY medium containing 10 or 20 mM Pi, and to a greater extent when supplemented with 40 or 60 mM Pi (Figure 6D). The kinetic expression of the augmented tent in TGY complemented with Pi is similar to that of the TGY control medium, occurring at the end of the exponential and beginning of the stationary growth phases. The expression of the tetR was also increased in cultures supplemented with Pi, but mainly within a short time between 24 and 32 h of culture (Figure 6E). This suggests that the regulation of TeNT expression by inorganic phosphate is independent of tetR. High CO_2 concentrations (70%) were found to increase the expression of of BoNT in C. botulinum group II in contrast to group I strains, where CO_2 has no significant stimulatory effect [27,28]. CO_2 also increases the toxin production in other bacteria such as Bacillus anthracis, Staphylococcus aureus and Vibrio cholerae [27]. However, the mechanism of the stimulatory effect of CO_2 is not well defined. Since C. tetani is phylogenetically related to C. botulinum [29], we tested the influence of carbonate as a nutritional requirement for TeNT synthesis. For that, the TGY medium was supplemented with various Na_2CO_3 concentrations, and addition of 50 or 100 mM Na_2CO_3 did not modify the growth of C. tetani CN655 (data not shown). We observed a significant stimulatory effect on TeNT synthesis in TGY medium supplemented with 100 mM Na_2CO_3 (Figure 7). No synergistic or cumulative effect was detected between the addition of carbonate and Pi (Figure 7). The pH at 72 h of culture was slightly higher (pH 7.6) in TGY supplemented with 100 mM Na_2CO_3 compared to control TGY (pH 7.0) or TGY with 50 mM Na_2CO_3 (pH 7.3). However, the pH in TGY cultures supplemented with 40 mM Pi or 100 mM Na_2CO_3/40 mM Pi was not significantly modified (pH 7.0 and 7.4, respectively), suggesting that the role of Pi and Na_2CO_3 on TeNT synthesis were not pH-dependent.Since the TCS CTC_RS05745/CTC_RS05750 seemed to be involved in TeNT secretion without controlling the synthesis level of toxin (see above and Figure 2), we investigated the morphology of the strain CN655/p1311 in comparison to the wt CN655 by electron microscopy (Figure 8). Electron microscopic analysis of wt CN655 and strain CN655/p1311 showed a marked alteration of the bacterial wall of the mutant strain (Figure 8). In the wt CN655 strain, the layers forming the bacterial wall are well organized. In contrast, in strain CN655/p1311, the bacterial wall appeared disorganized and an abundantly detected diffuse material

surrounded the bacteria. The altered bacterial ,revewoH .noitcudorp TNeT tneu101qesbus dna noisserpxe Rtet-tnet eht decneulfni 55101SR_CTC/05101SR_CTC 50831_CT eht eht eht eht et t et t e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e t e r e r e r e r e r e r e r e r e r e r e r e r e r e r e r e t e r e r e r e r e r e r e r e r e r e t e t e t e r TNeckllötttt. transcription were not decreased in the exponential growth phase despite a reduction of TeNT production. This might result from a delayed effect of the RNA anti-sense system and/or of unproductive RNA hybridized with the anti-sense RNA. They seem to indirectly control the expression of tent and tetR, since the respective RRs bound neither to Ptent nor PtetR.The TCS CTC_RS13810/CT_RS13805 is the only TCS located on the large tetR-tent operon-containing plasmid, and it is encoded about 25 kb upstream of tent. The CTC_RS13805 RR belongs to the OmpR family (Table 1) and shares 91% amino acid identity with a RR of Clostridium lundense but has no homolog in other clostridia or bacteria. The chromosomally encoded RR CTC_RS10155 is related to the LytR/AlgR family of regulators and is putatively involved in autolysis. This TCS has homologs in C. botulinum and other clostridia. However, the TCS homolog in C. botulinum has no influence on BoNT production [25].The C. botulinum TCS CBO0786/CBO0787 was previously found to repress BoNT synthesis [26]. This TCS has a homolog in C. tetani (CTC_RS07310/CTC_RS07315) with a 58% identity at the amino acid level of the RR, and it is predicted to be involved in cell division. This C. tetani RR was also found to repress TeNT synthesis. Indeed, secreted and total TeNT levels were increased in the anti-sense strain (CN655/p1419). Interestingly, the RR CTC_RS07315 bound to Ptent and PtetR, resulting in a significant decrease in tent expression at the stationary growth phase and a moderate impairment of tetR transcription at late exponential and early stationary growth phases. This suggests that the negative effect on tent transcription results from a direct interaction with Ptent, possibly independent of tetR since the effect on tetR transcription was weak. The mode of action of CTC_RS07315 RR is likely similar to that of CBO0786, which binds to the ntnh-bont/A and ha operon promoters in C. .C ni seneg 741 slortnoc RriV-SriV .snegnirfrep .C fo RriV rotcaf ecneluriv eht htiw ytitnedi dica onima %43 a sah RR 58740SR_CTC ,RtetP htiw ton tub tnetP htiw detcaretni RR 58740SR_CTC tiebla ,devresbo saw noisserpxe tnet ni esaerced thgils a ylno , revewoH .noisserpxe Rtet ni esaerced dekram a dna TNeT ralullecartxe fo tnuoma eht ni esaerced thgils a detibihxe ,detagrat saw RR 58740SR_CTC hcihw ni ,0131p/556NC niarts esnes-itna ehT.]52[sisehtnys TNoB fo lortnoc eht ni devlovni eb ot dnuof ton saw)sRR gnidnopserroc eht neewteb ytitnedi dica onima %37(5832_CLC/6832_CLC golomoh munilutob .C eht ,revewoH .munilutob .C gnidulcni ,aidirtsolc rehto ni sTCT detaler ot suogolomoh si 05750SR_CTC/54750SR_CTC .sisehtnys TNeT fo level latot eht gnitceffa tuohtiw TNeT ralullecartxe Desercni eht rof rof Elbisnopser Yekser Yaw 1131P/5566nc Noitacfidom ltlaw ltlec .]63[Muidem ralullecartxe eht by noititumucca tnet sisylou inatet .c tahk s. Eyton Noiterces Tnet Fo Msinahcem .C . llits era 05750SR_CTC/54750SR_CTC yb dellortnoc syawhtap yrotaluger ehT .llaw lairetcab eht fo ylbmessa eht gnidulcni syawhtap ciportoielp slortnoc ylbaborp SCT sihT .devresbo saw RtetP dna tnetP ot RR 05750SR_CTC fo gnidnib on ,51370SR_CTC RR eht ot tsartnoc ni ecnis rennam tceridni na ni ylbaborp , noitpircsnart Rtet dna tnet stibihni yletaredom 05750SR_CTC .ekatpu etahpsohp eht ni devlovni eb ot desoppus si ti dna ylimaf RpmO eht ot sgnoleb RR 05750SR_CTC ehT .level TNeT ralullecartxe desaercni na dewohs)1131p/556NC(niarts esnes-itna evitcepser eht ecnis ,noiterces TNeT no tceffe evitagen stbihxe osla 05750sr_ctc s ScT eht.]62[rennam tnednepni-tibob ,noitpircsnart rieht stibihni dna lauq on ,2053CCTA A opit munilutob .C ed a moc sodic¡Ãonima ed edaditnedi ed %18 ahlitrapmoc ydoc inatet .C .)5S arugiF seratnemelpus siairetam(yT ed oiem o moc o£Ã§Ãarapmoc me YGT me odazilaer ©Ã inatet .C ed ovitluc o odnauq roiam ©Ã sadnet ed o£Ã§Ãudorp A .esocilg alep adibini ©Ã o£Ãn inatet .C me sadnet ed esetnÃs a ,eliciffid .C moc etsartnoc mE .]74[oneg³Ãtap essen anixot ad aicnªÃluriv e esetnÃs ad ovitagen rodaluger mu ©Ã ydoC e ,]64[eliciffid .C me sanixot ed o£Ã§Ãudorp a memirper esocilg a omoc ,siev¡Ãzilobatem etnemadipar sotardiobrac sO .]54[RDCT rotomorp o moc odnigaretni ,eliciffid .C me anixot ad eneg od o£Ãsserpxe a etnemaretnidni emirper ydoC ,otnatne oN .]44,34,24,14,04[succocotpertS ed seic©Ãpse sair¡Ãv e senegotyconom airetsiL ,suerec sullicaB ,sicarhtna sullicaB omoc ,savitisop-marg sair©Ãtcab sartuo me aicnªÃluriv a e sanixot ed o£Ã§Ãudorp a avita m©Ãbmat ydoC .]02[PTG ed etnedneped arienam ed norepO atnoB-HNTN rotomorp oa o£Ã§Ãagil ad s©Ãvarta A/tnoB esetnÃs a etnemavitisop aluger ydoC ,A munilutob .C mE .]71[aicnªÃluriv e .omsirtet od o£Ã§Ãircsnart a ralumitse e adnet ad rotomorp oa ragil es arap uiv es 58740SR_CTC arobme ,adnet ad ,esetnÃs a etnemadaredom e o£Ã§Ãerces a etnemlapicnirp aludom SCT esse euq meregus sodatluser sossoN .inatet .C me savitagen e savitisop sair³Ãtaluger saiv ed axelpmoc eder amu me odivlovne ¡Ãtse m©Ãbmat inatet .C ed SCT 58740SR_CTC/08740SR_CTC O .]93[seneg sortuo ed o£Ã§Ãaluger an adasu ©Ã ,)ANR-RV(etnacifidoc o£Ãn ANR mu odniulcni ,air³Ãtaluger atacsac amu otnauqne ,sodalortnoc etnemaretid o£Ãs e rotomorp ues me RRIV oa o£Ã§Ãagil ed axiac amu mªÃtnoc ovla seneg sod snuglA .ovla seneg sues etnemavitagen uo raluger edop snegnirfrep .C ed RRIV-SRIV ametsis O .]83,73[aniseda-oneg¡Ãloc e 2ateb anixot a macifidoc euq seleuqa omoc ,oedÃmsalp moc adazilacol anixot ed seneg e anixot-afla e anisilognirfrep ,olpmexe rop ,macifidoc euq socim´Ãssomorc sodazilacol anixot ed seneg odniulcni a ,720 opit³Ãbir ed sapec sa omoc ,eliciffid .C ed sapec satrec mE .ocit©Ãneg odnuf od edneped euq ,sanixot ed esetnÃs a etnemetnerefid aludom A0OPS o ,eliciffid .C mE .556NC on lanoicnuf avatse osnes-itna A0OPS o euq odnacidni ,ocis¡Ãfib oiem on A0OPS/556NC sarutluc san sodatceted maref o£Ãn sorópse so e]25[.la te sillenA ed ocis¡Ãfib oiem on)lm/sorópse 01(lam sanepa sodalurópsE 556NC .essazitetnis o£Ãsnet asse arobme ,YGT me sorópse uomrof o£Ãn 556NC inateT .C .]15[A munilutob .C me sanixot ed o£Ã§Ãudorp e o£Ã§Ãalurópse ertne o£Ã§Ãalerroc somavresbo o£Ãn sam ,]32[munilutob .C me o£Ã§Ãalurópse a alortnoc A0OPS O .anixoT e rteT/RTOB ed esetnÃS ,A0opS ertne o£Ã§Ãaretni amu etsixe es lev¡Ãnoitseuq Ã .munilutob .C e inatet .C ed I opurg od anixotoruen ed seneg ed sotnemapurga so moc etsartnoc me ,RTET uo RTOB ed sogol³Ãmoh m©Ãtnoc o£Ãn e munilutob .C ed seneg ed retsulc o euq ratlasser elaV .]05[etnoB ed rotomorp oa o£Ã§Ãagil ad s©Ãvarta E munilutob .C me tnoB ed esetnÃs an ovitisop otcapmi ues moc etsartnoc me ,acarrab ad esetnÃs a ralortnoc arap adartnocne iof o£Ãn A0OPS a ,iuqA .]94[mucilytuboteca muidirtsolC omoc ,siatneibma sair©Ãtcab me o£Ã§Ãalurópse/esenªÃgotnevlos e esenªÃgodica ed saiv sa ertne acort an odivlovne ¡Ãtse A0OPS o ,olpmexe roP .]84[siatneibma seµÃ§Ãidnoc s Ã o£Ã§Ãatpada an sodivlovne so etnemlapicnirp ,seneg sortuo sosoremuN aluger m©Ãbmat ,o£Ã§Ãalurópse e sapatse sariemirp sa ralortnoc arap odacifitnedi etnemlaicini iof euq ertsem rodaluger mu ©Ã m©Ãbmat A0OPSA .socifÃcepse setneirtun ed edadilibinopsid ad o£Ã§Ãasnes ad s©Ãvarta acarrab ad esetnÃs a ralortnoc edop ale ,]91[emof Ã o£Ã§Ãatpada an latnemadnuf lepap mu ahnepmesed ydoC omoC .inatet .C me sanixot ed o£Ã§Ãudorp e omsilobatem ertne o£Ã§Ãaretni a adnia atropus ossI .adnet ad etneuqesbus esetnÃs Ã e acarrab ad adaromirpa o£Ã§Ãircsnart Ã e ,rteTP a o£Ãn sam ,TNETP a es- uogil inatet .C ed ydoC ,munilutob .C me edadivita aus Ã aigolana mE .]02[A/tnoB esetnÃs a etnemavitisop alortnoc negatively regulates production ofA and toxin B, while in other strains, Spo0A does not affect the production of toxins [53] .in C. difficile, the MFD protein is involved in the repair of excision and the elongation of the transcription of nucleotides. This protein has been identified as a positive regulator of toxin synthesis A and toxin B. MFD possibly prevents the inhibitory effect of Cody and CCPA (A carbon catabolite protein) by inhibiting its connection to the promoters of the toxin gene [24]. The MFD is kept in C. tetani. However, it did not have an impact on the synthesis of tents under the conditions tested. In contrast to C. difficile, the stem synthesis is not suppressed by rapidly fermentable carbohydrates [54]. Whenever numerous regulatory genes are kept in Clostridia, they have distinct pleiotropic effects in each species. Our data indicates that C. tetani maintains exclusive regulatory pathways to control the synthesis of tents, possibly as an adaptation to specific nutrients and/or environmental conditions. Regulatory components of the network. As some TCSs are involved in IP capture and/or metabolism, we test the influence of the PI. We observed that an ideal concentration of PI around 40 mM significantly increased the transcript of the tent and subsequent tent production. In C. perfringens, the concentrations of 20 to 50 mM of Pi increase the spulation and the production of enterotoxin of C. perfringens, whose gene expression depends on spulation. The PI induces the expression of Spo0A and subsequent spulation [55.56]. However, we do not observe the impact of Pi supplementation on the CN655 spur in the TGY medium. Pi also increased the production of toxins in Bacillus thuringiensis [57]. PI is a key element in bacterial metabolism; Notably, it is incorporated into the transcriptional regulatory proteins, and the IP homeostasis has a critical adaptive role in bacterial virulenceFor example, the availability of the PI controls motility, biofilm formation, colonization, virulence factors induction, such as phospholipases in aeruginous Pseudomonas, colonization factor and synthesis of toxins in vibrio cholerae, enteropatogenic and enterohemorrhia -escreichia coli (revision [58 years]. In Bacillus anthracis, Pi's hunger increases spore germination, the invasion of macrophages and the secretion of toxins [59]. It can be speculated that the availability of the PI can also facilitate the colonization of the wound by C. tetani and the production of in situ tent. The Pi mechanism in control of the transcript of the tent is not yet known. It is possibly mediated by TCs as CTC_RS05745/CTC_RS05750 and CTC_RS10030/CTC_RS10035, which are involved in phosphate pickup. In fact, the corresponding anti-sensus strains (CN655/P1311 and CN655/P1313) cultivated in TGY supplemented with PI (40 mM) showed slightly increased extracellular tent compared to the culture in TGY (additional materials Figure S6). However, these two TCSs may have pleiotropic effects and indirectly control the synthesis of tents, since they are homologous to Phop/Phor TCs, which was found to control various pathways of metabolism, such as homeostasis redox and the adaptation to acid pH in Mycobacterium, as well as the main and secondary pathways of metabolism under phosphate limitation [60,61]. High concentration CO_2 (70%) in the gas phase stimulates the expression of the BONT gene and the production of BONT, despite a reduced growth rate of strains of group II of C. botulinum, in which the BOTR regulatory gene is missing. The CO_2 action mode is still speculative: it should dissolve and increase the concentration of bicarbonate in the middle of TGY culture and subsequently to increase the carboxylation reactions [62]. It was found that increased concentrations of bicarbonate increase the production of toxins in C. difficile, possibly through o o ,seµÃ§Ãidnoc sasson mE .]36[anitoib ed etnedneped o£Ã§Ãalixobrac Tent's not increased in TGY TGY containing 100 mm Na_2CO_3 independently of pH, while no significant differences were observed with the supplementation of 50 mm Na_2CO_3. Carbonate involvement in carboxilation reactions is more probable responsibly by the regular effects on the tent so than the effect of carbonate cap. In conclusion, Tentani's tent is under the control of a complex network of regulatory elements, including TCSS and metabolism regulators, as tested by the anti-sense RNA system. However, it is necessary a more direct approach to the exclusion of putative target regulatory genes to confirm regular function over the tent of these genes. C. Tetani and C. Botulinum are related scalers that synthesize powerful neurotoxins regulated, and their genomes contain numerous TCS (30 in C. tetani and 39 in C. Botulinum A [25.35]). Interestingly, although most TCSS genes are homologists in both spories, C. tetani and C. Botulinum mainly use distinct SCS sets in the regularity of toxin samples. Among the nine RRs investigated in C. Tetani, only one system (CTC_RS07315 in C. tetani and the CBO0786 homolog in C. Botulinum) has a similar function in both samples, ie, serves as a regulator negative toxin sample [26]. Four other RRS modulate the tent (one acting as negative and three as positive regulators), while its homologous counterparts in C. Botulinum the no effect on toxin producing [25]. In addition, two TCSS, which positively control the production of bont in C. Botulinum, so homologs in C. tetani that do not impact on the tent. We suggest that C. Tetani has adapted to the somosis to try to nutritional and environmental requirements. In fact, C. tetani preferably uses spectable metabnlic paths [35]. Notably, ed ed socifÃcepse sotruc soedÃtpep e inatet .C arap socitÃrc sotartsbus o£Ãs sodic¡Ãonima e digestion is essential for the synthesis try [32.35]. here, we also found that pi and carbonate are additional nutritional requirements for the production of try. Thus, the synthesis of try is dependent on a complex network of regulation linked to the metabolism of c. tetani, in which carbohydrates and pi are important elements. a better understanding of the regulation of fin synthesis and the underlying environmental factors is necessary to optimize the production of toxins per c. tetani for the production of vaccines. Although tetanus is rare in the developed world, it is still an important cause of deaths in many underdeveloped countries, with 34,000 neonatal tetanus deaths estimated in 2015 [64]. tentative immunization is an efficient method of prevention of tetanus, and thus a 96% reduction of this disease has been achieved since 1988 [64]. the availability of a cheap and efficient tetanus vaccine is an important factor for the eradication of this disease. unraveling the regulation of the synthesis tries constitutes the basis for the construction of hyperproductive strains. the recombinant strains used in this study are presented in Table 2. escherichia coli strains were cultivated in the broth of Luria-Bertani (lb) and in the broth of c. tetani CN655 in the broth of tgy (ph 7.5) containing tripticase peptone (bd biosciences; 30 g/L), yeast extract (bacto yeast extract, bd biosciences; 20 g/L), glucose (5 g/h c. tetani was cultivated in tgy with different concentrations of inorganic phosphate (pi) by the addition of Na_2HPO_4, and/or with sodium carbonate na_2co_3 (merck, guyancourt, frança.) the growth kinetics of CN655 c. tetani strains in the middle tgy period culture complemented with erythromicin (5 µg/mL) were monitoredThe genes studied containing the region of the place of link to the ribosome (RBS) were amplified by PCR and inserted in the reverse orientation in Pat18, as already described in reference [14]. DNA segments for anti-sensus mRNA production were designed in the RR of three CTs, in the SHK gene of six CTs, as well as in three regulatory genes (Cody, Spo0A and MFD) (Table 2). The CN655 genome DNA PCR products contain a 3 TM NCOI site and a 5 € PSTI site and were cloned in PMRP306, a derivative of Pat19 containing the promoter of the iota toxin gene, the NCOI-PSTI cloning sites and the 3â TM part of the iota toxin gene [14]. The resultant anti-sense RNA plasmids were transformed into CN655 by electroporation. The recombinant plasmids were prepared in Escherichia coli Top10 strain (Dam+, Dcm+) and electroporated into C. tetani CN655, with an efficiency of 25â¢50 transformants per 1 Î1⁄4g DNA. Curiously, no transformer was obtained from plasmids prepared in E. coli BL21 (dam+, dcmâµ) or c2925 (dam , dcmâ'), indicating that C. tetani cn655 contains a functional dcmylase that harms the transformation of non-DCM-metilado DNA.at 8, 24, 32, 48, 56, 72, and 144 h. The cells were collected at 10000 rpm for 10 min to 4 ° C, and the overnadants corresponding to the extracellular toxin were filtered (0.22 °m) and stored at 20 ° C. For the intracellular toxin test, the granules were washed twice with distilled water and two osmotic lysms were stored with TGY containing 20 mg/ml Posses of microtitular plates (Nunc Maxisorp; Nunc, Roskilde, Denmark) were coated100 µl of equine anti-tetan serum (Sanofi) FA193727) in 0.05 M carbonate buffer pH 9.5 and incubated at 4 Â°ÂC overnight. Plates were washed 3 times with phosphate buffered saline (PBS)-Tween20 0.1% with an automatic plate washer (BioTek, Washer 120, BioTek France, Colmar, France). After blocking with 20 mg/ml BSA in carbonate buffer during 30 minutes under agitation, three washes were performed, and 100 ÂµÂl/well of serial two-fold dilutions in PBS-Tween20 0.1%-BSA 1% (PBS-T-BSA) of samples were added. The plates were incubated for 1 h at room temperature with shaking. Tetanus toxin (Sanofi Pasteur, Marcy l¢ÃÂÂEtoile, France) was used as standard. After three washes, the plates were incubated with 100 Â¼ÂL/well of rabbit anti-tetanus serum (1:6400; Sanofi Pasteur nÂ°Â 7078) for 1 h at room temperature, then with 100 ÂµÂl of goat anti-rabbit Ig peroxidase-linked (1:4000, 111-035-006, Jackson Immunoresearch) for 1 h at room temperature in PBS-T-BSA. For detection, 100 ÂµÂL of 1 mg/mL ortho-phenylene-diamine (OPD, Sigma) in citrate buffer (0.05 M, pH 4.5 containing 0.06% H_2O_2) was used. The color development was stopped after 8 min by adding 50 ÂµÂL 3 M HCl. The absorbance was read on a microplate reader (Biorad, model 680) at 490 and 655 nm. Total RNA from C. tetani strains were extracted at 8, 24, 32 and 48 h of growth. After centrifugation at 4000 rpm for 15 min at 4 Â°ÂC, the culture pellet was mechanically disrupted in the presence of silica beads (Lysing Matrix B, MP Biomedicals, Illkirch, France) and buffer RLT (MP Biomedicals) by shaking with a FastPrep apparatus (MP Biomedicals), and RNA total extracted using RNeasy mini kit (Qiagen, Courtaboeuf, France), according to the manufacturer¢ÃÂÂs instructions. The RNA preparations were stored at ¢ÃÂÂ80 Â°ÂC. A DNAse treatment with TURBO DNase (Ambion, Thermoscietific, Les Ulis, France was performed following the manufacturer¢ÃÂÂs instructions. The absence of DNA contamination on RNA extracts was checked by real-time PCR targeting targeting tent. Total RNA amount was monitored with NanoDrop ND-100 Spectrophotometer. cDNAs were then synthesized from 1 ÂµÂg of total RNA with random primers (pDN6 5 ÂµÂg/ÂµÂL, Roche, Meylan France) and RNase OUT¢ÃÂ¢Â Recombinant Ribonuclease Inhibitor (Invitrogen, ThermoFischer, Les Ulis, France) and with M-MLV Reverse Tanscriptase kit (Invitrogen), according to the manufacturer¢ÃÂÂs instructions. RT-PCR was performed in 25 ÂµÂl reaction volume containing 30 ng of cDNAs, 12.5 ÂµÂL of SYBR Green Supermix (Bio-Rad, 2ÃÂ; 1.25 U iTaq DNA polymerase, 0.4 mM each dNTP, 6 mM $MgCl_2$, 20 nM fluorescein, SYBR Green I) and 500 nM gene-specific primers (Table 3) in an iQ iCycler apparatus (Bio-Rad). The reaction was subjected to denaturation at 95 Â°ÂC for 3 min followed by 40 cycles of denaturation at 95 Â°ÂC for 10 s, annealing/elongation at 61.7 Â°ÂC for 30 s for rpoB and tent genes and 65.1 Â°ÂC for gyrA and tetR genes. Then, a dissociation stage of 65 to 95 Â°ÂC with a heating rate of 0.5 Â°ÂC per 10 s was performed to establish a melting curve to confirm the specificity of the RT-PCR reaction for each primer pair.The relative cDNA quantity of each sample was determined with the threshold cycle [ÃÂÂÂCT] method (Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2ÃÂÂÂCT method). cDNA quantity of the tent and tetR gene was normalized to the quantity of cDNA of the rpoB and gyrA gene.The genes ctc_p21, ctc_01979, ctc_1421, ctc_00935, codY and tetR were PCR-amplified from the genome of C. tetani CN655, with primers (Table 3), adding a BamHI site at the 5¢ÃÂÂend and an EcoRI at the 3¢ÃÂÂ end. PCR products were digested with appropriate restriction enzymes and the resulting products were cloned into a pCR2.1 vector and subcloned into pET28a (Novagen, Merck, Guyancourt, France) for the expression of N-terminal 6-histidine proteins. The resulting constructions were transformed in E. coli BL21DE3, according to the ? nworg erew senolc ,snietorp tnanibmocer fo noisserpxe ecudni ot ,ylfeirb for 30 min. The transferred DNAs were linked to membranes with UV light. The Biotin-Labered DNAs were detected with the Emsa Lightshift Chemiluminescent kit (Pierce, Thermofischer, Les Ulis, France) according to the manufacturer's specifications. The 18 h culture bactons were mixed V/V with a fixed solution containing 5% glutararaldean (GA) at 0.2 m phem pH 7.2 (60 mm tubes, 25 mm hepes, 10 mm EGTA, 2 mm mgcl2) and incubated to 1 h. The samples were washed twice in PBs before performing a high pressure (> 2000 bar) freezing in 1-hexadecan using a Bal-Tec HPM 010 (Leica). The replacement of the freezer was made with 2% OSO_4 and 0.5% uranil acetate in acetone followed by several steps: -90 ° C to 42 h, heated at © -30 ° C (5 ° C / h), incubation to 12 h, heated at © 0 ° C (10 ° C / h) and incubation to 1 h. Then the samples were washed with acetone on the ice and incubated with the increase in the low viscosity -embarrassed Momia kit (EMS Ref. 14300) / Mixture Deaceton (1: 4) to 3 h, 1: 1 at night, 3: 1 During the day, 9: 1 at night, Spurr resin for day and at night. The samples were placed on pure spurr -infiltrated flat tubes before polymerization at 60 ° C to 48 h. The sections (60-70 nm) were obtained from an ultramicrotome FC6/UC6 (Leica, Wetzlar, Germany), transferred in 200 formal and carbon-coated and carbon-covered copper grids (CF-200-Cu50, Microscopy Delta). The samples were stained with 4% Uranyl acetate and contacted with lead citrate. The images were recorded with Tecnai Spirit 120 kV (with a Mara Eagle 4K X 4K assembled at the bottom, Thermo Fisher Scientific, Waltham, MA, USA). For scanning electron microscopy (without), the dehydrated samples with ethanol were dried with CO_2, and then sprayed with a 20 nm gold paater with a Gatan Ion bundle coater and were examined with an electron microscrease field emission scan JEOL JSM 6700 F operating in 7 Kv. The images were acquired with the upper SE SE detectortsael ta fo MES Â±Ã seulav naem era ataD .91TAp/556NC ot derapmoc noisserpxe Rtet ni ecnereffid tnacifingis on dewohs 9031p/556NC dna 8031p/556NC)B(.91TAp/556NC ot derapmoc noisserpxe tnet ni ecnereffid tnacifingis on dewohs 3131p/ 556NC dna 2131p/556NC ,9031p/556NC ,8031p/556NC)A(.ecnereffid tnacifingis on gniwohs sniarts esnes-itna 556NC dna 81TAp/556NC ni Rtet)B(dna tnet)A(fo noisserpxE :4S erugiF .serutluc tnednepedni eerht tsael ta fo MES Â±Ã seulav naem era ataD .81TAp/556NC ot derapmoc slevel TNeT latot ni ecnereffid tnacifingis on dewohs 3131p/556NC dna 2131p/556NC ,1131p/556NC ,0131p/556NC ,9031p/556NC ,8031/556NC .ecnereffid tnacifingis on gniwohs sniarts esnes-itna 556NC dna)rotcev ytpme(81TAp/556NC yb decudorp)TNeT(nixot sunatet latoT :3S erugiF .serutluc tnednepedni eerht tsael ta fo MES Â±Ã seulav naem era ataD .81TAp/556NC ot derapmoc TNeT fo slevel ralullecartxe ni ecnerefffid tnacifingis on dewohs 0841p/556NC DNA 2741P/556NC, 3131p/556nc, 2131p/556nc, 9031 p/556NC ,8031p/556NC .ecnereffid tnacifingis on gniwohs sniarts esnes-itna 556NC dna)rotcev ytpme(81TAp/556NC yb decudorp)TNeT(nixot sunatet ralullecartxE :2S erugiF .serutluc tnednepedni eerht tsael ta fo MES Â±Ã seulav naem era ataD ,tw 556NC dna 81TAp/556NC htob ni ralimis erew noitcudorp TNeT latot dna scitenik htworg ,h 441 ot 27 morf esahp yranoitats eht nI .rehgih ylthgils saw)B(TNeT fo noitcudorp latot eht tub ,h 65 tsrif eht gnirud tw 556NC Fo Taht Naht Rewol YLTHGILS SAW 81TAP/556NC FO) A (Scitenik HTWORG .81TAP/556NC DNA TW 556NC NI NOITCUDORP) TNET (Nixot Sunatet DNA Scitenik HTWORG: 1S Erugif: 1S/823/2166-270. 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