Cloning and Sequencing of Some Genes Responsible for Porphyrin Biosynthesis from the Anaerobic Bacterium *Clostridium josui*

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The 6.2-kbp DNA fragment encoding the enzymes in the porphyrin synthesis pathway of a cellulolytic anaerobe, *Clostridium josui*, was cloned into *Escherichia coli* and sequenced. This fragment contained four *hem* genes, *hemA*, *hemC*, *hemD*, and *hemB*, in order, which were homologous to the corresponding genes from *E. coli* and *Bacillus subtilis*. A typical promoter sequence was found only upstream of *hemA*, suggesting that these four genes were under the control of this promoter as an operon. The *hemA* and *hemD* genes cloned from *C. josui* were able to complement the *hemA* and *hemD* mutations, respectively, of *E. coli*. The COOH-terminal region of *C. josui* HemA and the NH₂-terminal region of *C. josui* HemD were homologous to *E. coli* CysG (Met-1 to Leu-151) and to *E. coli* CysG (Asp-213 to Phe-454) and *Pseudomonas denitrificans* CobA, respectively. Furthermore, the cloned 6.2-kbp DNA fragment complemented *E. coli cysG* mutants. These results suggested that both *C. josui hemA* and *hemD* encode bifunctional enzymes.

Metal-chelating tetrapyrrole derivatives are contained in several essential components of most organisms, such as respiratory chain complexes, light-harvesting complexes, catalases, and peroxidases, and their biosynthesis routes have been studied in many organisms (3, 17, 24, 34, 47).

Recently, the genes involved in tetrapyrrole biosynthesis have been cloned by using *Escherichia coli* auxotrophs requiring some intermediates such as 5-aminolevulinic acid (ALA) and hemin for porphyrin synthesis from facultatively anaerobic bacteria such as *E. coli* (10, 11, 19, 23, 41, 50) and *Salmonella typhimurium* (12, 13) and from strict aerobes such as *Bacillus subtilis* (20, 36). Nothing is known, however, about the genes involved in porphyrin biosynthesis from strictly anaerobic bacteria. We have attempted to isolate interesting clones by a



FIG. 1. Fluorescence of the transformants harboring pOR1 (1) and pBR322 (2). After overnight cultivation on an LB-ampicillin plate, cells were exposed to visible light (A) and UV light (B).

simple means: exposing *E. coli* transformants to long-wave UV light. Since porphyrins are excited by light of approximately 400 nm to exhibit pink fluorescence, organisms which overproduce porphyrins exhibit such fluorescence.

In this paper, we describe the cloning and nucleotide se-

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FIG. 2. Physical map of pOR1. Shaded and open bars show the cloned fragment. The shaded region was sequenced on both strands. Open arrows (ORF1 to ORF5) show the localization of each gene and the orientation of coding sequences. The regions encoding ORF1 and ORF5 are shown as rectangles with ragged left and right sides, respectively. The genes encoding homologous enzymes are indicated in parentheses. The symbol between ORF1 and ORF2 indicates the presence of a palindromic structure. pOR101 carries a 2.3-kbp *Pst1-Pst1* fragment at the *Pst1* site in pUC119. pOR105 carries a 3.4-kbp *Xba1-Pst1* fragment at the *Xba1-Pst1* site in pUC118. Arrows indicate the direction of *lacZ'* transcription. B, *BamH1*; D, *Dra1*; E, *Eco*R1; Ev, *Eco*RV; H, *Hind*III; P, *Pst1*; Sa, *Sau3*AI; SI, *Sal1*; X, *Xba1*.

quence of the gene cluster responsible for porphyrin biosynthesis in a cellulolytic anaerobe, *Clostridium josui* (14, 15, 49).

C. josui FERM P-9684 (49), isolated from compost in Thailand, was cultivated in GS medium (16) with cellobiose as the

1 GATCAGGCTTCCATTGAAGCATGGTACTGTAATTAAACCGTAACGATAAGGGAATAGAAGATACTACTATATGG <ORF1>D Q A S I E A W Y S E L N R N D K G I E D T T I W 76 CAAAATATAGAAATACCTTCCTATTGGGAAGACGAGGGCTTGGGCAACTTTAACGGTGTTGTCTGGTTCAGAAAG Q N I E I P S Y W E D E G L G N F N G V V W F R H 226 ACCTATATCAATGGTGTAGAGGTAGGAACAACGCCAAATCAGTATTATCTCTTGAAAATATAGTGTTCAGGAGGGC T Y I N G V E V G T T P N Q Y I S R K Y S V Q E G L L K E G K N T I L L R V I N I S G K G G F Y K G 451 ACCGCCCCTATGCCCGGCCCGGCCCTTGTTCAGTGGCGTCCCCTAGGATGTACAACGGAATGATGCCCCGCTTGTTCAGTGGCGGCCCCTTAGGATGTACAACGGAATGATGCCCCTGTT S G P M P G P A F V Q W R P L G L Y N G M I A P V 526 ACAAGTTATGCGATAAAAGGCTTTATATGGTATCAAGGGGAAGCTAATACCAAGAATCCTGTAGGGTATGAAAAT SYAIKGFIWYQGEANTKNPVGYE 601 CTCCTAAAGGCATTGATTTCAGACTGGAGACAGAAATGGGGAAATCTGCCGTTTTTGTATGTTCAATTG L L K A L I S D W R Q K W G M G N L P F L Y V Q L 676 CCGAATTTCATGGAGGCCTCAGAAATACCTGTGGAAAGCAAGTGGGCAGAGTTGAGGGAAGCCCAGCGAAAGACA P N F M E A S E I P V E S K W A E L R E A Q R K T LSVPNTGMIVTIDLGEWNDIHPSNK 901 CCAATGTATATATTATCCTTTACTGATACTGGAAGTGGGTTGATTGTTAACAGTGGAGAACGGCCGGGAGCTTTT YILSFTDTGSGLIVNSGERPGAF 976 GCTATATCAGGGCCAGATAGAATATTTGTTCGTGCTGATACAGAACTTATAGGAAATGATGTGGCAGTCTGGAGT A I S G P D R I F V R A D T E L I G N D V A V W S 1051 GAAAAGATAGCTCACCCGGCTTACGTTAGGTATGCATGGCGCGATAATCCACAGATGCAAACCTTTATAACCGTG E K I A H P A Y V R Y A W A D N P Q M Q T F I T V 1276 TGAAAAGTAATGAGCAGTTTACAGTAAATTTA<u>TGGGCA</u>GTAGATGTAGGCTGCGG<u>TATAAT</u>GAATATTGATATGA 1351 TCCTARATGCAGCTGGGACGGCATARUAGCGGCAGAING <ORF2> M Q L G R H N S G S I K K R L . EcoRV . ECGRV 1426 GATGTATATTTGASTATTATATCAGCAAGTTAGACATATAAACGGCGAAGGATTCAG 17 M Y I L S I I S A S L D Y K S A A I D I R E R F S 1501 CTATACATCCACAAGAATCAGAGAAATACTTAGAAGGATAAAAGGGGCTGAGGGGTGTTTCAGGAGGTGTACTCCT 42 Y T S T R I R E I L R R I K A A D G V S G A V L L sole carbon source, and its chromosomal DNA was isolated by the method of Saito and Miura (38). C. josui DNA was partially digested with Sau3AI, and 4- to 10-kbp fragments were fractionated by agarose gel electrophoresis. The C. josui gene bank was constructed by ligating the Sau3AI fragments with the vector pBR322 (39), which had been digested with BamHI and dephosphorylated with bacterial alkaline phosphatase by using T4 DNA ligase. E. coli HB101 (39) was transformed with the chimera plasmids, plated onto Luria-Bertani (LB) agar medium containing ampicillin (100 µg/ml), and kept at 37°C overnight. One transformant fluorescing pink on UV irradiation at 375 nm was isolated (Fig. 1). It harbored a plasmid designated pOR1 with the 6.2-kbp Sau3AI fragment of C. josui at the BamHI site in pBR322. The restriction map of the cloned fragment is shown in Fig. 2. Subcloning was performed with E. coli JM103 (58) and XL1-Blue (Stratagene, La Jolla, Calif.) as hosts and plasmids pUC118 and pUC119 as vectors. In Southern hybridization analysis, the 4.6-kbp XbaI-DraI fragment hybridized with the XbaI-and-DraI digest of chromosomal DNA of C. josui at the position corresponding to kbp 4.6 (data not shown), indicating that the cloned fragment originated from C. josui chromosomal DNA without any rearrangement.

FIG. 3. Nucleotide and deduced amino acid sequences of the 6.0-kbp fragment of the *C. josui* chromosome. The underlined nucleotide sequences marked -35 and -10 refer to the sites for recognition and binding of RNA polymerase. SD indicates a possible ribosome-binding site. The stop codons are indicated by three asterisks. Palindromic sequences between ORF1 and ORF2 are shown by horizontal arrows.

¹⁵⁷⁶ GTGTACCTGCAACAGAACCGAACTTTATATTTCGGGAGATAATATTGAAAATATGAATCCTGCCCTGCTTTTG 67 C T C N R T E L Y I S G D N I E N M N P A L L L C 1651 CCAGTTGTCAGGTGAGGAAGACCATAAATCCTTAATGACTTTATTAGTATAAGACATGATTCAGAGGCAATATT Q L S G E E D H K S L M T L F S I R H D S E A I 1726 CCATCTGATGGAAGTAGCTTGCGGGCTTCAGTCTATGGTACTTTTTGAGGATCGAGTAATAACACAGGTAAAAAA 117 H L M E V A C G L Q S M V L F E D R V I T Q V K N 1801 TGCGCTGCTATTTCCCCGAGAGGAGAAACCATTGATCAACATTGGAGACACTGTTTTGGACTGTGTATTACAGC 142 A A A I S R E E X T I D S T L E T L F R L C I T A 1876 TGCCAAGAAAGCCAAAAACCGAAATTAAGGTAAAGGCAGTCCCTACTTCGGCAGCAGAAAGAGCAATAACGGAATT $\begin{array}{c} \textbf{Bcorr}\\ \textbf{1951} \\ \textbf{1951} \\ \textbf{ATCAMAAAAGTATTGTTTTACTGATAAAA<u>GAATCT</u>TGTAATCGGTAATGGCGAAATAGGACGGCTGTGTTGCAA$ $192 S K K Y C F T D K R I L V I G N G E I G R L C C K \\ \end{array}$ 2026 AAAACTTATTGAACTGGGGGGGGGAAATAACAATGACAACGACGAAAGTATAACATGGGGGAAATAATTATTCCTTT 217 K L I E L G A E I T I T L R K Y K H G E I I I P V 2101 AGGCTGCAATACAATTCCCTATGACGAAAGAGAAGAGGGTTCTTCCTCTTTCAGATGTGGTTATAAGTGCAACTAC G C N T I P Y D E R E E V L P L S D V V I S A DYSVLNQKEVSKIREIINHFILQFE 2401 AAAAATGGAAAGATTATCGTGAAGAAGCAGCATTTACAAAAATTCCCGATTTACATAATGATACTCTATATGGAAG KWKDYREEAAFTKIPDLHNDTLYGH 2551 AAAAACACTACTGAGATTCGGGGCAGATATTTATCTGGTAGCTCCACATCTTACATCGGAGCTTCAAGAAATGTT K T L L R F G A D I Y L V A P H L T S E L Q E M 2626 GAATTGTAAGCTGATTAATTACAGAGAAGGATATTATGAATCACAGGATATTCAAAATATGTTTCTGGTGATTGC N C K L I N Y R E G Y Y E S Q D I Q N M F L V I A 2701 TECTACAAATGATAGAGAGAGAAACCATAAGGTATATCTGGACGCCAAGGAAAAGGGCATACAAATGAGTATAGC 442 A T N D R E T N H K V Y L D A X E K G I Q M S I A 2776 GGATTGCAGAGAAGAGTGTAGCTTTTACTTTCCTGCAATATTCGAATTTGATGGTATAGTCGGTGGGCTGGTTTC D C R E E C S F Y F P A I F E F D G I V G G L V S 2851 CCAAAATGGAGATAATCACAGTCTTGTAAAATCTGTTGCTGAACAGATTGGAAAAATTGGACAGGCTACAGATTG 492 Q N G D N H S L V K S V A E Q I R K I G Q A T D ** 2926 AGAATTTTATCATTTGAAGGAAAGCTATGGTATTTGACATGAAAAAAATCAGAATAGGCAGGAGCAGGGACAGCAAG $1 \star $SD < crF3 > M \ V \ F \ D \ M \ K \ K \ I \ R \ I \ G \ S \ R \ D \ S \ K \ Hindiii$ $\begin{array}{c} \underline{ring} \\ \underline{ring}$ 3001 3076 ACAATGAAAACTACAGGAGATAAGATTTTGGACAAAACTCTTGACAAAATAGAAGGAAAGGGACTTTTCGTCAAG 42 T M K T T G D K I L D K T L D K I E G K G L F V K

The DNA sequence of the 6.0-kbp *Sau*3AI-*Pst*I fragment of pOR1 from *C. josui* was determined by the dideoxy-chain termination method (40) by using single-stranded DNA templates and a Sequenase DNA sequencing kit (United States Biochemical Co., Cleveland, Ohio) according to the supplier's protocol (Fig. 3). The deletion-bearing plasmids for DNA sequencing determination were constructed by exonuclease III and mung bean nuclease digestion as described by Henikoff (22), with some modifications, and single-stranded DNAs were prepared by infecting *E. coli* MV1184 harboring pUC118 or pUC119 derivatives with M13KO7 (53). Sequence data were analyzed by using the program GENETYX-MAC, version 5.0 (Software Development Co., Ltd., Tokyo, Japan).

As a result, five open reading frames (ORFs) (ORF1 to ORF5) were found in the 6.0-kbp fragment (Fig. 3). ORF1 (Fig. 2 and 3), encoding 389 amino acid residues, was incomplete, i.e., the initiation codon was not contained in this fragment. Immediately downstream of ORF1, two palindromic structures, which were followed by a putative promoter sequence and four ORFs (ORF2 to ORF5) of 1,545, 885, 1,512, and 616 bp, were detected. ORF5, the last ORF, did not contain any stop codon (Fig. 2 and 3) in the 6.0-kb fragment, indicating that ORF5 was also incomplete. Each ORF was preceded by a typical ribosome-binding site upstream of its ATG initiation codon. Only one putative promoter sequence,

3151 GAACTGGATAATGCATTATACAATAATGAGGTGGATATAACCGTACATAGTTATAAGGATATGCCTTTGGAAGAA 67 E L D N A L Y N N E V D I T V H S Y K D M P L E E 3226 AATCCGGAGGTGCCTGTTGTAGGCCTGTAAAAGCGTGAAGACCCTAGAGATGCCTTATTTTGCCTCAAAATGGT 92 N P E L P V V A L S K R E D P R D A F I L P Q N G 3301 GAAAATGGTGAACCAATAGGAAGTCCAAGTCCAAGACGACGCCCCAGCTGAAGAAGAATTATTCCCGGGTCTAAG 117 E N G E P I G S S S L R R Q L Q L K E L F P G C K 3376 ACTGCTCCTATCCGGGGAAATGTACAAACCCGACTTAAAAAACTTGACAGCGGTGAGTTTCCGCCATAGTACTT 142 T A P I R G N V Q T R L K K L D S G E F S A I V L 3451 GCTGCTGCTGGAATTAAAAGGCTTGGTCTTGAAAGTCGTATCGGCAGATATTTTTCAGTTGATGAAATTCTTCCT A A A G I K R L G L E S R I G R Y F S V D E I L P 3526 GCGCCARGTCAGGGCATTATAGCGGTACAGGGCAGAGCGCGGGGAGAACTTTGATTTTCTAAAGCTGTTTCATAGT 192 A A S Q G I I A V Q G R V G E N F D F L K L F H S .psti 3676 GCGGCTTATGCAACCATTCAGGGAAGTGAAATAATTCTAAAGGGCTTATACTGCAATGAAACCACAGGAGAGTTA 3901 TRAGGGGCTTATTACARTRAGGGGAGCAGARCTGCTCTCACAGGCTGATGTGGTAGTTTATGACAGACTGGTATC K G L I T I R G A E L L S Q A D V V V Y D R L V S 3976 CCAAGAAATTATTAAAATGATTCCAACTACGGCAGAAAAGAATAAATGTGGGAAAAGAAGAATAAATTCCACCTCTT 41 $\,$ Q E I I K M I P T T A E K I D V G K E N K F H P V 4051 TAAACAGGAAGAAATTAACCATATACTCCTAGAAAAGTCATTAGAAGGAAAGAAGGATATAAGGCTTAAAGGAGG KQEEINHILLEKSLEGKKVIRLKGG D P F V F G R G G E E L E L L Y E N N I P F E V V 4201 TCCCGGAGTAACTTCGGCAGTGGCAGCATTATGCTACGGGGGAATACCGGCGTACCCACAGGGATTTTGGCTCTTC 116 P G V T S A V A A L C Y G G I P A T H R D F C S S L H I I T G H A R E G G Q L S I P F H E L K E L N 4351 TGGAACCATTGTTTTTCTTATGGAGATTCTTCACTATCATATAAATGGGCTTATAAATGGGGGATGGA 166 G T I V F L M G D S S L S Y L M N G L I N A G M E 4501 ACTGGAGCAAAAGGCTTTGGAGATGGAAATAAAATCTCCTGCCATTATTGCTGTAGGTAAGGTCTGTTCTCTATC L E Q K A L E M E I K S P A I I A V G K V C S 4576 TGAAAAGTTCAGTTGGTTATGAAAAAGCCTCTTTTGGGTACAAAAATCTAGTTCAAGACCCAAAGAATCTTC 241 E K F S W F M K K P L F G T K I L V T R P K E S S Hindlii 4651 TGGCACACTTGTAGAAAAGCTTCGCCAACTGGGTGCGGGAGCCTGTAGAGTATCCCTGTATAGAGGTAGTACCTAT 266 G T L V E K L R Q L G A E P V E Y P C I E V V P I

¹³⁰⁸TGGGCA¹³¹³ as the -35 region (consensus for *E. coli*, TTGACA) and ¹³³¹TATAAT¹³³⁶ as the -10 region (consensus for *E. coli*, TATAAT), was found with the consensus distance of 17 bp upstream of ORF2, and no other promoter sequence was identified in the nucleotide sequence, suggesting that ORF2 to ORF5 are transcribed from this promoter in a polycistronic mRNA, i.e., the genes form an operon.

Amino acid sequences deduced from ORF2, ORF3, ORF4, and ORF5 were homologous to those of HemA, HemC, HemD, and HemB, respectively, of *E. coli* and *B. subtilis*, as described below (Fig. 2).

The NH₂-terminal region of the ORF2 protein (Leu-20 to Trp-343) was highly homologous to HemA proteins of *B. subtilis* (identity, 29%) (36), *E. coli* (33%) (10, 29, 52), and *S. typhimurium* (31%) (12) (Fig. 4A), which synthesize ALA via the C₅ pathway, but not homologous to HemA proteins of *Rhizobium meliloti* (27), *Agrobacterium radiobacter* (9), *Bradyrhizobium japonicum* (32), *Saccharomyces cerevisiae* (51), chickens (7), rats (48), mice (43), and humans (4), which synthesize ALA via the C₄ pathway. The plasmid pOR105 (Fig. 2), containing ORF2, complemented *E. coli hemA* mutants AN344 (provided by Y. Murooka) and SASX41B (provided by B. Bachmann; CGSC4806). These results indicate that ORF2 encodes HemA protein, NAD(P)H-dependent glutamyl-tRNA reductase (20), which is involved in ALA synthesis via the C₅

⁴⁷²⁶ ACCACAAAATGAAAAGCTCTACCATGCATGTGAAAATATCAGGGAATATGGCTGGATTTTGCTTACCAGTAAAAA 291 P Q N E K L Y H A C E N I R E Y G W I L L T S K N 4801 CGGTATACAGATATTTTTTGATTACTTAAATTCCAAAGGATTAGATGCAAGAGTTCTTGCAAATACAAAAATCGG GIQIFFDYLNSKGLDARVLANTKIG HindIII Drai $\begin{array}{c} \text{Algebra} \\ \text{A876 CACGGTAGGAAGTCAGACAGCGAAAGCCTTTAAAAGAAGTGGGACTGATTTCTGATTCCACCCCTGAAATCTTTGA 341 T V G S Q T A K A L K E V G L I S D F T P E I F D \\ \end{array}$ 5026 AGCAAGTGACGATATTGTTAACATTTTGAGAAGTAATAATATTAAATTTGATAGAGTTCCATTGTACAATACGAA A S D D I V N I L R S N N I K F D R V P L Y N T N 5101 TTATATAAATGAAAACAGTAATAAGGTCAAAAAATCGATTGTCCATGGTGAACTCAAGTACATAACCTTTACAAG 416 YINENSNKVKKSIVHGELKYITFTS 5176 TGCATCCACAGTTGAAGGATTTATAGCATCTATGAAAGACATTCCTTTGGAAAGTCTGACGGCTGTCTGCATTGG 441 A S T V E G F I A S M K D I P L E S L T A V C I G 5251 AAACAAGACAGCGGAAGCTGCTAAAAAGTATAACCTGAGATATGTAGTAGTGAAAAGTCAACAATTGATTCCAT N K T A E A A K K Y N L R Y V V A E K S T I D 5326 GATAGATAAGCTATTAGAAAT<u>AGGAGG</u>CGGCAATATTTATGATTAAGAGACCAAGAAGATTGAGAACCAATGAAG IDKLLEIGGGNIYD*** SD <0RF5>MIKRPRRLRTNE 491 5401 TOCTGCGAAAAGCAGTCAGGGAAACCAGACTATCAACGGATTCACTTATTTGGCCTCTTTTTATAGTAGAGGGTA 13 V L R K A V R B T R L S T D S L I W P L F I V E G 5476 AAAATATAAAAAAAGGAAATCAGCTCCCCTCCCCGGACAGTATCATTTAGTCCGGATATGGTTGGGAAAGCAATTG 38 K N I K K E I S S L P G Q Y H F S P D M V G K A I 5551 AAGCTGCATTAAAAGCAGATGTAAAATCCGTATTGCTATTTGGACTTCCTAAGCATAAGGATGAAAAAGGCTCTG 63 E A A L K A D V K S V L L F G L P K H K D E K G S 5701 TAACAGATATITGCATGTGCGAATACACATCTCCGCGCATTGCGGAATTTTGGAGGGGGGAAAGGGTTGATAATG 113 I T D I C M C E Y T S H G H C G I L E G E R V D N 5776 ACAGAACCCTTCCCTATCTGGAAAAAATAGCTTTGTCCCATGTAATGGCAGGAGCGGACATGATTGCTCCGTCGG 138 D R T L P Y L E K I A L S H V M A G A D M I A P S 5851 ATATGATGGATGGTAGGATATATGGTCTTAGGTCAACTCTGGACAAAACGGGTTTACAGACATTCCTATTATGT 163 D M M D G R I Y A L R S T L D K N G F T D I P I M PotI 5926 CATATGCCGTAAAAATATGCATCGTCATTTTATGGACCATTCCGTGAGGC<u>CTGCAG</u> 188 S Y A V K Y A S S F Y G P F R E A A

А

C. josui	HemA 1 MQLGRHNSGSIKKRLEMYILSIISASLDYKSAATDIRERFSYTSTRIREIIRRIKAA
B. subtil.	is HemA 1 MHILVVGVDYKSAPIEIREKVSFQPNELAEAMVQLKEE
E. coli	HemA 1 MTLLALGINHKTAPVSLRERVSFSPDKLDQALDSLLAQ
S. typhim	irium HemA 1 MTLLALGINHKTAPVSLRERVTFSPDTLDQALDSLLAQ
C.j 58	DGVSGAVLLCTCNRTELYISGDNIENMNPALLLCQLSGEEDHKSLMTLFSIRHDSEAIFHLMEVACGL
B.s 39	KSILENIIVSTCNRTEIYAVVDQLHTGRYYIKKFLADWFQLSKEELSPFLTFYESDA-AVEHLFRVACGL
E.C 39	PMVQGGVVLSTCNRTELYLSVEEQDNLQEALIRWLCDYHNLNEEDLRKSLYW-HQDNDAVSHLMRVASGL
S.t 39	PMVQGGVVLSTCNRTELYLSVEEQDNLQEALIRWLCDYHNLNEDDLRNSLYW-HQDNDAVSHLMRVASGL
C ÷ 126	OF MUT PEDDUT MOUPHIES AT CORPUTE CONTRACT ON ANY AVAILABLE
E g 109	CONVERSION OF CONTRACTOR CONTRACTOR OF CONTRACTOR CONTR
E.G 108	DELUCERDIL COVERAFADEORCEMENTER FOR FEVERENTER FOI CACAVEVERAFADE C
S ± 108	DELVICEDOTLCOVER FEDEOROR CHINA SAT DEMEOR SESURE DEPENDICA SAVSVAFAACI-D-ARQ
0.0 100	DELAGET VIIG VARATAGE KOIMAGAETOTI VARAVAISID TORSAVSVATAROS-1-ARU
C.j 196	CFTDKRILVIGNGEIGRECCKKLIELGAEI-TITLRKYKHGEIIIP-VGCNTIPYDEREEVLPLSDV
B.s 176	IFGNLSSKHILILGAGKMGELAAENLHGOGIGKVTVINRTYLKAKELADRFSGEARSLNOLESALAEADI
E.C 176	IFESLSTVTVLLVGAGETIELVARHLREHKVOKMITANRTRERAOILADEVGAEVIALSDIDEREREADI
S.t 176	IFESLSTVTVLLVGAGETIELVARHLREHKVOKMIIANRTRERAOALADEVGAEVISLSDIDARLODADI
C.j 261	VISATTSPHFTITYDMIEKLER-KPEIFVDLALPRDIESSISNFTGVELYNLDRFYTDYS-VLNQ-K
B.s 246	LISSTGASEFVVSKEMMENANKLRKGRPLFMVDIAVPRDLDPALNDLEGVFLVDIDDLEGIVEANMKERR
E.C 246	11SSTASPLPIIGKGMVERALKSRRNQPMLLVDIAVPRDVEPEVGKLANAYLYSVDDLQSIISHNLAQRK
S.t 246	IISSTASPLPIIGKGMVERALKSRRNQPMLLVDIAVPRDVEPEVGKLANAYLYSVDDLQSIISHNLAQRQ
	01 - 01 0000 W W W.
C.j 325	EVSKIRE-IINHFILQFEKWKDYREEAAFTKIPDLHNDTLY
B.S 316	BTAEKVELLIEETIVERRQWMNTLGVVPVISALREKALAIQ
E.C 316	AAAVEAETIVAQETSEFMAWLRAQSASETIREYRSQAEQVR
S.T 310	ARAVEABTIVEQEASEIMAWLRAQGASETIREYRSQSEQIR
-	
В	
C. josui	Hem A 361 DTLYCREPTETDLSGKKULUUGGGETATERUKTLIDEGADTYLUADULTSELOEMI
E. coli	
5. 5011	0/00 I HEREE SECTORE SOUTHER FRANKLEND AGAIN I FUT IAWA

417 NCKLINYREGYYESODIONMFLVIAATNDRETNHKVYLDAKEKGIOMSIADCREECSFYFPAIFEFDGIV c.i 54 DAGMLTLVEGPFDESLLDTCWLAIAATDDDALNQRVRQAAEARRTFCNVVDAPKAASFIMPSTI

C.j 487 GGLVSQNGDNHSLVKSVAEQIRKIGQATD

124 VA-VSSGGTSPVLARLLREKLESLLPLHL..... E.c

FIG. 4. Homology analysis of the predicted amino acid sequence from ORF2 (hemA) from C. josui. (A) Alignment of the predicted amino acid sequences in the NH_2 -terminal region in HemA of *C. josui* (C.j) and NAD(P)H-dependent glutamyl-tRNA reductases (HemA) of *B. subtilis* (B.s), *E. coli* (E.c), and *S. syphimurium* (S.t). (B) Alignment of the predicted amino acid sequences in the COOH-terminal region in HemA of *C. josui* (C.j) and in the NH₂-terminal region of CysG of *E. coli* (E.c). The putative NADP⁺-binding site is underlined. Shaded residues represent amino acids which are identical to those in C. josui HemA

pathway. ORF2 was termed hemA. In addition to having similarity with other HemA proteins, C. josui HemA had similarity (23%) in its COOH-terminal region (Asp-361 to Asp-515) with the NH₂-terminal region of CysG protein of E. coli (35, 55, 56) (Fig. 4B). Recently, M. J. Warren et al. have reported that the NH₂ terminus of E. coli CysG was involved in the dehydrogenation of dihydrosirohydrochlorin (precorrin-2) and ferrochelation, which convert precorrin-2 into siroheme (54). The NADP⁺-binding site (21, 45) identified as Asp-14 to Asn-41 in E. coli CysG (54) was conserved in C. josui HemA as Lys-377 to Val-404 (Fig. 4B). These results suggest that the hemA gene of C. josui is responsible for two different steps in porphyrin biosynthesis, i.e., the synthesis of ALA from glutamate and siroheme from precorrin-2.

The amino acid sequence predicted from ORF3 displayed a high degree of homology with sequences of porphobilinogen deaminases (PBG-Ds) (hydroxymethylbilane synthase [HMB-S]; EC 4.3.1.8) which are encoded by the *hemC* genes of B. subtilis (36), E. coli (1, 50), humans (37), S. cerevisiae (25), and Euglena gracilis (46) (Fig. 5). An extract from E. coli BL21(DE3) cells (Novagen, Madison, Wis.) harboring pER1 (constructed by inserting PCR products containing the hemC region into pET-16b vector purchased from Novagen) had PBG-D activity (data not shown). These results indicate that ORF3 corresponds to the hemC gene. The cysteine residue in the dipyrromethane cofactor-binding site which was identified as Cys-242 in E. coli PBG-D (44) was conserved in C. josui HemC as Cys-237 and is present in all other PBG-Ds reported so far (Fig. 5). One Asp and six Arg residues which were identified as catalytic sites for tetrapyrrole synthesis in PBG-D from E. coli (30) are conserved in C. josui HemC as Asp-86, Arg-13, Arg-128, Arg-129, Arg-146, Arg-152, and Arg-173 and are also conserved in the other PBG-Ds (Fig. 5).

ORF4 encodes a polypeptide of 504 amino acids, and its COOH-terminal region downstream of Met-247 revealed 24% identity with the HemD protein, uroporphyrinogen III (UroIII) synthase (EC 4.2.1.75), from B. subtilis (20) (Fig. 6A). pOR101 (Fig. 2) complemented E. coli hemD mutant SASZ31 (provided by B. Bachmann; CGSC7153). On the basis of these results, ORF4 was identified as hemD. The NH₂-terminal region (Met-1 to Phe-246) of C. josui HemD revealed 49 and 39% identities with the COOH-terminal region (Asp-213 to Phe-454) of the E. coli CysG protein (35, 55, 56) and with the whole of the Pseudomonas denitrificans CobA protein (8) (Fig. 6B), respectively. Both proteins are S-adenosylmethionine-dependent UroIII methylases. Therefore, HemD of C. josui might catalyze sequential reactions to synthesize UroIII from HMB and then precorrin-2, which are intermediate compounds in both vitamin B_{12} and siroheme biosyntheses.

C. jos B. sub E. col human yeast	ui tili i	HemC HemC HemC HEM3	1 1 1 1	MVFDMKK MMRT MLDNV MRV MGPET	IRIGSRDSKL IKVGSRRSKI LRIATROSPL IRVGTRKSOL LHIGGRKSKL	AIIQSELIM AMTQTKWVI ALWQAHYVK ARIQTDSVV AVIQSNHVL	SAIRKYDPDII QKLKEINPSFJ DKLMASHPGLV ATLKASYPGLQ KLIEEKYPDYI	ELELITMKTTG AFEIEKIVTKG AVELVPMVTRG AVELVPMVTRG AFEIIAMSTTG ACKVFTLQTLG	DKILDK DRIVDV DVILDT DKILDT DQIQFK
E. gra	10111	s Henc	140	STIGSNIGAGET	VEVATERSPE	AMWQAEFIQ	SELERLWRGE.	IVELOPMSTRG	DKILDS
c.j	54	TLOKIEGI	(GLF)	KELDNALYN	-NEVDITVES	* YKOMPLEEN	PELPVVALSKI	EDPRDAFI	
B.s	51	TLSKVGGR	(GLF\	KEIEQALLN	-EEIDMAVHS	MKDMPAVLP	EGLVIGCIPE	REDPRDALISK	NRV
E.c	52	PLAKVGGE	GLF	KELEVALLE	-NRADIAVHS	MKDVPVEFP	QGLGLVTICE	REDFRDAFVSN	NYD
Hum	50	ALSKIGER	(SLF)	RELEHALEK	-NEVDLVVHS	LKOLPTVLP	PGFTIGAICK	RENPHDAVVFH	PKFVGK
Yea	52	ALYSFGGR	(ALW)	KELEDHLYHDDP	SKKLDLIVHS	LKOMPTLLP	EGFELGGITK	RVDPTDCLVMP	FYSAYK
E.g	198	PLAKVGGE	GLF	KELETALLE	-NRSDIAVHS	TKOVPMELP	EGLVLGVICK	RHDPCDAIVFP	KGSNLK
c	112	TOONCER	Renŝ	COORDED DOOT OF 7	PT NDCCTOND				* 57.07300
2.1	114	ATCHWKK-	GLFI	Gegenterending	TEDBOLTTEN	TECHTOMET		TTINNGIN	REGERO
E.C	115	SLDALPA-	.osti	GTSSIRROCOLA	ERREDLITES	LRCNVCTRL	SKLDNG_RVD	-TTLANAGUS	RIGHRO
Hum	116	TLETLPE-	-KSVI	GTSSURRAAOLO	REPRISERS	TRONTINTET	RELDEOORES	-TILATAGLO	RMGWHN
Yea	122	SLDDLPD-	GGIN	GTSSVRRSAOLK	RKYPHLEFES	VRGNIOTRE	OKLODPKSPY	OCTIDASAGLM	RMGLEN
E.g	264	SL-EDLPH	GAR	GTSSLEROCOLL	LKRPDLKFLE	LRGNVNTRL	AKLDSG-DYD	-TILAAAGLK	RLGFSD
		30					0.000273-06400	2 30 10000000000000	
									*
C.j	179	RIG-RY	rsv-	DEILPAASQGII	AVQGRVGEN-	-FDFLKLFH	SEESLCISLA	RTFVREMNGG	CSTPIA
B.s	181	DVVTH	FLEI	ERCLPAVGQGAL	AIECRESDEE:	LLALFSQFT	DEYTKRTVLA	RAFLNAMEGG	CQVPIA
E.C	182	RIRA	ALPI	EISLPAVGQGAV	GIECRLDDSR	FRELEAALN	HETALRVTA	RAMNTRLEGA	CONSIG
Bum	184	RVG()ILHI	EECMYAVGQGAL	GVEVRAKDQD	ILDLVGVLH	DPETLLRCIAL	RAFLRHLEGG	CSVPVA
Yea –	191	к1ТÇ	JRFHS	TDMYHAVGQGAL	GIEIRKGDTK	MMKILDEIC	DLNATICCLS	RALMRTLEGG	CSVPIG
E.g	331	RVLPGETN	IT T DE	'NVMCPAAGQGAL	SIELRTNDPE	IAALLEPLE	HIPDAVTVAC	RAMNRRLNGG	COVPIS

- C.j B.s 243 AYATIOGS-EIILKGLYCNETTGELRKECV-----SGNRNNPVELGYELVKKMESSEST
- 248 GYSVLNGQDEIEMTGLVASPDGKIIFKETV-----TGNDFEEVGKRCAALMADKGAKDLIDRVKRELD E.c 248 SYAELIDG-EIWIRGEVGAPDGSOII------BGERGAPODAEOMGTSLAFELINNGARETLAE
- 250 VHTAMK-DGQLYDTGGVWSLDGSDSIQETMQATIHVPAQHEDGPEDDPQLVGITARNIPRGPQLAAQNLG Hum
- Yea 257 VESKYN-EETKKILLKAIVVDVEGTEAVEDEIEMLIENVKEDSMACGKILAERMIADGAKKILDEINLDR
- E.g 401 GFAQLKDG-QLRMEARVGSVTGKGPLIIQSKTFRLPWSGRTWPQLQKESEALGVEVADMLLADGAQAYLD
- B.s 311 EDGK
- E.c 306 VYNGDAPA 319 ISLANLLLSKGAKTILDVARQLNDAH Hum
- 326 IK
- 470 EAYASRTLGWA E.q

FIG. 5. Alignment of the predicted amino acid sequence from ORF3 (hemC) of C. josui (C.j) and the amino acid sequences of PBG-Ds (HMB-S) (HemC) of B. subtilis (B.s), E. coli (E.c), humans (Hum), S. cerevisiae (Yea), and E. gracilis (E.g). Shaded residues represent amino acids which are identical to those in C. josui HemC. Conserved amino acids which are candidates for the catalytic sites discussed in a previous paper (30) are marked by asterisks.

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C. josui	HemD 247 MKKPLFGTKILVTRPKESSGTLVEKLRQLGAEPVEYPCTEVVPIPQNEKLYHA
B. subtilis	HemD 1 MENDFPLKGKTVLVTRNKAQAASFQQKVEALGGKAVLTSLITFRRALPNDVAEQV
C.j 300 C	NIREYGWILLTSKNGIQIFFDYLNSKGLDARVLANTKIGTVGSQTAKALKEVGLISDFTPEIFDGRHL
B.S 56 RE	DLAAPGWLVFTSVNGADFFFSYLKENQLILPAHKKIAAVGEKTARRLKMENVSVDVMPQEYIAEQL
c.j 370 ÅI	GIAERVGENEKVLICDAAIASDDIVNILRSNN-IKFDRVFLYNTNYINENSNKVKKSIVHGELKYITF
B.s 124 AD	alkqhaefgetitvmkGnlsrdvikqelvflg-fevkewvlysetipdebgiealkdaagqysfdyvtf
a - 100 m	
8.s 193 TS	ASTVECTASMEDIPLESTAVCIGRATABAARKINLRVVASKSTIDEMIDKLESIGGONID SSTVHTFMHVLGEELKKWKANGTACISIGPLINDALLTYGITSHTPDTFTDGMLKLMCSMSREEERI
В	
C. josui	HemD 1 MEHGFVALVGAGPGDKGLITIRGAELLSQADVVVYDRLVS
5. coli	CysG 213 DHRGEVVLVGAGPGDAGLLTLKGLQQIQQADVVVyDRLVS
P. denitrifi	cans Coba 1 MIDDLFAGLPALEKGSVWLVGAGPGDPGLLTLHAANALRQADVIVHDALVN
C.j 41 QE	IIKMIPTTAEKIDVGKENKPHPVKQEEINHILLEKSLEGKKVIRLKGGDPPVPGRGGEELELLYENNI
5.c 253 DD	IMNLVRRDADRVFVGKRAGYHCVPQEEINQILLREAQKGKRVVRLKGGDPFIFGRGGEELETLCNAGI
9.0 52 EL	CLELARPGAVLEFAGERGGEPSPROEDISLEDVELARAGNEVIELERGGDPFVFGRGGEEALTEVEHQV
.j 111 PF	evvpgvtsavaalcyggipathrofcsslhiitghareg-gqlsipphelkelngtivplmgdsslsy
323 FF	SVVPGITAASGCSAYSGIPLTHRDYAQSVRLITGHLKTG-GELDWENLAAEKQTLVFYMGLNQAAT
e.d 122 PE	RIVPGITAGIGGLAYAGIPVTHREVNHAVTFLTGHDSSGLVPDRINWQGIASGSPVTVMYMAMKHIGA
С.ј 180 цм	NGLINAGMEKDMPAAIVENGTRPNORKLVATVGTLEOKALEMEIKSPATIAVGKVCSLSEKFSWF
5.e 390 IQ	QKLIEHGMPGEMPVAIVENGTAVTQRVIDGTLTQLGELAQQMNSPSLIIIGRVVGLRDKLNWF
o al 100 T/	AND TAKEN DODDENIAN STATISTICS STATE FOR A DATE AND A D

FIG. 6. Homology analysis of the predicted amino acid sequence from ORF4 (*hemD*) from *C. josui*. (A) Alignment of the predicted amino acid sequences in the COOH-terminal region in HemD of *C. josui* (C.j) and UroIII synthase (HemD) of *B. subtilis* (B.s). (B) Alignment of the predicted amino acid sequences in the NH₂-terminal region in HemD of *C. josui* (C.j) and S-adenosylmethionine-dependent UroIII methylases (CysG and CobA) of *E. coli* (E.c) and *P. denitrificans* (P.d). Shaded residues represent amino acids which are identical to those in *C. josui* HemD.

The NH₂-terminal region of the amino acid sequence predicted from ORF5 (205 residues) showed a high degree of similarity with PBG synthases (ALA dehydratase) (EC 4.2.1.24) of *B. subtilis* (20), *E. coli* (11, 28), *S. cerevisiae* (31), humans (57), and rats (6) (Fig. 7), whereas the 6.0-kbp fragment sequenced in this study did not contain the region encoding the COOH-terminal moiety. The amino acid sequence of *C. josui* HemB contained a short motif (Cys-117 to Cys-127) similar to a zinc-binding domain, including two cysteines and two histidines in a zinc finger (5, 26), and this motif was highly conserved in all PBG synthases (Fig. 7). This incomplete gene, however, was not sufficient for complementing *E. coli hemB* mutant RP523 (provided by B. Bachmann; CGSC7199), probably because of the defectiveness of the *C. josui hemB* gene.

The arrangement of the gene cluster responsible for porphyrin biosynthesis in *C. josui* (Fig. 2 and 8) was similar to that of the gene cluster in *B. subtilis*, although a gene corresponding to *hemX* was not found between *hemA* and *hemC*. Homology analysis of HemAs suggested that in *C. josui*, ALA was possibly synthesized via the C₅ pathway, which was also found to be the case in *Clostridium thermoaceticum* (33). Therefore, some clostridia seem to use the C₅ pathway for ALA synthesis. The *hemL* gene, encoding glutamate-1-semialdehyde-2,1-aminotransferase (EC 5.4.3.8), which is involved in ALA synthesis via the C₅ pathway, was not included in the fragment cloned from *C. josui* in this study, although the *hemL* genes of several organisms, such as *S. typhimurium*, *E. coli*, *B. subtilis*, and plants, have been cloned and sequenced (13, 18, 19, 20). Since the *hemL* gene is located downstream of the *hemB* gene in *B*. *subtilis*, the *hemL* gene of *C. josui* might also occur downstream of *hemB* (ORF5).

In addition, HemA and HemD might be involved in the biosynthesis of vitamin B_{12} or siroheme (Fig. 8). We examined the vitamin B_{12} productivity of *C. josui* by performing a microbiological assay with vitamin B_{12} auxotrophic E. coli 215 (42) according to the method of the Association of Official Analytical Chemists (2). When C. josui was cultivated at 45°C for 4 days in 20 ml of GS medium (16) containing biotin (0.2 mg/ liter), p-aminobenzoic acid (0.4 mg/liter), and $CoCl_2 \cdot 6H_2O$ (20 mg/liter) instead of yeast extract, it accumulated 30 ng of CN-vitamin B_{12} in total. Furthermore, pOR1 (Fig. 2) was able to complement E. coli cysG mutants AT718 and AT2455 (provided by A. Nishimura; ME5358 and ME5461). Homology analysis and complementation experiments indicated that the HemA and HemD proteins of C. josui each contained two putative catalytic domains with different functions and therefore may be bifunctional enzymes (Fig. 2, 4, and 6).

Our results showed that these genes responsible for porphyrin synthesis were arranged in a more compact organization in *C. josui* than in the other bacteria and suggested that the gene cluster might be involved in the synthesis of vitamin B_{12} and siroheme. To our knowledge, this is the first report describing the genes responsible for porphyrin biosynthesis from a strictly anaerobic bacterium.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the GSDB,

C. josui	HemB	1	MIKRPRRIRTNEVIRKAVRETRISTDS-LIWPLFIVEGKNIKK		
B. subtil	i HemB	1	MSQSFNRHRRIRTSKAMREMVKETRLHPSD-FIYPIFVVEGLEGEK		
E. coli	HemB	1	MTDLIQRPRRLEKSPALPRMFEETTLSLND-LVLPIFVEEEIDDYK		
yeast	HEM2	1	MHTAEFLETEPT-EISSVLAGGYN-HPLLRQWQSERQLTK-NMLIFPLFISDNPDDFT		
human	HemB	1	MQPQSVLHSGYFHPLLRAWQTATTTLNASNLIYPIFVTDVPDDIQ		
rat	HemB	1	MHHQSVLHSGYFHPLLRAWQTTPSTVS-ATNLTYPIFVTDVPDDVQ		
	2010000000				
C.j 43	EISSLPGQ	YHF	SPDMVGKAIEAALKADVKSVLLFGLPKHKDEKGSEAYNENGVLQQGIRETKQRYPQ		
B.s 46	AVPSMPDV	HHV	SLDLLKDEVAELVKLGIQSVIVFGIPEEKDDCGTQAYHDHGIVQKAITEIKEHFPE		
E.C 46	AVEAMPGV	MRI	PEKHLAREIERIANAGIRSVMTFGISHHT-DETGERAWREDGLVARMSPICKQTVFE		
Yea 56	EIDSLPNI	NRI	GVNRLKDYLKPLVAKGLRSVILFGVPLIPGTKDPVGTAADDPAGPVLQGIKFIREYFPE		
Hum 46	PITSLPGV	ARY	GVKRLEEMLRPLVEEGLRCVLIFGVP-SRVPKDERGSAADSEESPAIEAIHLLREYFPN		
Rat 46	PIASLPGV	ARY	GVNQLEEMLRPLVEAGLRCVLIFGVP-SRVPKDEQGSAADSEDSPTIEAVRLLRKTFPT		
C.i 110	MOVITETO	MCR	YTSHGACGTT_KGERWINNERT, DYLEKTAT, SHUMAGADMTADEDMMNCDT VAT DOTT.DV		
B.8 113	MVVVADTO	LCE	YTOHCHCGLVKD-GUILNDESTELLAOTAVSOAKACABUTTAPSNMMDCFUTUTREALDE		
E.C 113	MIVMSDTC	TOT	YTSHCHCCUTCERC_UDNDATEENI CKOAUUAAAAGADETADSAAMDCOUDATEOATDA		
Yea 126	LVTTCDUCTOFVESHCHCCUUVDDCFINDEPSUSDEANUAUVAVACAUCUADCDATDATDATDATA				
Hum 115	LINACOVC	T.CP	VTSHCHCGLI SENCAFRAFESROPLAEVALAVAKACCOVVAPSDMMCCRUFATCHCLCN		
Bat 111	LINACOVC	TOP	V7SHGHCGLISENGAFLAFESPORTAEVALAVAKAGCOV/ADSDMNDCDVFATVAALLE		
100 111		-184			
C.j 179	NGFTD-IF	IMS	YAVKYASSFYGFFREAA		
B.s 182	EGFVN-IF	IMS	YAVKYSSEFYGPFRDAANSTPQFGDRKTYQMDPANRMEALREAQSDVEEGADFLIVKPS		
E.c 182	AGFKD-TA	IMS	YSTKFASSFYGPFREAAGSALK-GDRKSYQMNPMNPREAIRESLLDEAQGADCLMVKPA		
Yea 196	ANLAHKTF	VLS	YAAKFSGNLYGPFRDAACSAPSNGDRKCYQLPPAGRGLARRALERDMSEGADGIIVKPS		
Bum 185	HGLGNRVS	VMS	YSAKFASCFYGFFRDAAKSSFAFGDRRCYQLPPGARGLALRAVDRDVREGADMLMVKPG		
Bat 185	EGLGNRVS	VMS	YSAKFASCFYGPFRDAAOSSPAFGDRRCYOLPPGARGLALRAVARDLOFGADTLMWRDG		

- B.s 251 LSYMDIMRDVKNEFT-LPLVAYNVSGEYSMVKAAAQNGWIKEKEIVLEILTSMKRAGADLIITYHAKD--
- E.c. 250 GAYLDIVRELRER-TELPIGAYQVSGEYAMIKFAALAGAIDEEKVVLESLGSIKRAGADLIFSYFALDLA
 Yea 266 TFYLDIMRDASEICKDLPICAYHVSDEYAMLHAAAEKGVVDLKTIAFESHQGFLRAGARLIITYLAPEFL
- Bum 255 MPYLDIVREVKDKHPDLPLAVYHVSGEFAMLWBGAQAGAFDLKAAVLEAMTAFRRAGADIIITYYTPOLL
- Rat 255 LPYLDMVQEVKDKHPELPLAVYQVSGEFAMLWHGAKAGAFDLRTAVLESMTAFRRAGADIIITYFAPQLL
- B.s 318 AAKWLAE
- E.c 319 EKKILR Yea 336 -- DWLDEEN
- Yea 336 --- DWLDEEN Hum 325 --- QWLKEE
- Rat 325 --- WEREE Rat 325 --- KWEKEE

FIG. 7. Alignment of the predicted amino acid sequence from ORF5 (*hemB*) of *C. josui* (C.j) and amino acid sequences of PBG synthases (ALA dehydratase) (HemB) of *B. subtilis* (B.s), *E. coli* (E.c), *S. cerevisiae* (Yea), humans (Hum), and rats (Rat). Shaded residues represent amino acids which are identical to those in *C. josui* HemB. A short motif similar to a zinc-binding domain is underlined.



FIG. 8. Proposed pathway of porphyrin biosynthesis in *C. josui.* -N and -C show the NH_2 -terminal region and the COOH-terminal region, respectively. The genes encoding homologous enzymes are given in parentheses. The *C. josui* proteins correspond to the following enzymes: HemA-N, NAD(P)H-dependent glutamyl-tRNA reductase; HemB, PBG synthase; HemC, HMB-S; HemD-C, UroIII synthase; HemD-N, *S*-adenosylmethionine-dependent UroIII methylase; HemA-C, siroheme synthase. The broken arrow shows that vitamin B_{12} is synthesized in several steps from precorrin-2. tRNA-Glu, glutamyl-tRNA; GSA, glutamate-1-semialdehyde...

DDBJ, EMBL, and NCBI nucleotide sequence databases with the accession number D28503.

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