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Escherichia coli Competence Gene Homologs Are Essential for Competitive Fitness and the Use of DNA as a Nutrient

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Natural genetic competence is the ability of cells to take up extracellular DNA and is an important mechanism for horizontal gene transfer. Another potential benefit of natural competence is that exogenous DNA can serve as a nutrient source for starving bacteria because the ability to "eat" DNA is necessary for competitive survival in environments containing limited nutrients. We show here that eight *Escherichia coli* genes, identified as homologs of *com* genes in *Haemophilus influenzae* and *Neisseria gonorrhoeae*, are necessary for the use of extracellular DNA as the sole source of carbon and energy. These genes also confer a competitive advantage to *E. coli* during long-term stationary-phase incubation. We also show that homologs of these genes are found throughout the proteobacteria, suggesting that the use of DNA as a nutrient may be a widespread phenomenon.

Natural genetic competence and transformation in bacteria refers to the ability to take up extracellular DNA and stably maintain it either on the chromosome or as a plasmid. It requires the expression of specialized proteins that assemble into a DNA-uptake complex to bring DNA inside the cell. There are three proposed roles for this process: (i) genetic transformation, (ii) DNA repair, and (iii) to provide a source of nutrients (7, 14, 15, 19, 25, 48, 49, 54, 56). The latter role was proposed long ago; however, it has only recently been shown that *Escherichia coli* is capable of consuming double-stranded DNA (dsDNA) as a sole source of carbon and energy and that this ability to "eat" DNA is necessary for the competitive survival of *E. coli* during long-term incubation (19). This process is considered to be a form of natural competence that we refer to as "nutritional competence," as opposed to the process of genetic competence associated with bacterial genetic transformation.

Even though *E. coli* is not known to be naturally competent for genetic transformation, several *E. coli* genes have been identified that are homologous to genes known to be involved in natural competence and genetic transformation in other bacteria such as *Haemophilus influenzae* and *Neisseria gonorrhoeae* (1, 4, 15, 16, 19, 59). One of these genes, *comE*, involved in the uptake of extracellular DNA, is part of a gene superfamily involved in the movement of macromolecules across membranes, including pilus biogenesis, protein secretion, competence-transformation, and twitching motility (15).

Extracellular DNA is abundant in virtually all environments encountered by bacteria (31, 47). In the mammalian gut DNA released from eukaryotic cells, foodstuffs, and other bacteria can reach concentrations up to hundreds of micrograms per milliliter. In normal human lung mucus DNA is found at levels of 100 to 200 μg/ml, reaching levels as high as 4 mg/ml in the lungs of cystic fibrosis patients (47). DNA levels in various marine and freshwater environments reach hundreds of micrograms per liter and DNA in association with soil, including various clay minerals, can reach hundreds of micrograms per gram of substrate (31). Importantly, whether or not this DNA is used as genetic material or as a nutrient, genetic and nutritional competence activities are not mutually exclusive.

Given that in natural environments extracellular DNA is highly abundant, it is important to understand the mechanisms that allow the acquisition of dsDNA as a nutrient. To date, only two putative competence gene homologs were tested for their role in "eating" DNA. We have now mutated all eight *E. coli com* gene homologs and show that each mutant is unable to use DNA as a sole carbon and energy source. We also show that *com* gene mutants show a reduction in competitive fitness and that the gene family is found in many other species, especially within the proteobacteria.

MATERIALS AND METHODS

Bacterial strains and plasmids. All experiments were performed with strains derived from *E. coli* K-12 strain ZK126 and are listed in Table 1.

Gene disruptions. Each gene of interest was replaced with a cassette expressing chloramphenicol resistance (Cam^r) using a Red recombinase-mediated system (11), with the following modifications. Template DNA for PCRs was not digested before electroporation, and a 15-ml portion of cells (optical density at 600 nm [OD_{600}] of ~0.6) per knockout was made electrocompetent just prior to electroporation. Table 2 lists the primers used to generate PCR fragments containing the Cam^r cassette flanked by regions of homology for each particular gene. PCR products were purified prior to electroporation by using a QIAquick PCR purification kit (QIAGEN). All mutants were colony purified and tested for the absence of the Red recombinase-expressing plasmid pKD46 by plating for antibiotic sensitivity on agar plates containing 150 μg of ampicillin/ml. To verify each mutation, PCRs were performed with one primer specific to a region upstream of the insertion point and another primer complementary to the sequence of P1 (P1-C [CGAAGCAGCTCCAGCCTACAC]) to amplify a band of specific size (Table 2; see also Fig. 2).

Since each knockout mutation was constructed via insertion of a Cam^r cassette, for several of the mutations polar effects cannot be explicitly ruled out. Evidence supporting a direct role for these genes in the use of DNA as a nutrient is discussed in Results.

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TABLE 1. Bacterial strains and plasmids

Strain	Relevant genotype or phenotype[a]	Source or reference
ZK126	W3110 Δ*lacU169 tna*-2	65
ZK1142	ZK126 Nal^r	65
ZK1143	ZK126 Str^r	65
SF2437	ZK126 + pKD46	This study
SF2438	ZK126 *yrfD*::Cam^r	This study
SF2439	ZK126 *yrfC*::Cam^r	This study
SF2440	ZK126 *yrfB*::Cam^r	This study
SF2441	ZK126 *yrfA*::Cam^r	This study
SF2442	ZK126 *hofQ*::Cam^r	This study
SF2443	ZK126 *yhgH*::Cam^r	This study
SF2444	ZK126 *yhgI*::Cam^r	This study
SF2445	ZK126 *yhiR*::Cam^r	This study
SF2486	ZK126 *nupC*::Cam^r	This study
SF2487	ZK126 *nupG*::Cam^r	This study
pKD46	Red recombinase-expressing plasmid; Amp^r	11
pKD3	Chloramphenicol-resistance gene; Cam^r	11

[a] Nal^r, nalidixic acid resistance; Str^r, streptomycin resistance; Amp^r, ampicillin resistance.

Long-term survival and batch culture competition assays. *E. coli* wild-type (ZK1142 Nal^r or ZK1143 Str^r) and mutant strains were separately incubated overnight in 5 ml of Luria-Bertani (LB) broth (Difco) at 37°C with aeration. Cultures were then inoculated 1:1,000 (vol/vol) into fresh LB, either individually or in coculture; 5 μl of each strain was inoculated into the same 5 ml of culture (19). Subpopulation titers were determined by periodic sampling of the cultures, serial dilution, and plating on medium containing appropriate antibiotics (nalidixic acid at 20 μg/ml, streptomycin at 50 μg/ml, or chloramphenicol at 20 μg/ml), allowing the determination of relative fitness (17, 20). All antibiotics were purchased from Sigma-Aldrich. Experiments were performed at least three times per strain. The limit of detection in all experiments is <1,000 CFU/ml.

Preparation of minimal medium supplemented with purified DNA. M63 medium was prepared as described previously (40, 45). Sonicated salmon sperm DNA (Sigma-Aldrich) was prepared as previously described (19), including multiple rounds of organic extraction (phenol, phenol-chloroform, chloroform, and ether) to remove protein. For each "DNA-eating" experiment, purified salmon sperm DNA was freshly precipitated and added to a final concentration of 0.1% (wt/vol). All media were prepared in and all experiments were performed in acid-treated glassware to eliminate the possibility of contamination by other nutrients. Cultures were inoculated 1:1,000,000 (vol/vol; ~10^3 CFU/ml), and viable cell counts were determined periodically for 24 h. Experiments were performed at least three times.

Multiplex PCR assay to determine relative fitness. Individual cultures of mutant strains were inoculated into LB from frozen stocks and incubated at 37°C overnight. The following day, 5 μl of each of the eight mutant strains were coinoculated (1:1,000) into 5 ml of fresh LB broth. Cultures were incubated at 37°C and periodically sampled by spotting 10 μl onto LB-agar plates. Cells collected from these spots were used as a template for PCRs (MasterMix PCR kit; Promega), performed as follows: initial incubation at 95°C for 2 min and then 30 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 1 min, followed by 72°C for 5 min. Table 2 lists primers used for multiplex PCR and indicates the fragment sizes used to distinguish individual mutant strains. Bands were separated on 8% acrylamide gels and scored for the presence or absence of each corresponding mutant strain.

Growth using nucleobases, nucleosides, or dNMPs as a carbon and energy source. *E. coli* strains were inoculated 1:1,000,000 into M63 minimal medium supplemented with 1 mg of vitamin B_1/ml, 1 mM $MgCl_2$, and one of the four nucleobases, nucleosides, or deoxynucleotide monophosphates (dNMPs) (Sigma), all at a concentration of 20 mM (except for guanosine, which was supplemented at 2 mM due to insolubility at higher concentrations). After inoculation, cultures were sampled periodically, and cell titers were determined by serial dilution and plating on LB-agar plates. In all experiments, acid-treated glassware was used.

DNA-protein sequence analyses. Homologs of *E. coli com* genes were identified by using the Microbial BLAST (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi)

TABLE 2. Primers used to generate null deletions of *E. coli com* genes and for multiplex PCRs

E. coli gene	Gene length (bp)	Primers (sequences) used for KO strains construction[a]	Primer for multiplex	Size (bp)
yrfD	780	H1P1 (5′-ACGGGTGATAAGGAGATCATCACAATGGCATTTAAGGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-CCTTCCCAAGCGCCAGCCCCAGCGCGATGGCAAAGTCATATGAATATCCTCCTTAG-3′)	5′-CGCGCGGTAGTATAAAGGCAAGC-3′	110
yrfC	540	H1P1 (5′-CCCGCCAATTAATTTTTTGCCCTGGCGACAGCAACGCCGGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-ACCTTCCTTGTTAACTGATACTCAAATTGCCAGCGTCCCCATATGAATATCCTCCTTAG-3′)	5′-CGGATTTGATCCCTGGGAGGC-3′	167
yrfB	441	H1P1 (5′-CTGGTGGTTCGCCACATCACCCCGCCTCCGCCAGCTTTGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-CCACGCTTAACGAAAAACGGCTCACGCTGACGTTGCGCTCATATGAATATCCTCCTTAG-3′)	5′-GCGTTCGCAACGGCAGCG-3′	353
yrfA	405	H1P1 (5′-CGCTGGTTGTTGGCAGGTATTGCATTGTGCCTTTTAACCGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-ATCTCCTTGCCGTTGCCACAACCATTGTGGCGGTTCGCACATATGAATATCCTCCTTAG-3′)	5′-GCTCCCTTCAGCGAGGAAAAAACGC-3′	297
hofQ	1239	H1P1 (5′-CGTTCCGGTAGCTCAGGTGTTGCAGGCGCTGGCTGAACAGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-CGTGGCGTGATAAACACCACTAACTCGCGTCGTTCATCTCATATGAATATCCTCCTTAG-3′)	5′-GCTGGGTACCGGGACAAACTG-3′	205
yhgH	684	H1P1 (5′-TTATTCATCCGCTATTGTTCTCTTTTGACTTACAAGGATGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-GGTTCGACAAAGGCACCAGACCTGGACAGCCGCCGCCATATGAATATCCTCCTTAG-3′)	5′-TCATCACTGAGTTGCTGCTGAAATCC-3′	432
yhgI	576	H1P1 (5′-ATCCGTATTTCCGATGCTGCACAAGCGCACTTTGCCAAAGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-GTAGGAGTGTTCGCCGCGCTGGTGTTCGGTGAGATCGCGCATATGAATATCCTCCTTAG-3′)	5′-GTCTTGAATTGCCCGTGCAAGGTCG-3′	262
yhiR	843	H1P1 (5′-CTCAGTTATCGCCACAGCTTTCACGCTGGCAACCACGCCGTGTAGGCTGGAGCTGCTTC-3′) H2P2 (5′-CACGATCCAGCTTACGGTGGCGTGCCCGGTGCCTGCCATATGAATATCCTCCTTAG-3′)	5′-TTCAGGTGGCTGACCGGGGAG-3′	480

[a] H1 corresponds to approximately the first 12 codons, and H2 corresponds to the last 12 codons of each particular gene. P1 and P2 are underlined and correspond to the regions flanking the antibiotic resistance cassette located on plasmid pKD3 (11).

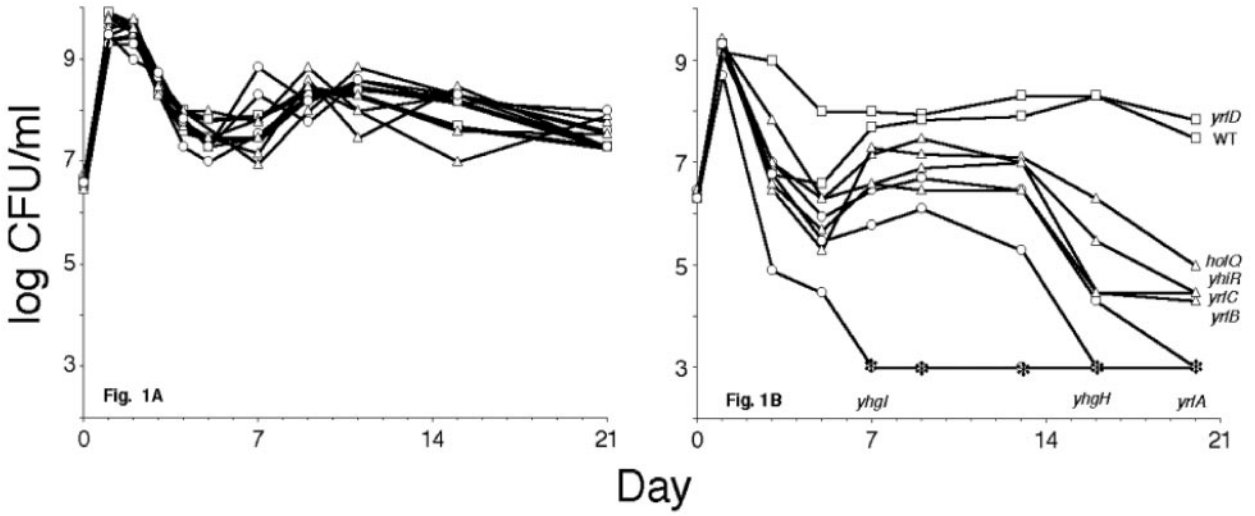


FIG. 1. Long-term survival and competition patterns of *com* gene mutants. (A) Superimposition of nine growth curves of wild-type and mutant strains grown separately as monocultures. (B) Superimposition of competition assays of cells grown in coculture. Each mutant strain was competed against the wild type. A single representative wild-type strain is shown. Mutant strains (solid lines) are grouped by their competition phenotype and indicated by symbols as follows: *yrfD*, squares; *hofQ*, *yhiR*, *yrfC*, and *yrfB*, triangles; and *yhgI*, *yhgH*, and *yrfA*, circles. Asterisks indicate no detectable cells. Representative data are shown.

and ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) algorithms at the National Center for Biotechnology Information Web site. Multalign (http://prodes.toulouse.inra.fr/multalin/multalin.html) and CLUSTAL W 1.8 (http://www.ebi.ac.uk/clustalw/) were used for multiple protein sequence alignments. Protein functions were predicted by using EBI-InterProScan (http://www.ebi.ac.uk/InterProScan/). Subcellular localization predictions were made by using PSORT (http://psort.nibb.ac.jp/form.html), SOSUI (http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0.html), and InterProScan.

RESULTS

Long-term survival and SPCD phenotypes of *com* mutants. Eight competence gene homologs were identified in *E. coli* based on sequence similarity to genes in *H. influenzae* (13, 19). Null mutants were created by replacing each gene with a Cam^r gene cassette. Each constructed mutant showed no growth deficiency phenotype during exponential phase when incubated in LB and displayed long-term survival patterns identical to those of wild-type cells (Fig. 1A), which was consistent with previous findings that these genes are not essential (21, 50). However, when mutants were cocultured with wild-type cells in LB, seven mutants show decreased competitive fitness to various degrees (Fig. 1B), expressing the stationary phase-specific competition-defective (SPCD) phenotype (19). The strongest SPCD phenotype is found in *yhgI* strains, where mutants are eliminated from the culture after 3 days of coincubation. Mutants with a deletion of the *yrfD* gene usually do not express the SPCD phenotype, competing as well as wild-type cells.

Relative fitness of *com* mutants during long-term stationary phase. After observing that different mutants showed differential SPCD phenotypes when competed against wild-type cells, we decided to determine the relative fitness of the mutants with respect to each other. Rather than performing pairwise competitions, we applied a multiplex PCR approach (9, 23) wherein each mutant is identifiable by a PCR fragment of unique size. Since each mutant is constructed by using the same Cam^r cassette, eight primers were designed to anneal at different distances upstream of the Cam^r cassette insertion point, yielding products of unique size (Fig. 2A and Table 2). Simultaneous competition of eight different mutant strains in a single culture allowed us to establish a hierarchy of relative fitness by scoring whether a particular mutant is present or absent at a specific time point. Figure 2B shows a representative multiplex PCR experiment where the *yhgI* mutant shows the weakest fitness and is eliminated from the culture by day 3. This result is consistent with our observation that this mutant is the least fit during competition with wild-type cells (Fig. 1B). Other mutants gradually disappear over time, with the exception of *yrfD*, which is present at the end of each experiment, a finding consistent with the results observed during competition with the parental strain (see Fig. 1B). Occasionally, the *yhiR* or *yrfC* mutant strains are also present at the end of an experiment. Mutants in each of these genes abolish the ability of *E. coli* to consume dsDNA as a nutrient and yet result in differential relative fitnesses in complex media, suggesting that other genes play other roles in long-term stationary-phase competitive survival.

Ability of *com* mutants to utilize salmon sperm DNA as a sole source of carbon and energy. To address the ability of *com* mutants to consume dsDNA, a "DNA-eating" assay was performed wherein DNA was provided as the sole source of carbon and energy. Highly purified sonicated salmon sperm DNA was added to M63 minimal medium at a concentration of 0.1% (wt/vol). Wild-type or mutant cells were inoculated from fresh overnight LB cultures at ~1,000 CFU/ml and incubated at 37°C. Cell titers were determined for 24 h, and final growth yields were calculated (Fig. 3). Wild-type cells show an increase in cell density of ~120-fold compared to all mutant strains which show growth yields of ≤3-fold.

Utilization of DNA components as nutrients. When DNA is released into any environment, various chemical processes, as well as endo- and exonucleases, can break it down. Gram-negative bacteria can transport nucleosides and nucleotides into the cell using specific outer membrane porins (61). Exogenous

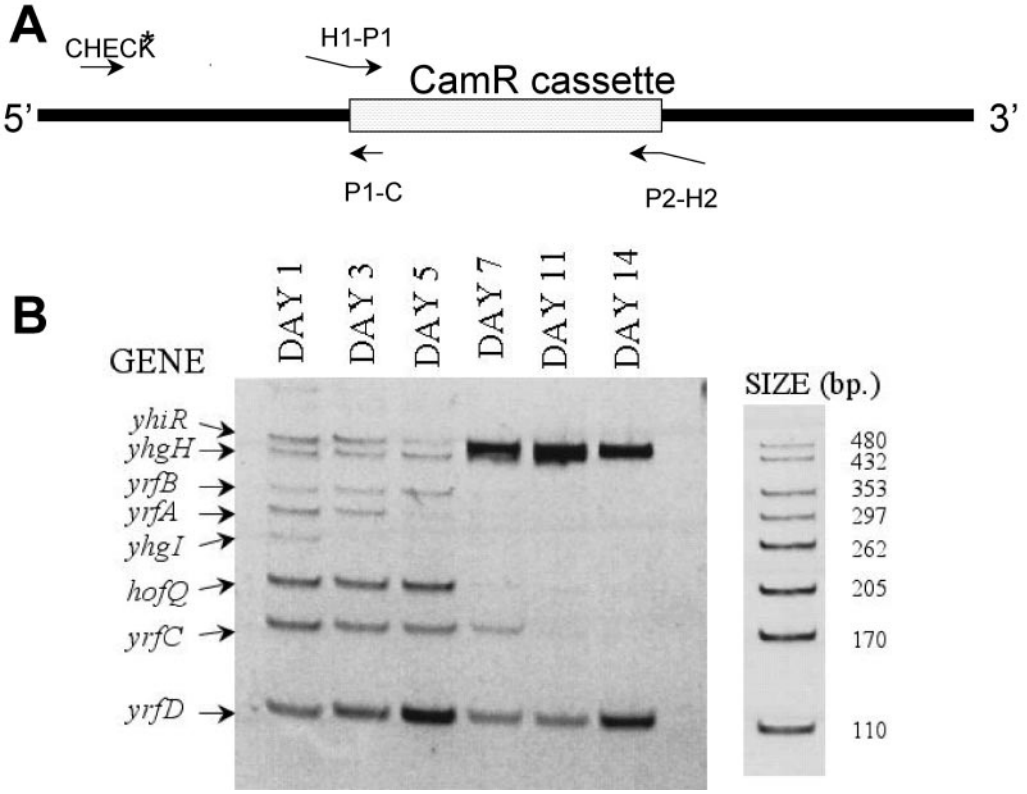


FIG. 2. Relative fitness of *com* mutants. (A) Schematic representation of the construct used for multiplex experiments; the asterisk indicates that the position of the upstream "check" primer varies for each gene mutated; (B) The eight knockout mutants were coinoculated in a single LB culture. The culture was sampled periodically, and PCR was performed to identify bands corresponding to each mutant. The presence of each product indicates the survival of a particular mutant strain. The bands in the right panel show the band sizes for each mutant. Representative data are shown.

nucleosides are transported by Tsx, which functions as a nucleoside-specific channel (5, 6, 22, 33, 34). In the periplasm nucleotides are dephosphorylated to nucleosides and are transported through the cytoplasmic membrane by proteins encoded by *nupC* and *nupG* (43, 64). Once inside the cell, nucleosides can serve as carbon and energy sources (30, 35, 36, 42, 52). Therefore, it was of particular interest to investigate the ability of the eight *com* mutants to consume different DNA components (Fig. 4).

M63 medium containing either of the four nucleobases, nucleosides, or dNMPs at 20 mM were tested for their ability to support growth as the sole carbon and energy source. The data are shown only for adenine, adenosine, and dAMP. However, except where noted, similar results are obtained for all other compounds. None of the nucleobases support bacterial growth (Fig. 4A), and high concentrations of adenine caused cell death, most likely by inhibiting de novo purine biosynthesis (29, 46). However, the addition of nucleosides and deoxynucleotide monophosphates support the growth of all mutants to the same levels as wild-type cells (Fig. 4B and C). Cells grown in deoxynucleotide monophosphates reach densities of $\sim 10^6$ CFU/ml, ca. 1% of the level reached by cells grown in the presence of nucleosides, except for TMP, where cultures reach a density of 10^7 to 10^8 by day 2 (data not shown).

To verify that growth was due to the consumption of nucleotides or nucleosides, growth profiles were determined for *nupC* and/or *nupG* mutants. NupG is involved in the intracellular transport of all four nucleosides, whereas NupC cannot transport guanosine (43, 64). *nupC nupG* double mutants reach cell densities at least 1,000 times lower than for wild-type cells after 48 h of incubation when grown on any of the nucleosides (data not shown). Such growth might be due to nonspecific

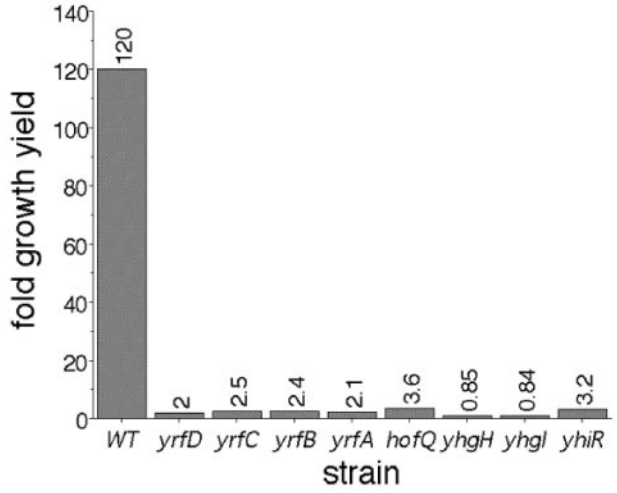


FIG. 3. Average growth yields of wild-type (WT) or *com* mutant cells in minimal medium supplemented with 0.1% ultrapure sonicated salmon sperm DNA as the sole source of carbon and energy. Growth yields (indicated above each bar) were determined by dividing the number of cells after 24 h of incubation by the number of cells at inoculation.

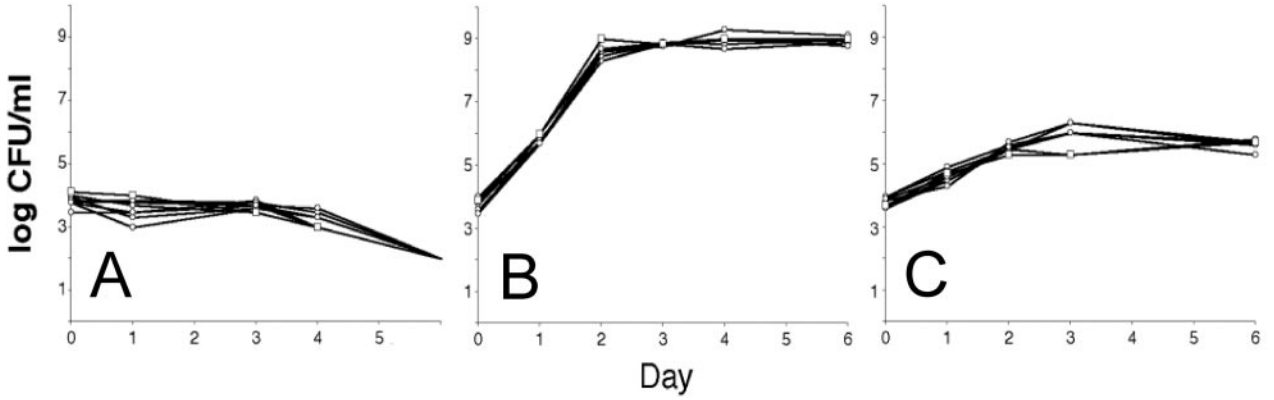


FIG. 4. Catabolism of nucleobases, nucleosides, and dNMPs by wild-type (□) and *com* mutant (○) strains. Long-term survival data are shown for the wild-type and eight *com* mutant strains grown in adenine (A), adenosine (B), or dAMP (C) as the sole source of carbon and energy. Representative data are shown.

uptake or catabolism of nucleosides and/or spontaneous hydrolysis or degradation of nucleosides outside of the cytoplasm.

In all of the experiments performed above, potential polar effects cannot be ruled out for all mutants. However, a large amount of data supports our contention that most, if not all, of these genes are involved in the nutritional competence process. These genes are found in three distinct locations on the *E. coli* chromosome: five genes (*yrfD-hofQ*) at 75.9′, two genes (*yhgH* and *yhgI*) at 76.4′, and *yhiR* located as a monocistronic gene at 78.5′. Mutation in each of them abolishes the ability of *E. coli* to catabolize dsDNA. Other evidence that suggests that strong polar effects may not be an issue include the following: (i) the *yrfD*::*cam* mutation, knocking out the first gene of the putative five-gene operon, has the weakest effect on competitive fitness of all five mutations in this cluster. In fact, there is no correlation between gene order in the five-gene operon and any mutation's effect on relative fitness. However, each mutant is unable to "eat" DNA; (ii) the *yhgH* and *yhgI* genes are most likely both transcribed as a bicistronic message (V. Palchevskiy and S. E. Finkel, unpublished data), and yet the *yhgI* mutation in the second gene has a much stronger effect on relative fitness than the *yhgH* mutation; and (iii) the *yhiR* locus is far from the other seven genes, and yet all mutations eliminate the ability of the cell to catabolize dsDNA.

Nucleic acid substrate preferences. In addition to linear dsDNA molecules, we were interested in determining whether other configurations of DNA or RNA are substrates of the *com*-mediated nucleic acid catabolism system. As shown in Table 3, only dsDNA substrates, either linear or circular, can serve as sole sources of carbon and energy. *E. coli* can efficiently catabolize dsDNA molecules as small as 24 bp. However, *E. coli* cannot utilize either single-stranded DNA (ssDNA) or RNA as a nutrient.

TABLE 3. Nucleic acid substrate preferences of *E. coli*

Substrate	Length[a]	Ability to utilize
RNA (whole cell)	Variable	–
ssDNA oligonucleotides	24–87 nt	–
dsDNA oligonucleotides	24–87 bp	+
Sonicated chromosomal dsDNA (*E. coli* or salmon sperm)	0.5–2 kb	+
dsDNA-circular plasmid	2.7 kb	+
dsDNA-linear plasmid	2.7 kb	+
dsDNA-plasmid restriction fragments	10–500 bp	+

[a] nt, nucleotides.

DISCUSSION

Null mutants of *E. coli*'s eight putative nutritional competence genes have been created by replacing each gene with an antibiotic resistance gene cassette. These genes are similar in

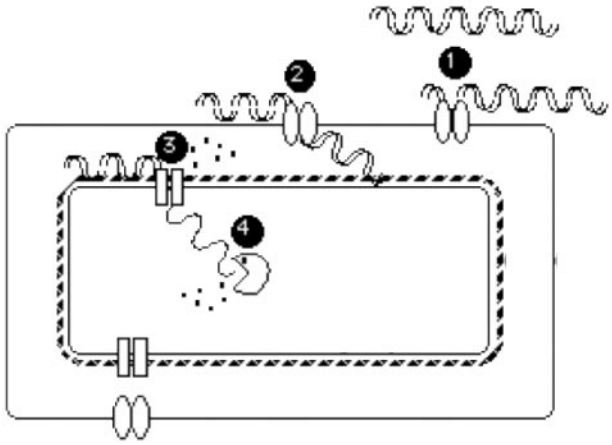
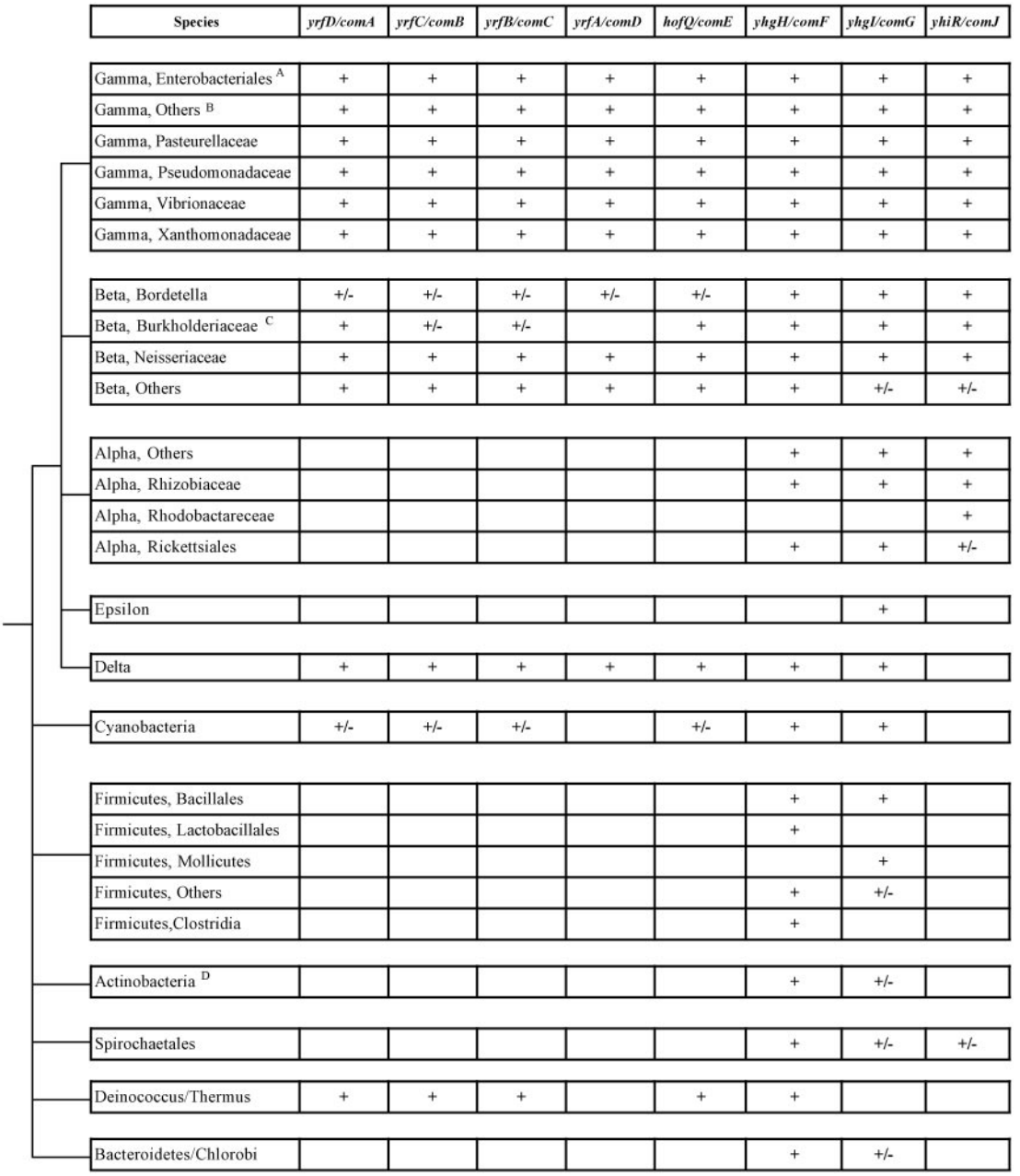


FIG. 5. Model of DNA uptake and catabolism in *E. coli*. The outer membrane, inner membrane, and cell wall (dashed line) are depicted, defining the borders of the extracellular space, periplasm, and cytoplasm. Ovals represent putative cell surface dsDNA binding and transport complexes. Rectangles represent putative inner membrane transport and processing complexes. The "Pac-Man" represents cytoplasmic ssDNA processing enzymes, including nucleases. Small dots represent degraded DNA or nucleotides. (Step 1) Extracellular dsDNA is bound by a surface receptor on the outer membrane, and dsDNA is transported across the outer membrane to the periplasm, probably through a porin encoded by *hofQ*; (step 2) dsDNA is processed to ssDNA, in the periplasm or possibly during transit across the inner membrane; (step 3) nucleotides from the degraded strand are released into the periplasm; and (step 4) ssDNA is processed to nucleotides in the cytoplasm which are further metabolized.



Species	*yrfD/comA*	*yrfC/comB*	*yrfB/comC*	*yrfA/comD*	*hofQ/comE*	*yhgH/comF*	*yhgI/comG*	*yhiR/comJ*
Gamma, Enterobacteriales [A]	+	+	+	+	+	+	+	+
Gamma, Others [B]	+	+	+	+	+	+	+	+
Gamma, Pasteurellaceae	+	+	+	+	+	+	+	+
Gamma, Pseudomonadaceae	+	+	+	+	+	+	+	+
Gamma, Vibrionaceae	+	+	+	+	+	+	+	+
Gamma, Xanthomonadaceae	+	+	+	+	+	+	+	+
Beta, Bordetella	+/-	+/-	+/-	+/-	+/-	+	+	+
Beta, Burkholderiaceae [C]	+	+/-	+/-		+	+	+	+
Beta, Neisseriaceae	+	+	+	+	+	+	+	+
Beta, Others	+	+	+	+	+	+	+/-	+/-
Alpha, Others						+	+	+
Alpha, Rhizobiaceae						+	+	+
Alpha, Rhodobactareceae								+
Alpha, Rickettsiales						+	+	+/-
Epsilon							+	
Delta	+	+	+	+	+	+	+	
Cyanobacteria	+/-	+/-	+/-		+/-	+	+	
Firmicutes, Bacillales						+	+	
Firmicutes, Lactobacillales						+		
Firmicutes, Mollicutes							+	
Firmicutes, Others						+	+/-	
Firmicutes,Clostridia						+		
Actinobacteria [D]						+	+/-	
Spirochaetales						+	+/-	+/-
Deinococcus/Thermus	+	+	+		+	+		
Bacteroidetes/Chlorobi						+	+/-	

FIG. 6. *com* gene homologs. The presence (+) or absence (−) of each of the eight *com* gene homologs is indicated to the right of a phylogenetic tree based on small-subunit rRNA. Genera or groups that have members with or without homologs are indicated by "+" or "−", respectively. Distances are not drawn to scale.

sequence, as well as in genetic organization, to *H. influenzae* and *N. gonorrhoeae* competence genes. All mutants are unable to utilize dsDNA as a nutrient, and most exhibit significant fitness reductions when they are competing with wild-type cells. This loss of competitive ability is observed during the stationary phase of the growth cycle, a time of severe nutrient depletion. We propose that DNA is one of several macromolecules, along with proteins, lipids, and peptidoglycan, which *E. coli* utilizes as a nutrient source during periods of nutrient shortage. Both wild-type and "non-DNA-eating" mutant cells can consume the same repertoire of "standard" nutrients, including small carbohydrates and amino acids (data not shown), as well as nucleic acid components (see Fig. 4). However, an inability to metabolize DNA incurs a significant fitness load on the cell during periods of competition. That is, all else being equal, cells that can consume DNA in addition to "standard" nutrients are more fit. This is undoubtedly of great importance in natural environments where nutrients are scarce and competition for them is intense (18).

This finding is particularly intriguing due to the fact that *E.*

coli is not generally thought to be naturally competent for genetic transformation. However, several research groups proposed that natural transformation exists in *E. coli* when it is grown on agar plates or in aquatic environments with physiological concentrations of calcium, but natural transformation is not observed in LB liquid cultures under laboratory conditions (2, 3, 32, 60, 63).

Our working model of dsDNA uptake and metabolism (Fig. 5) is based on current models of genetic competence and transformation proposed for the two best-studied naturally transformable gram-positive and gram-negative organisms: *B. subtilus* and *H. influenzae*, respectively (7, 15, 54).

In our model there are at least four distinct steps in the nutritional competence process. Initially, extracellular dsDNA is bound by a receptor located at the outer membrane and is transported into the periplasm, probably through the porin encoded by *hofQ* that might serve as the receptor as well. In the periplasm, or possibly during passage across the inner membrane, dsDNA is processed to ssDNA. One intact single strand enters the cytoplasm while the second strand is degraded and nucleotides are released into the periplasmic space. Some of these degraded nucleotides are then processed and transported into the cytoplasm, probably through the NupC and NupG systems located in the inner membrane (44, 57). Upon entering the cytoplasm, ssDNA is processed to single nucleotides that are further metabolized and used as sources of carbon and energy. It is likely that distinct pathways exist for the uptake of nucleotides and dsDNA because none of the *com* mutants show any significant growth defect when nucleosides or nucleotides are utilized as the sole sources of carbon and energy (Fig. 4B and C).

Specific functions for most of the *com* genes have not been assigned in either of the best-studied gram-negative organisms, *H. influenzae* and *N. gonorrhoeae*. More progress has been made in the gram-positive organism *B. subtilis*. Dubnau and coworkers have assigned functions to proteins necessary for dsDNA binding, transport across the cell membrane, and processing of Com proteins (15, 16, 27). In addition, one-for-one sequence homologs for several of the genes investigated here (*yrfD-yrfA* and *yhiR*) are not found in *B. subtilis* and other naturally competent gram-positive organisms (Fig. 6). While the fundamental mechanism of DNA uptake, where dsDNA is bound but only a single strand enters the cytoplasm intact, seems to be conserved between gram-positive and gram-negative organisms, the genes involved in competence and transformation appear to be very different between the two groups at the sequence level. To us this suggests convergent evolution of this process.

The programs PROSITE and EBI-InterProScan, as well as published reports, predict functions for *hofQ*, *yhiR*, *yhgH*, and *yhgI* (Table 4). HofQ was identified as a putative outer membrane porin/secretin based primarily on its homology to the *pilQ* gene of *P. aeruginosa*, involved in type IV pilus synthesis and twitching motility (37, 38). YhiR contains a motif found in *N*-6-adenine-specific DNA methyltransferases (8). YhgH is predicted to encode a purine/pyrimidine-phosphoribosyl transferase based on homology to proteins involved in the biosynthesis and salvage of purine samples and pyrimidines (24). We noticed that the motif CXXC-(X_{7-12})-CXXC-(X_{9-11})-CXXC-(X_{10-13})-CXXC is conserved among all YhgH homologs we have identified (Table 4 and Fig. 6); this motif bears a resemblance to a zinc-finger DNA-binding domain. YhgI contains the NifU/HesB-like domain that has been associated with proteins involved in nitrogen fixation (12, 13, 26). *yhgI* is the only gene of the eight that has been reported to be highly expressed (10, 50) and shows induction under conditions of heat shock or the presence of misfolded proteins (28), iron depletion (39), and the addition of kanamycin (53). The fact that it is induced under a variety of conditions might explain why mutation of *yhgI* shows the greatest effect during long-term stationary-phase survival (Fig. 1 and 2).

TABLE 4. Predicted subcellular localization and function of *E. coli com* gene products

E. coli	Predicted subcellular location[a]	Predicted function[b]
HofQ	Outer membrane	Outer membrane porin
YrfD	Periplasm	Unknown
YrfC	Inner membrane	Unknown
YrfB	Inner membrane	Unknown
YrfA	Inner membrane	Unknown
YhgH	Cytoplasm	Pur/Pyr-phosphoribosyl transferase
YhgI	Cytoplasm	Unknown; contains Nif-like domains/nitrogen fixation
YhiR	Cytoplasm	*N*-6-adenine-specific DNA methyltransferase

[a] Subcellular locations are predicted by PSORT, SOSUI, and InterProScan.
[b] Functions are predicted by PROSITE and InterProScan.

No functions are predicted for four genes: *yrfD*, *yrfC*, *yrfB*, and *yrfA*. However, in almost every organism where these four genes are found, they comprise the first four genes of a putative five-gene operon ending with a *hofQ* homolog. These genes also show the largest degree of sequence divergence (19).

Subcellular localization can be predicted using the PROSITE and SUSUI algorithms. These programs predict that HofQ is in the outer membrane, a periplasmic localization for YrfD, an inner membrane localization for YrfC, YrfB, YrfA, and cytoplasmic localization for the YhgH, YhgI, and YhiR proteins. Although we currently have no definitive functions assigned to these gene products, it is satisfying to note that these predictions assign at least one protein to each subcellular location: outer membrane, periplasm, inner membrane, and cytoplasm. Current studies are directed toward determining protein locations biochemically. We have purified seven of the proteins and antibodies are being generated for each. Preliminary data based on fractionation experiments supports the cytoplasmic localization predicted for YhgI (Palchevskiy and Finkel, unpublished).

Transcriptional profiles of the *com* genes have been determined by using genomic microarrays, *lacZ* fusions, and reverse transcription-PCR. Several studies have reported that the *yrfD-hofQ* operon is poorly transcribed (41, 50, 51, 58). Moreover, no conditions have been identified that increased the expression levels of these genes. In one study, mRNA for *yrfD*, *yrfC*, *yrfB*, and *hofQ* was determined to be "absent," whereas *yrfA*, *yhgH*, *yhgI*, and *yhiR* were considered to be "present," but only YhgI protein was detected (10). Based on preliminary reverse transcription-PCR analyses, we speculate that the *yrfD-hofQ* operon is initially transcribed as a five-gene transcript and is

TABLE 5. Gene names in several organisms

E. coli gene(s)			*H. influenzae* gene	*N. gonorrhoeae* gene
b3395	*yrfD*	*hofM*	*comA*	*pilM*
b3394	*yrfC*	*hofN*	*comB*	*pilN*
b3393	*yrfB*	*hofO*	*comC*	*pilO*
b3392	*yrfA*	*hofP*	*comD*	*pilP*
b3391	*hofQ*	*hofQ*	*comE*	*pilQ*
b3413	*yhgH*	*gntX*	*comF*	NGO1725
b3414	*yhgI*	*gntY*	*comG*	NGO1426
b3499	*yhiR*		*comJ*	NGO0859

degraded or processed, resulting in decreased levels of the flanking *yrfD* and *hofQ* mRNA. We have observed that by mid-log phase (i.e., an OD_{600} of ~0.3), it is difficult to obtain a cDNA of the five-gene operon; however, individual regions such as *yrfD-yrfA* or *yrfC-hofQ* can be amplified (data not shown).

Figure 6 shows *com* gene homologs identified in a wide variety of genera. It had been previously reported that *com* gene homologs only occur in a small subset of bacterial species (21). This is most likely due to the low degree of sequence identity between genes of this family. However, the organization of these genes is highly conserved. Whenever the five homologs *comA* through *comE* are found, their gene order is identical to that of *E. coli*. In addition, the five-gene cluster is frequently found between the genes encoding shikimate kinase (*aroK*) and penicillin-binding protein 1a (*mrcA*). We used the Microbial Genome BLAST algorithm (with the expect value set at 0.01) and determined that *hofQ* homologs were found in 101 bacterial species, *yhiR* homologs were found in 106 bacterial species, *yhgH* homologs were found in 190 bacterial species, and *yhgI* homologs were found in 158 bacterial species. Figure 6 shows that *yrfD*, *yrfC*, *yrfB*, and *yrfA* genes are found in many bacterial species, albeit with various degrees of sequence divergence.

The genes studied here, all homologs of genes shown to play a role in natural competence in *H. influenzae* and *N. gonorrhoeae*, have been assigned different names by different investigators (Table 5). It appears that *E. coli com* gene homologs comprise a DNA uptake apparatus that allows the consumption of dsDNA as a nutrient, rendering almost all of these genes essential under conditions of competition. Ongoing work is directed toward determining the specific roles of the eight *com* gene products in the "DNA-eating" process. In addition, we are studying the ability of other species to consume DNA as a nutrient. For example, preliminary experiments indicate that *Pseudomonas aeruginosa* can use DNA as a sole source of carbon and energy (S. Finkel, unpublished observation). A role for extracellular DNA in biofilm formation has been shown in *P. aeruginosa* (55, 62). Preliminary data indicate that *yhgI* mutants are hyper-biofilm formers in *E. coli* (data not shown). Together, these studies will lead to a better understanding of the mechanisms of "nutritional" competence.

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