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An Epitope of Elongation Factor Tu Is Widely Distributed within the Bacterial and Archaeal Domains

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A monoclonal antibody (MAb), MAb 900, which detects a 43-kDa protein present on *Escherichia coli* was found. Subsequently, more than 90 organisms, belonging to either the bacterial, archaeal, or eucaryal domain, were tested for reactivity to this MAb. Of the bacterial and archaeal domains, almost all species proved to be positive, whereas all organisms from the eucaryal domain gave negative results. The 43-kDa protein was purified by affinity chromatography and subsequently analyzed by microsequencing methods. Two peptide sequences which showed a high degree of homology (>99%) to the prokaryotic elongation factor Tu (EF-Tu) were obtained. Western blot (immunoblot) analysis using both purified EF-Tu and EF-Tu domains confirmed that the unknown protein was EF-Tu. The panbacterial distribution of EF-Tu, which is present in large amounts in every prokaryotic cell, renders this protein a good candidate for a diagnostic approach. In consequence, we have used the anti-EF-Tu MAb 900 to design both a dot blot assay and an enzyme-linked immunosorbent assay. From either blood culture, urine, or gall-bladder fluid, bacterial contamination could be detected. The sensitivity of these tests is currently 10^4 bacteria per ml.

Elongation factor Tu (EF-Tu) is one of the most abundant proteins in prokaryotes, representing about 5% of the total cellular protein of *Escherichia coli* (18). During protein biosynthesis, that is, the elongation process, EF-Tu catalyzes the binding of each aminoacyl-tRNA to the ribosome (30). As a multifunctional protein, it interacts with several macromolecules and guanine nucleotides, including EF-Ts, GDP, GTP, and some ribosomal proteins.

More than 10 years ago, both the complete amino acid sequence for EF-Tu and the nucleotide sequence of the two EF-Tu genes, *tufA* and *tufB*, were described for *E. coli* (1, 40). However, until now, sequences of only a panel of 22 genes encoding bacterial and two archaeal EF-Tu sequences have been determined (4, 9, 10, 23–26, 36). In addition, EF-Tu genes from seven chloroplasts (5, 6, 11, 21, 27, 35) and the mitochondria of *Saccharomyces cerevisiae* were recorded (28). Alignment of the amino acid sequences deduced from the bacterial EF-Tu gene sequences revealed a high degree of similarity. Previously we described a monoclonal antibody (MAb), MAb 900, which we obtained by immunization of BALB/c mice with *E. coli* K-12 C600 Rif^r(pKT146) bacteria (3, 19). This antibody recognized a 43-kDa band from *E. coli* and other members of the family *Enterobacteriaceae* on Western blots (immunoblots). Further studies revealed a broad cross-reactivity with more than 90 species from either the bacterial or the archaeal domain. Here we report (i) the purification of the 43-kDa protein by affinity chromatography, (ii) the characterization of the protein as EF-Tu by partial sequencing after *Asp*N digestion, and (iii) its application for a panbacterial detection assay.

MATERIALS AND METHODS

Chemicals. CNBr-Sepharose 4B was purchased from Pharmacia-LKB, Uppsala, Sweden. Growth media were obtained from Difco, Augsburg, Germany, and GIBCO-BRL, Eggenstein, Germany. 5-Bromo-4-chloro-indolylphosphate (BCIP) and Nitro Blue Tetrazolium were from Sigma Chemie, Deisenhofen, Germany. 4-Nitrophenylphosphate was from Boehringer, Mannheim, Germany. All buffer ingredients were from Merck, Darmstadt, Germany.

Microorganisms. Organisms either were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, or the National Collection of Type Cultures, London, United Kingdom, or were patient isolates. The latter strains were isolated by routine culture techniques and confirmed by fermentation and enzyme patterns according to international standards by using the commercial API system (API bioMérieux, Marcy l'Etoile, France).

Sulfur- and sulfate-reducing bacteria, as well as archaea, were kindly provided by Herb Frederickson (Gesellschaft für Biotechnologische Forschung mbH, Braunschweig, Germany). *Rhodospirillum rubrum*, *Azospirillum brasilense*, and a *Myxococcus* sp. were kindly provided by K.-H. Schleifer and R. Amman (Lehrstuhl für Mikrobiologie, TU München, Munich, Germany). Purified elementary bodies of *Chlamydia trachomatis* serovar K were a kind gift from L. Köhler (Abteilung I Rheumatologie, Medizinische Hochschule Hannover, Hannover, Germany).

Generation of MAb 900. MAb 900 was generated as described previously (19). Briefly, BALB/c mice were immunized with 2.5×10^8 bacteria of *E. coli* K-12 strain C600 Rif^r(pKT146) with five intraperitoneal booster injections.

Release of EF-Tu from different bacterial strains. Bacterial strains were all grown in tryptic soy broth (TSB), Luria-Bertani (LB) medium, or Schaedler anaerobic medium according to their growth conditions or as indicated by the guidelines of the Deutsche Sammlung von Mikroorganismen und Zellkulturen and the National Collection of Type Cultures.

At an optical density at 600 nm of about 1.0, bacteria were harvested, centrifuged, and resuspended in 100 μl of 10 mM phosphate-buffered saline (PBS), pH 7.5. To disrupt bacterial cell walls, a loopful of glass beads with a diameter of 100 μm (Sigma, Munich, Germany) was added. The sample was processed for 2 min in a tissue disintegrator at maximum speed (Mickle Laboratory, Gomshall, United Kingdom). After centrifugation, 15 μl of the supernatant was added to an equal amount of sodium dodecyl sulfate (SDS) sample buffer and used for SDS-polyacrylamide gel electrophoresis (PAGE).

Affinity chromatography. *E. coli* K-12 C600 Rif^r(pKT146) cells (100-ml culture) were grown overnight in TSB containing tetracycline at a concentration of 10 μg/ml. Cells were centrifuged for 10 min in a JA-10 rotor ($3,000 \times g$) and washed three times with PBS. Subsequently they were resuspended in 10 ml of PBS and broken as described above. Cytoplasmic extract was incubated overnight at room temperature with MAb 900, which was coupled to CNBr-Sepharose 4B according to the manufacturer's instructions. After the Sepharose beads had been washed three times with PBS, the 43-kDa protein was eluted with 0.5 M acetic acid. Sepharose beads and eluant were separated by centrifugation through a 0.22-μm-pore-size filter (Millipore). The eluant was freeze-dried in a Speed Vac Concentrator and finally suspended in SDS sample buffer (0.0625 M Tris hydrochloride [pH 6.8], 5% 2-mercaptoethanol, 2% SDS, 12.5% glycerol).

SDS-PAGE and immunoblotting. The affinity chromatography-purified 43-kDa protein was checked for contamination by SDS-PAGE, which was per-

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TABLE 1. Reaction patterns of purple bacteria with MAb 900

α Subdivision			β Subdivision			γ Subdivision			δ and ε Subdivisions		
Subgroup	Organism	Result[a]	Subgroup	Organism	Result	Subgroup	Organism	Result	Subgroup	Organism	Result
1	*Rhodospirillum rubrum*[b]	−	1	*Comamonas testosteroni*	+	2	*Legionella pneumophila*	+		*Campylobacter jejuni*	+
	Azospirillum brasilense	+		*Comamonas acidovorans*	+					*Helicobacter pylori*	+
											+
2	*Pseudomonas diminuta*	+	2	*Chromobacterium violaceum*	+	3	*Vibrio parahemolyticus*	+	Myxococcus group	*Myxococcus* sp.[b]	−
	Agrobacterium tumefaciens	+		*Alcaligenes faecalis*	+		*Pseudomonas putida*	+	Sulfur and sulfate reducers	*Desulfovibrio desulfuricans*[b]	−
				Pseudomonas (*Burkholderia*) *cepacia*	+		*Pseudomonas aeruginosa*	+		*Desulfovibrio vulgaris*[b]	−
							Xanthomonas maltophilia	+		*Desulfovibrio salexigens*[b]	−
							Aeromonas hydrophila	+		*Desulfotomaculum ruminis*[b]	+
3	*Rhodobacter capsulatus*	+	3	*Neisseria meningitidis*	+		*Yersinia enterocolitica*	+		*Desulfotomaculum* sp. strain 43[b]	+
				Neisseria gonorrhoeae[b]	+		*Citrobacter freundii*	+		*Desulfomicrobium aspheronum*[b]	−
							Enterobacter aerogenes[b]	+			
							Enterobacter cloacae	+			
							Klebsiella pneumoniae[b]	+			
							Klebsiella oxytoca	+			
							Escherichia coli	+			
							Proteus mirabilis	+			
							Salmonella enteritidis[b]	+			
							Salmonella bovis[b]	+			
							Serratia marcescens	+			
							Shigella flexneri type I	+			
							Shigella sonnei[b]	+			
							Haemophilus influenzae	+			
							Bordetella bronchiseptica	+			

[a] +, detected in Western blot; −, not detected in Western blot.
[b] Data not shown in figures.

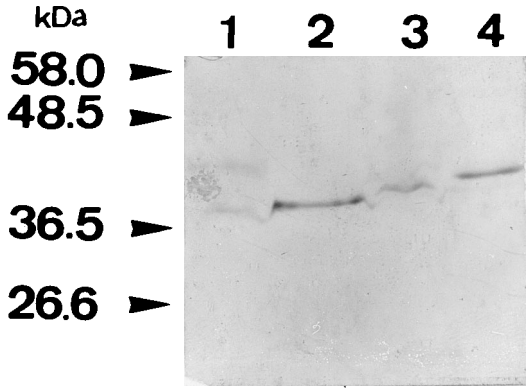

FIG. 1. Western blot of purple bacteria, α subdivision. Lanes: 1, *Azospirillum brasilense*; 2, *Pseudomonas diminuta*; 3, *Agrobacterium tumefaciens*; 4, *Rhodobacter capsulatus*.

formed according to the method of Laemmli (22). After addition of SDS sample buffer, the protein solution was boiled for 5 min and then run on an SDS–12.5% polyacrylamide gel. Subsequently, proteins were stained either with Coomassie brilliant blue R-250 or with silver nitrate. For immunoblotting, the proteins were electrotransferred to a nitrocellulose (NC) membrane (0.43-μm pore size; Schleicher & Schüll, Dassel, Germany) (7) and then incubated for 2 h with MAb 900. Binding was detected by using a biotinylated anti-mouse immunoglobulin antibody (Amersham, Braunschweig, Germany) and an avidin alkaline phosphatase conjugate with BCIP-NBT as the substrate.

Protein sequencing. The purified protein was applied to a 10% polyacrylamide gel, electroblotted onto Glassybond (Biometra), and stained with Coomassie blue as described previously (12). The 43-kDa protein was sequenced in an Applied Biosystems 477 A pulsed liquid phase sequencer equipped with an on-line 120A PTH analyzer according to the instructions of the manufacturer. For internal sequence analysis, 3 μg of the protein was subjected to SDS–10% PAGE. The 43-kDa band in the stained polyacrylamide gel was excised and cleaved with proteinase *Asp*N (Boehringer, Tutzing, Germany) by using an enzyme-to-substrate ratio of 1:10 as described earlier (13). The peptides were eluted and separated by reversed-phase high-performance liquid chromatography by using SMART System (Pharmacia, Freiburg, Germany) and a Supersphere Select B RP 18 column (125 by 2 mm; Merck). Trifluoracetic acid (0.1%) in water was used as solvent A, and a gradient of 0 to 50% of solvent B (0.1% trifluoracetic acid in acetonitrile) was applied in 50 min. The flow rate was 70 μl/min, and the detection wavelength was 206 nm. Fractions were sequenced as specified above.

Amino acid alignment. The amino acid alignment was done with the PC GENE program (IntelliGenetics, Inc.) and the SWISS-PROT protein sequence data library.

Dot blot assay. *E. coli* K-12 strain C600 Rif^r(pKT146) was grown in LB medium until it reached an optical density at 600 nm of 1.0. One milliliter of the culture was taken and centrifuged, and the pellet was resuspended in 100 μl of PBS. Clinical samples were treated similarly. From either blood culture (Hémoline; bioMérieux, Marcy l'Etoile, France), urine, or gall bladder fluid, a 1-ml sample was taken. Bacterial cell walls were broken as described above. The cytoplasmic solution was diluted to a final volume of 1.4 ml. Subsequently, serial dilutions were prepared. One hundred microliters of each dilution was loaded on an NC sheet that was fixed in a blot chamber (SCR 96 D Minifold I; Schleicher & Schüll). Filtration was performed by using vacuum pressure. Subsequently, the NC was removed and blocked with PBS–2% bovine serum albumin (BSA). Immunodetection of EF-Tu was done as described above.

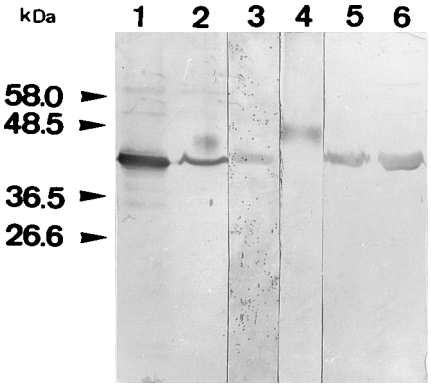

FIG. 2. Western blot of purple bacteria, β subdivision. Lanes: 1, *Comamonas testosteroni*; 2, *Comamonas acidovorans*; 3, *Chromobacterium violaceum*; 4, *Neisseria meningitidis*; 5, *Pseudomonas* (*Burkholderia*) *cepacia*; 6, *Alcaligenes faecalis*.

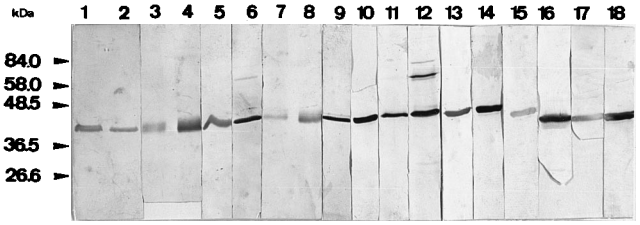

FIG. 3. Western blot of purple bacteria, γ subdivision. Lanes: 1, *Yersinia enterocolitica*; 2, *Serratia marcescens*; 3, *Shigella flexneri*; 4, *Bordetella bronchiseptica*; 5, *Pseudomonas aeruginosa*; 6, *Enterobacter aerogenes*; 7, *Citrobacter freundii*; 8, *Enterobacter cloacae*; 9, *Salmonella enteritidis*; 10, *K. oxytoca*; 11, *Legionella pneumophila*; 12, *Vibrio parahemolyticus*; 13, *Proteus mirabilis*; 14, *Pseudomonas putida*; 15, *Haemophilus influenzae*; 16, *Xanthomonas maltophilia*; 17, *Aeromonas hydrophila*; 18, *E. coli*.

TABLE 2. Reaction patterns of gram-positive bacteria with MAb 900

Group and organism(s)	Result[a]
High G+C	
Mycobacterium gordonae	+
Corynebacterium pseudotuberculosis	+
Actinomyces naeslundii	+
Propionibacterium acnes	+
Nocardia farcinica	+
Micrococcus kristinae	+
Low G+C	
Bacillus cereus	+
Listeria monocytogenes	+
Staphylococcus aureus	+
Staphylococcus epidermidis	+
Viridans group streptococci	+
Streptococcus pyogenes[b]	+
Peptostreptococcus sp.	+
Streptococcus pneumoniae	+
Pediococcus sp.	+
Erysipelothrix rhusiopathiae	+
Clostridium perfringens	+
Enterococcus faecalis	+
Ureaplasma urealyticum[b]	−
Mycoplasma hominis[b]	−
Spirochetes	
Leptospira sp.[b]	+
Treponema hyodysenteriae	+
Cytophaga-Flexibacter-Bacteroides	
Bacteroides fragilis	+
Flavobacterium multivorum	+
Capnocytophaga canimorsus[b]	−
Haliscomenobacter hydrossis	+
Fusobacteria	
Fusobacterium varium	+
Planctomyces-Chlamydia	
Chlamydia trachomatis	−

[a] +, detected in Western blot; −, not detected in Western blot.
[b] Data not shown in figures.

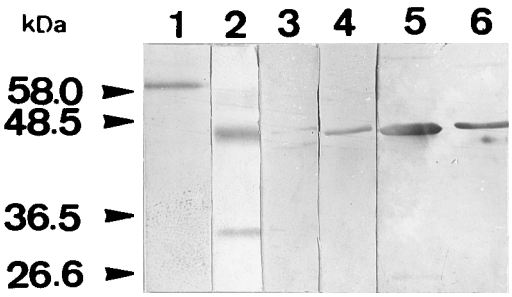

FIG. 4. Western blot of gram-positive bacteria, high-G+C subdivision. Lanes: 1, *Mycobacterium gordonae*; 2, *Corynebacterium pseudotuberculosis*; 3, *Actinomyces naeslundii*; 4, *Propionibacterium acnes*; 5, *Nocardia farcinica*; 6, *Micrococcus kristinae*.

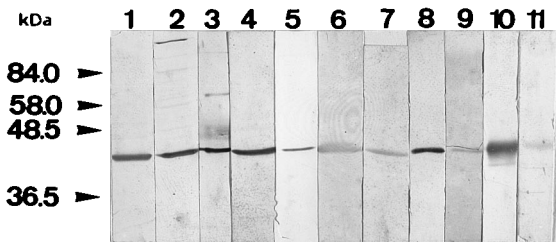

FIG. 5. Western blot of gram-positive bacteria, low-G+C subdivision. Lanes: 1, *Bacillus cereus*; 2, *Listeria monocytogenes*; 3, *Staphylococcus aureus*; 4, *Staphylococcus epidermidis*; 5, viridans group streptococci; 6, *Peptostreptococcus* sp.; 7, *Streptococcus pneumoniae*; 8, *Pediococcus* sp.; 9, *Erysipelothrix rhusiopathiae*; 10, *Clostridium perfringens*; 11, *Enterococcus faecalis*.

EF-Tu ELISA. Serial dilutions, which were prepared for the dot blot assay, were also used for an EF-Tu enzyme-linked immunosorbent assay (ELISA). Two hundred microliters of each dilution was incubated for 2 h on a microtiter plate at room temperature. Plates were subsequently blocked with PBS–2% BSA. Fifty microliters of MAb 900 (4.8 μg/ml) was diluted 1:1,000 and incubated for 1 h at room temperature. Binding was shown by using a biotinylated anti-mouse immunoglobulin antibody (Amersham) and an avidin alkaline phosphatase conjugate with 4-nitrophenolphosphate as the substrate.

RESULTS

Immunization of BALB/c mice with an *E. coli* K-12 strain resulted in MAb 900, which specifically recognized a 43-kDa band on Western blots with *E. coli* as the substrate. Subsequent analysis of other enterobacterial strains gave the same strong signal at 43 kDa.

Because of this reaction pattern, we started to evaluate systematically the reaction profiles of other bacterial strains. For this purpose, the classification scheme of Woese et al., who suggested a new highest-level taxon called domain above the kingdom level (for further information see references 29, 38, and 39), was used. On the basis of this taxon, the living world comprises three domains, namely, the *Bacteria*, the *Archaea*, and the *Eucarya*. Within these domains, several kingdoms, phyla, and their subdivisions can be differentiated according to their 16S rRNA relationships. From each subdivision, a minimum of one strain was tested. However, since organisms were considered mainly on the basis of their medical importance, some phyla and their subdivisions were omitted (see below). Within the bacterial domain, the following phyla were tested: (i) purple bacteria, (ii) low-G+C-content gram-positive bacteria, (iii) high-G+C-content gram-positive bacteria, (iv) spirochetes, (v) the *Cytophaga-Flexibacter-Bacteroides* group, (vi) fusobacteria, and (vii) the *Planctomyces-Chlamydia* group. In addition, archaea were evaluated.

Purple bacteria. Purple bacteria can be divided into four subdivisions (α, β, γ, δ, and ε) containing various subgroups. Western blot analysis of bacteria of every subdivision was performed, with at least one strain from every subgroup being considered (Table 1). From the α subdivision, all strains, belonging to either subgroup 1, 2, or 3, were positive (Fig. 1) except *Rhodospirillum rubrum*. The same results were obtained by using strains of either the β or γ subdivision as the substrate for MAb 900 (Fig. 2 and 3). *Neisseria meningitidis* showed a positive reaction at a molecular mass of about 47 kDa, which may be due to either a real or an apparent shift in the molecular mass of *Neisseria* EF-Tu. Such anomalous behavior in SDS-PAGE has been described for proteins with high proline contents, very basic proteins, or very acidic proteins. Even exchange of a single amino acid may change the mobility of proteins in SDS-PAGE up to ±10% (17). Recognition of almost all tested bacteria belonging to the γ subdivision is important with respect to their medical relevance. Pathogenic microorganisms belonging to the family *Enterobacteriaceae*, *Legionella* species and *Vibrio* species, as well as nonfermenters, are summarized within this subdivision. Within the δ and ε subdivisions, the important enteric pathogens *Helicobacter pylori* and *Campylobacter jejuni* were detected. Some sulfate- and sulfur-reducing bacteria within this subdivision of the proteobacteria gave negative results.

Gram-positive bacteria. These gram-positive bacteria can be divided into subdivisions based on a high or low G+C content. From the first group, all six species tested gave a positive signal on Western blot analysis (Table 2; Fig. 4). *Actinomyces naeslundii* repeatedly showed two weak signals of nearly the same molecular weight, the smaller of which probably is a degradation product. From the second group, 18 species were evaluated. All strains showed a positive signal (Fig. 5), except *Ureaplasma urealyticum* and *Mycoplasma* species, five of which have been analyzed (*Mycoplasma hominis*, *Mycoplasma salivarium*, *Mycoplasma orale*, *Mycoplasma pneumoniae*, and *Mycoplasma fermentans* (data not shown). Viridans group streptococci repeatedly gave a faint signal.

Other bacterial phyla. Within the bacterial domain, several other phyla can be distinguished according to the aforementioned criteria of Woese et al. and Olsen et al. (29, 38, 39). From those phyla, spirochetes, members of the *Cytophaga-Flexibacter-Bacteroides* group, and members of the *Planctomyces-Chlamydia* group, as well as fusobacteria, were tested. Most species proved to be positive (Table 2; Fig. 6). However, *Capnocytopha canimorsus* (*Cytophagales* branch) and *Chlamydia trachomatis* gave negative results. Strains from cyanobacteria

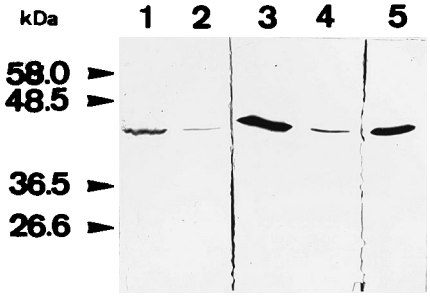

FIG. 6. Western blot of members of other bacterial kingdoms. Lanes: 1, *Treponema hyodysenteriae* (spirochetes); 2 to 4, *Cytophaga-Flexibacter-Bacteroides* group; 2, *Bacteroides fragilis*; 3, *Haliscomenobacter hydrossis*; 4, *Flavobacterium multivorum*; 5, *Fusobacterium varium* (fusobacteria).

TABLE 3. Reaction patterns of archaea with MAb 900

Organism	Result[a]
Methanobacterium thermoautotrophicum[b]	−
Halococcus morrhuae[b]	+
Halobacterium trapanicum[b]	+
Halobacterium marismortui	+
Halobacterium halobium	+
Thermococcus celer[b]	+/−
Natronobacterium pharaonis	+
Natronococcus occultus	+

[a] +, detected in Western blot; −, not detected in Western blot; +/−, very faint signal.
[b] Data not shown in figures.

and chloroplasts, green sulfur and nonsulfur bacteria, radioresistant micrococci and relatives, as well as fibrobacteria, were not tested as they do not represent medically relevant pathogens.

Eucarya. To demonstrate that the binding of MAb 900 is specific for prokaryotic EF-Tu, the following eucarya were tested: the promyelocytic cell line U937 and a protozoon (*Toxoplasma gondii*). Both gave repeatedly negative results. In addition, some fungi were evaluated. Elongation factor from both members of the subdivision Ascomycotina such as *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* and members of the subdivision Deuteromycotina such as *Candida albicans*, *Candida lusitaniae*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis* could not be detected.

Archaea. The archaeal domain can be divided into two distinct kingdoms, which represent a rather diverse collection of phenotypes (29, 38, 39). First are the methanogens and their relatives, which comprise five different phyla and are summarized as the kingdom *Euryarchaeota*. Second are organisms which are extremely thermophilic and which constitute the kingdom *Crenarchaeota*. From the euryarchaeotal kingdom, three groups have been considered in this study (Table 3). EF-Tu from *Methanobacterium thermoautotrophicum*, a member of the *Methanobacterium* group, was not detected by MAb 900. However, all tested halophiles (four species) gave positive results. From *Thermococcus celer*, only very weak signals were obtained. In addition, two strains, one each of *Natronobacterium* and *Natronococcus*, gave positive signals on Western blots (Fig. 7). The kingdom *Crenarchaeota*, comprising the phyla *Thermoproteus* and *Pyrodictium* as well as some marine bacteria from environmental samples (29, 39) was not considered.

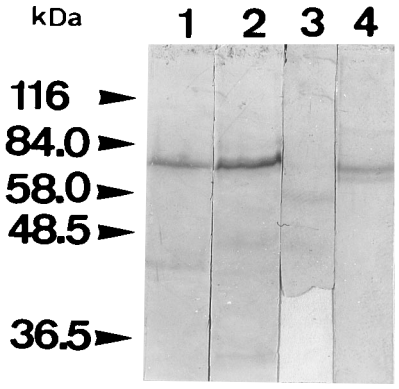

FIG. 7. Western blot of archaea. Lanes: 1, *Natronobacterium pharaonis*; 2, *Natronococcus occultus*; 3, *Halobacterium halobium*; 4, *Halobacterium marismortui*.

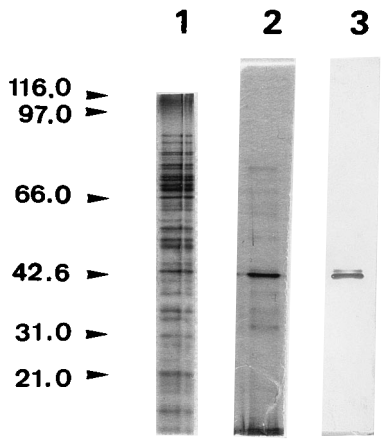

FIG. 8. SDS-PAGE (silver stained gel) and Western blot of affinity-purified EF-Tu. Lanes: 1, *E. coli* lysate before affinity purification; 2, purified EF-Tu after affinity chromatography; 3, Western blot of purified EF-Tu protein with MAb 900. Numbers indicate molecular mass in kilodaltons.

Identification of the 43-kDa protein as EF-Tu. Because of the broad distribution of the 43-kDa protein within the bacterial and the archaeal domains, we started to elucidate the identity of this protein. The unknown protein was purified by using MAb 900 in an affinity chromatographic procedure. Cytoplasmic lysate of *E. coli* was prepared and incubated with the affinity matrix as described in Materials and Methods. A highly purified 43-kDa protein could be obtained with only some low-molecular-mass contaminants, which reacted specifically with MAb 900 (Fig. 8). These contaminants probably represent degradation products, which were generated during the purification process.

Protein sequencing revealed that the N terminus of the protein was blocked. Therefore, the protein was digested with endoproteinase *Asp*N, which yielded a panel of cleavage products. Sequencing of the two most prominent peaks revealed sequences with lengths of 20 and 12 amino acid residues, respectively. A homology search of the SWISS-PROT protein sequence data library showed a 100% sequence identity of fragment DEGRAGENVGVL and one amino acid exchange in peptide DHGKTTCTAAITTVLAKTYG in comparison with the prokaryotic EF-Tu (Fig. 9). To confirm the sequencing result, Western blot analysis with either purified EF-Tu or different strains expressing a truncated *tufA* gene coding for domain 1 (amino acids 1 to 201), domains 1 and 2 (amino acids 1 to 296), and domains 2 and 3 (amino acids 1 to 12 and 199 to 393) (kindly provided by A. Pingoud [31]) was performed. With purified EF-Tu, MAb 900 showed the expected strong signal. In addition, all truncated proteins were detected (Fig. 10). For domain 1 and domains 2 and 3, MAb 900 recognized a single band in Western blot analysis, whereas two bands with slightly different molecular weights were observed for domains 1 and 2. Silver staining of the purified domains revealed that, especially with domains 1 and 2, one smaller degradation product exists (Fig. 10B).

EF-Tu assays. Since EF-Tu sequences of almost all tested species, which are pathogenic in humans, were detected by MAb 900, we wondered whether this antibody is suitable for use in a panbacterial detection assay. For this purpose, two methods were checked: (i) a dot blot assay and (ii) a direct ELISA.

Dot blot and ELISA of *E. coli* lysate and clinical samples. An *E. coli* lysate of approximately 10^9 cells per ml was prepared,

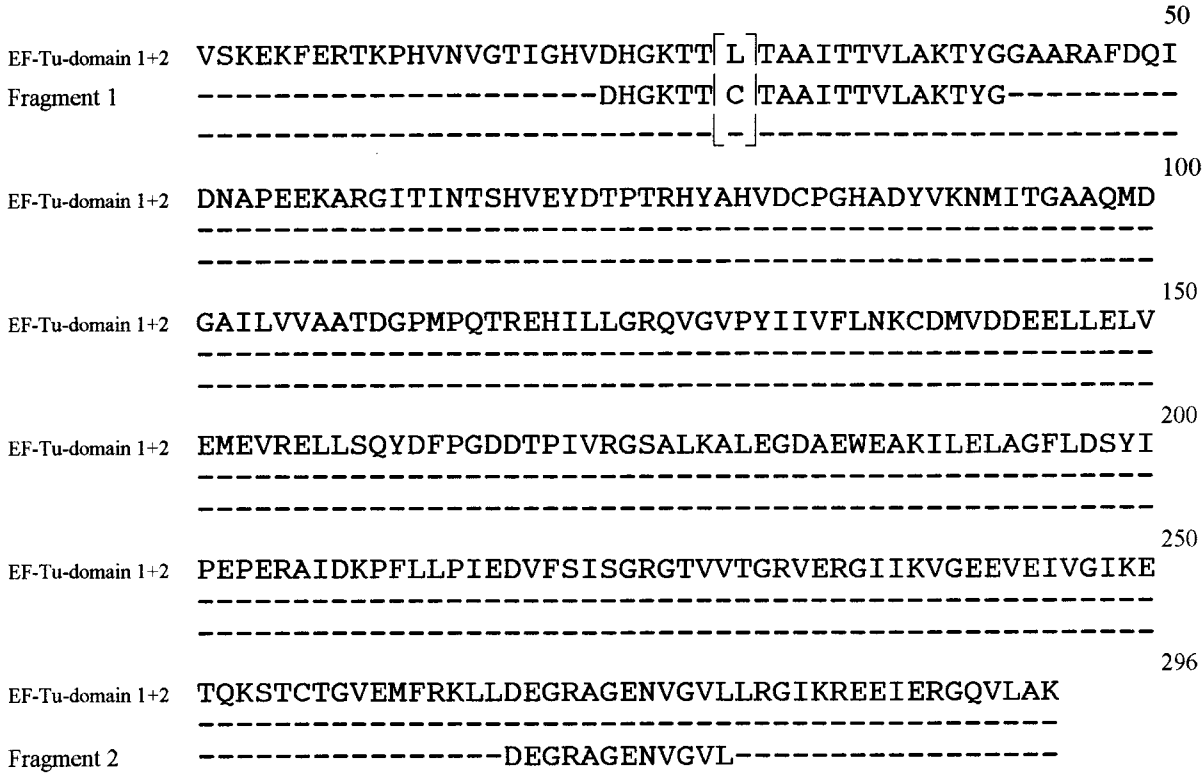

FIG. 9. Alignment of affinity-purified protein fragments after *Asp*N digestion and EF-Tu. Sequence exchanges are marked with brackets.

and 100-μl volumes of serial dilutions were dotted onto an NC sheet. MAb 900 was added, and binding was detected by a subsequent enzymatic reaction. The highest dilution of the *E. coli* lysate that was considered positive was 1:1,024 (Fig. 11). This dilution corresponds to a bacterial count of approximately 10^4/ml. To determine the sensitivity in another immunological assay, an ELISA was performed with the same dilutions as described for the dot blot assay. Sensitivity in comparison with that of the dot blot assay was essentially unchanged (data not shown). To investigate whether the results obtained from an in vitro isolate could be transferred to the in vivo situation, clinical samples from different sources were used (blood cultures [$n = 3$], urine samples [$n = 3$], and gall bladder fluid [$n = 1$]). *Staphylococcus aureus*, *E. coli*, *Klebsiella oxytoca*, or *Pseudomonas aeruginosa* could be isolated from these materials. MAb 900 recognized EF-Tu in all clinical samples (Fig. 11).

DISCUSSION

A MAb (MAb 900) was found which recognized a 43-kDa prokaryotic protein present in a broad variety of organisms within the bacterial and archaeal domains. Partial amino acid sequencing of the protein revealed two cleavage products showing high degrees of homology to EF-Tu of *E. coli*. The antibody detects purified EF-Tu as well as purified domains 1, 1 and 2, and 2 and 3 of EF-Tu, as described by Pieper et al. (31). Therefore, we conclude that EF-Tu is the unknown protein. As EF-Tu represents about 5% of the total cellular proteins, it seems reasonable that immunization of mice with a bacterial lysate reveals antibodies against that protein.

Parts of the EF-Tu genes are highly conserved in prokaryotic organisms, which is due mainly to strong selective pressure, as proposed by Filer et al. (14–16). The coexistence of both conserved and variable parts within the EF-Tu sequence rendered this molecule an interesting tool for phylogenetic studies. These properties were used by Ludwig et al. to compare amino acid sequences of both EF-Tu and elongation factor 1 alpha from organisms belonging to either the bacterial, archaeal, or eucaryal domain. The calculated phylogenetic tree proved to be comparable to the relationships deduced from 16S rRNA data (25). Furthermore, the conservation and duplication of the *tuf* gene were used to define new phylogenetic relationships among bacteria (34). A new phylogenetic tree based on the nucleotide and amino acid sequences of the EF-Tu gene from *Chlamydia trachomatis* was compared with those of other organisms and was found to be not completely congruent with rRNA gene-based phylogenetic trees (10). In addition, *tuf* sequences of single species have been compared with that of *E. coli*. The EF-Tu genes of *Saccharomyces cerevisiae* (28), *Mycoplasma hominis* (26), *Thermus thermophilus* HB8 (33), *Chlamydia trachomatis* serovar F (10), and *Mycobacterium leprae* (36) share between 60 and 70% similarity with the *E. coli tuf* gene. Apart from the 16S rRNA genes, systematic microbiologists seem to have another powerful tool to clarify the phylogenetic relations among bacteria: comparing the conserved sequences of EF-Tu and other elongation factors.

Recently, a MAb against mycoplasmal EF-Tu which was used to detect and differentiate members of the class *Mollicutes* has been described (8, 20). A similar MAb specific for a 43-kDa membrane protein of *Mycoplasma fermentans* detected 14 other mycoplasma species and some gram-negative and gram-positive bacteria (32). Although the nature of this protein was not further specified, we assume it to be EF-Tu because of its size and its distribution. In this study, the reactivity of a MAb directed against the prokaryotic EF-Tu has been investigated systematically. To our knowledge, it is the first time that bac-

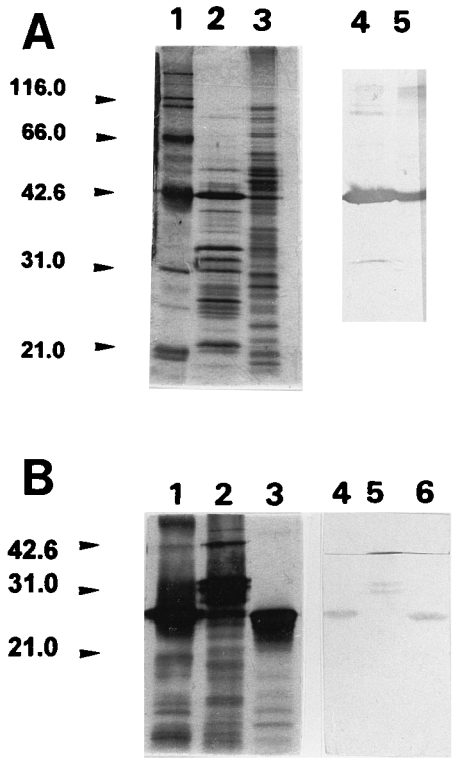

FIG. 10. SDS-PAGE and Western blot of purified EF-Tu (A) and purified EF-Tu domains (B). (A) Lanes: 1, molecular weight marker; 2, purified EF-Tu; 3, *E. coli* lysate; 4 and 5, corresponding Western blots. (B) Lanes: 1, EF-Tu domain 1; 2, EF-Tu domains 1 and 2 with contaminating EF-Tu at 43 kDa; 3, EF-Tu domains 2 and 3; 4 to 6, corresponding Western blots. Numbers indicate molecular mass in kilodaltons.

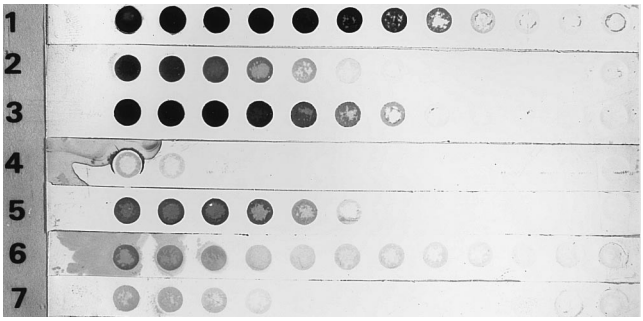

FIG. 11. Dot blot of *E. coli* lysate and clinical samples. From left to right, each spot denotes a separate dilution step from 1:2 to 1:4,096. Rows: 1, *E. coli* lysate (the reaction was considered positive up to a dilution of 1:1,024); 2, urine sample infected with 10^5 *Staphylococcus aureus* cells per ml; 3, urine sample infected with 10^6 *E. coli* cells per ml; 4, sterile urine sample (weak reactivity at dilutions of 1:2 and 1:4); 5, gall bladder fluid infected with *E. coli* and *K. oxytoca*; 6, blood culture infected with *Staphylococcus aureus* (positive from dilutions of 1:2 to 1:8; the other spots are colored by the blood culture medium); 7, sterile blood culture (the spots are colored by medium).

teria and archaea, defined by 16S rRNA sequence similarity (29, 38, 39), have been screened for reactivity to a common, almost panbacterial epitope. At least two representative strains from each subdivision were tested. Most medically important bacteria are found within the β and γ subdivisions of purple bacteria and in the gram-positive bacterial phylum. The γ subdivision includes all members of the family *Enterobacteriaceae*, including important pathogens causing human diarrhea (*Salmonella* spp., *Yersinia* spp., and *Vibrio* spp.) or nosocomial infections (*Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, and *Legionella* spp.), which play an important role in human pneumonia. All tested pathogens were detected by MAb 900. Within the β subdivision, positive results could be obtained with *Neisseria meningitidis*, an important pathogen in meningitis; *Neisseria gonorrhoeae*, a pathogen in sexually transmitted disease; *Comamonas* spp., which could be isolated from nosocomial infections; and *Pseudomonas cepacia*, which plays a role as a pathogen in patients with cystic fibrosis. Furthermore, EF-Tu of almost all gram-positive bacteria was detected by MAb 900.

However, a panel of 10 different species belonging to either the proteobacteria, the gram-positive bacteria, the *Cytophaga-Flexibacter-Bacteroides* group, or the archaea was not detected. This result raises the question of why this epitope is shared by remotely related species (archaea and proteobacteria) but not by more closely related ones (e.g., *Rhodospirillum rubrum* and *Azospirillum brasilense*). Sequential epitopes of MAbs span two to eight amino acid residues, which are either continuous or discontinuous (2). However, replacement of only one amino acid residue can severely affect affinity. With respect to this reaction profile, it is conceivable that EF-Tu molecules from different species may differ in a particular epitope although they are closely related. On the other hand, remotely related species may share a particular surface region, which results in cross-reactivity. The latter phenomenon has been reported by Wenzig and Schleifer (37), who found strong cross-reactivities between antisera raised against either *E. coli* or *Bacillus subtilis*. Thus, it is useful to screen a given epitope for its distribution within the system of organisms, but one must be cautious about postulating phylogenetic relationships based only on immunological cross-reactivities.

Sequence analysis of EF-Tu from different species revealed a molecular mass of 43 to 47 kDa. However, on Western blots performed with *Mycobacterium gordonae*, a single band at 60 kDa was detected. Strains from the archaeal domain gave positive bands at an apparent molecular mass of either 80 or 60 kDa (*Halobacterium marismortui*). The molecular weight shift of archaeal EF-Tu in SDS-PAGE has also been observed by Baldacci et al. (4), who relate the higher molecular weight in SDS-PAGE either to the halophilic character of the protein or, more likely, to an anomalous running behavior in SDS-PAGE. The latter explanation is also conceivable for the mycobacterial EF-Tu. Furthermore, viridans group streptococci repeatedly gave only faint signals. A possible explanation might be the only partial epitope identity of viridans group streptococci EF-Tu and EF-Tu of *E. coli*. Sequence alignment (*Streptococcus oralis* versus *E. coli*) supports this hypothesis. Both N- and C-terminal stretches of 10 to 15 different amino acid residues exist, and these could serve as putative epitopes. Other explanations may be that (i) viridans group streptococci are less susceptible than is *E. coli* to the cell disruption method applied (because of their heterogeneous cell wall composition) or (ii) the amounts of EF-Tu differ substantially between the two species.

Reactivity in Western blots indicates that the denatured epitope is sequential. The lack of reactivity to mycoplasmal EF-Tu might be helpful in determining the EF-Tu epitope of MAb 900. Amino acid alignment of *Mycoplasma hominis* and *Mycoplasma pneumoniae* versus *E. coli* revealed 56.8% identity. Six stretches of 6 to 16 nonconserved amino acid residues were found to serve as putative epitopes for MAb 900. However, since MAb 900 recognized EF-Tu from both *E. coli* and

Halobacterium marismortui, one would expect some residues from these stretches to be conserved between *E. coli* and *H. marismortui*. However, this is not the case. Unfortunately, no sequence information is available from other species, which are not detected by MAb 900. Thus, sequence comparisons currently fail to give any clue to elucidate the epitope of MAb 900 on EF-Tu.

MAb 900 reacts with domains 1, 1 and 2, and 2 and 3 of EF-Tu. Since only domain 1 has been inspected individually, no statement can be given about reactivity with domain 2 or 3 at the moment. Although these EF-Tu parts share the first N-terminal 12 amino acid residues (because of the vector construct used), sequence alignment with different species makes it most unlikely that this is the clue to the epitope. Work is in progress to elucidate this epitope.

Whereas most investigators used the conservation of the EF-Tu sequences for phylogenetic studies, we wondered whether this conservation could serve diagnostic purposes. A MAb detecting a highly conserved epitope on the EF-Tu molecule may prove useful for a panbacterial detection assay. The occurrence of EF-Tu in all bacteria and in large amounts renders this protein a good candidate for this diagnostic purpose. In consequence, we designed both an ELISA and a dot blot assay which can be used to detect bacterial contaminants in fluids. To get access to the cytoplasmic EF-Tu, bacterial cell walls were broken with glass beads. Subsequently, the cytoplasmic supernatant was either dotted onto NC strips or applied to a microtiter plate. Results can be obtained within 4 to 6 h, which in comparison with the time necessary for standard culture techniques is a remarkable time saving. Taking clinical samples from different sources like blood cultures, urine specimens, and gall bladder fluid, we were able to demonstrate that the assay works under clinical conditions. Because MAb 900 exclusively recognizes prokaryotic but not eukaryotic elongation factor, even heavy contamination by eukaryotic cells (as shown for blood cultures) will not interfere with the assay. However, at the moment only random samples have been tested. Sensitivity, specificity, and reliability of the assay must be assessed in further studies. Possible applications are rapid screening of blood-derived products (e.g., erythrocyte concentrates and platelet concentrates), food, or other fluids.

The sensitivity of the tests currently available is only approximately 10^4 organisms per ml, rendering the assay suitable only for gross bacterial contamination. However, sensitivity would be substantially improved by using a sandwich ELISA instead of the direct ELISA currently used. Work is in progress to search for other MAbs which share the same broad reactivity as found for MAb 900 but which recognize a different epitope on EF-Tu. In addition, the ELISA detection system could be changed to a more sensitive one, e.g., a chemiluminescent system, which would improve the sensitivity 10- to 100-fold. In consequence, a detection limit of 10^1 to 10^2 bacteria per ml seems to be an accessible aim.

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REFERENCES

1. **An, G., and J. Friesen.** 1980. The nucleotide sequence of *tuf B* and four nearby tRNA structural genes of *Escherichia coli*. Gene **12:**33–39.
2. **Appel, J. P., C. Pinilla, H. Nimann, and R. Houghton.** 1990. Elucidation of discontinuous linear determinants in peptides. J. Immunol. **144:**976–983.
3. **Bagdasarian, M., R. Lenz, B. Rückert, F. C. H. Franklin, M. M. Bagdasarian, A. J. Frey, and K. N. Timmis.** 1981. Specific purpose plasmid cloning vectors. II. Broad host range, high copy number RSF 1010-derived vectors, and a host vector system for gene cloning in pseudomonas. Gene **16:**237–247.
4. **Baldacci, G., F. Guinet, J. Tillit, G. Zaccai, and A.-M. de Recondo.** 1990. Functional implications related to the gene structure of the elongation factor EF-Tu from *Halobacterium marismortui*. Nucleic Acids Res. **18:**507–511.
5. **Baldauf, S. L., J. R. Manhart, and J. D. Palmer.** 1990. Different fates of the chloroplast *tufA* gene following its transfer to the nucleus in green algae. Proc. Natl. Acad. Sci. USA **87:**5317–5321.
6. **Baldauf, S. L., and J. D. Palmer.** 1990. Evolutionary transfer of the chloroplast *tufA* gene to the nucleus. Nature (London) **344:**262–265.
7. **Beisiegel, U.** 1986. Protein blotting. Electrophoresis **7:**1–18.
8. **Blazek, R., K. Schmitt, U. Krafft, and U. Hadding.** 1990. Fast and simple procedure for the detection of cell culture mycoplasmas using a single monoclonal antibody. J. Immunol. Methods **131:**203–212.
9. **Carlin, N. I. A., S. Löfdahl, and M. Magnusson.** 1992. Monoclonal antibodies specific for elongation factor Tu and complete nucleotide sequence of the tuf gene in *Mycobacterium tuberculosis*. Infect. Immun. **60:**3136–3142.
10. **Cousineau, B., C. Cerpa, J. Lefebvre, and R. Cedergren.** 1992. The sequence of the gene encoding elongation factor Tu from *Chlamydia trachomatis* compared with those of other organisms. Gene **120:**33–41.
11. **Douglas, S. E.** 1991. Unusual organization of a ribosomal protein operon in the plastid genome of cryptomonas phi: evolutionary considerations. Curr. Genet. **19:**289–294.
12. **Eckerskorn, C., and F. Lottspeich.** 1989. Internal amino acid sequence of proteins separated by gel electrophoresis after tryptic digestion in polyacrylamide matrix. Chromatographia **29:**92–94.
13. **Eckerskorn, C., W. Mewes, H. Goretzki, and F. Lottspeich.** 1988. A new siliconized glass fiber as support for protein chemical analysis of electroblotted proteins. Eur. J. Biochem. **176:**509–519.
14. **Filer, D., R. Dhar, and A. V. Furano.** 1981. The conservation of DNA sequences over very long periods of evolutionary time: evidence against intergeneric chromosomal transfer as an explanation for the presence of *Escherichia coli tuf* gene sequences in taxonomically-unrelated prokaryotes. Eur. J. Biochem. **120:**67–77.
15. **Filer, D., and A. V. Furano.** 1980. Portions of the gene encoding elongation factor Tu are highly conserved in prokaryotes. J. Biol. Chem. **255:**728–734.
16. **Filer, D., and A. V. Furano.** 1981. Duplication of the *tuf* gene, which encodes peptide chain elongation factor Tu, is widespread in gram-negative bacteria. J. Bacteriol. **148:**1006–1011.
17. **Hames, B. D.** 1990. One-dimensional polyacrylamide gel electrophoresis, p. 16–22. *In* B. D. Hames and D. Rickwood (ed.), Gel electrophoresis of proteins: a practical approach. Oxford University Press, Oxford.
18. **Jacobson, G. R., and J. P. Rosenbusch.** 1976. Abundance and membrane association of elongation factor Tu in *E. coli*. Nature (London) **261:**23–26.
19. **Jürs, M., H. Peters, K. N. Timmis, and D. Bitter-Suermann.** 1985. Immunoblotting with monoclonal antibodies: a highly specific system for detection and identification of bacterial outer membrane proteins, p. 94–102. *In* K.-O. Habermehl (ed.), Rapid methods and automation in microbiology and immunology. Springer-Verlag, Berlin.
20. **Kamla, V., B. Heinrich, and U. Hadding.** 1992. Species differentiation of mycoplasmas by EF-Tu specific monoclonal antibodies. J. Immunol. Methods **147:**73–81.
21. **Kraus, M., M. Goetz, and W. Löffelhardt**. 1990. The cyanelle str operon from cyanophora paradoxa: sequence analysis and phylogenetic implications. Plant Mol. Biol. **15:**561–573.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
23. **Lechner, K., and A. Böck.** 1987. Cloning and nucleotide sequence of the gene for an archaebacterial protein synthesis elongation factor Tu. Mol. Gen. Genet. **208:**523–528.
24. **Ludwig, W., G. Wallner, A. Tesch, and F. Klink.** 1991. A novel eubacterial phylum: comparative nucleotide sequence analysis of a *tuf*-gene of Flexistipes sinusarabici. FEMS Microbiol. Lett. **78:**139–144.
25. **Ludwig, W., M. Weizenegger, D. Betzel, E. Leidel, T. Lenz, A. Ludvigsen, D. Möllenhoff, P. Wenzig, and K. H. Schleifer.** 1990. Complete nucleotide sequences of seven eubacterial genes coding for the elongation factor Tu: functional, structural and phylogenetic evaluations. Arch. Microbiol. **153:** 241–247.
26. **Lüneberg, E., V. Kamla, U. Hadding, and M. Frosch.** 1991. Sequence and expression in *Escherichia coli* of a *Mycoplasma hominis* gene encoding elongation factor Tu. Gene **102:**123–127.
27. **Montandon, P. E., and E. Stutz.** 1983. Nucleotide sequence of a *Euglena gracilis* chloroplast genome coding for the elongation factor Tu: evidence for a spliced mRNA. Nucleic Acids Res. **11:**5877–5892.
28. **Nagata, S., Y. T. Tsunetsugu-Yokota, A. Naito, and Y. Kaziro.** 1983. Molecular cloning and sequence determination of the nuclear gene coding for mitochondrial elongation factor Tu of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80:**6192–6196.
29. **Olsen, G. J., C. R. Woese, and R. Overbeek.** 1994. The winds of (evolutionary) change: breathing new life into microbiology. J. Bacteriol. **176:**1–6.
30. **Parmeggiani, A., and G. W. M. Swart.** 1985. Mechanism of action of kirro-

mycin-like antibiotics. Annu. Rev. Microbiol. **39:**557–577.
31. **Pieper, U., H. J. Ehbrecht, A. Fliess, B. Schick, F. Jurnak, and A. Pingoud.** 1990. Genetic engineering, isolation and characterization of a truncated *Escherichia coli* elongation factor Tu comprising domains 2 and 3. Biochim. Biophys. Acta **1087:**147–156.
32. **Sasaki, T.** 1991. Evidence that mycoplasmas, gram-negative bacteria, and certain gram-positive bacteria share a similar protein antigen. J. Bacteriol. **173:**2398–2400.
33. **Satoh, M., T. Tanaka, A. Kushiro, T. Hakoshima, and K. Tomita.** 1991. Molecular cloning, nucleotide sequence and expression of the *tuf B* gene encoding elongation factor Tu from Thermus thermophilus HB8. FEBS Lett. **288:**98–100.
34. **Sela, S., D. Yogev, S. Razin, and H. Bercovier.** 1989. Duplication of the *tuf* gene: a new insight into the phylogeny of eubacteria. J. Bacteriol. **171:**581–584.
35. **Siemeister, G., C. Buchholz, and W. Hachtel.** 1990. Genes for the plastid elongation factor Tu and ribosomal protein S7 and six tRNA on the 73 kb DNA from Astasia longa that resembles the chloroplast DNA of euglena. Mol. Gen. Genet. **220:**425–432.
36. **Silbaq, F., and H. Bercovier.** 1993. Nucleotide sequence of *Mycobacterium leprae* elongation factor (EF-Tu) gene. Nucleic Acids Res. **21:**3327.
37. **Wenzig, P., and K.-H. Schleifer.** 1989. Unusually strong immunological cross-reaction between elongation factor Tu of Escherichia coli and Bacillus subtilis. Arch. Microbiol. **152:**258–262.
38. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51:**221–271.
39. **Woese, C. R., O. Kandler, and M. L. Wheelis.** 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. USA **87:**4576–4579.
40. **Yokota, T., H. Sugisaki, M. Takanama, and Y. Kaziro.** 1980. The nucleotide sequence of cloned *tufA* gene of *Escherichia coli*. Gene **12:**25–31.