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Proteomic analysis of the cell envelope fraction of *Escherichia coli*

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Summary. We applied proteomics technologies to analyze a membrane preparation of *Escherichia coli*, wild type strain and of transformants expressing human cytochrome P450s. The proteins were analyzed by two-dimensional electrophoresis and identified by matrix-assisted laser desorption ionization mass spectrometry. The membrane proteins were solubilized with both mild detergents such as CHAPS and strong detergents, such as sodium and lithium dodecyl sulfate, sodium cholate and sodium deoxycholate. In the *E. coli* membrane fraction, 394 different gene products were identified. Approximately 28% of them were predicted to be integral membrane proteins, of which 100 proteins have been predicted to carry one transmembrane region, ten proteins to carry two, and two proteins to include three transmembrane domains. The remaining are probably membrane-associated and cytosolic proteins. Cytochrome P450s did not enter the immobilized pH gradient strips but were efficiently analyzed in a two-dimensional, two-detergent system. Use of strong solubilizing agents resulted in the detection of about 20 membrane proteins, which were not detected following extraction with mild detergents and chaotropes. The present database is one of the largest for membrane proteins.

Keywords: Proteomics – Membrane proteins – *Escherichia coli* – Matrix-assisted laser desorption ionization mass spectrometry – Hydrophobicity

Introduction

A proteomic analysis usually involves the separation of a protein mixture by two-dimensional (2-D) gel electrophoresis and identification of the protein spots by mass spectrometry techniques, mainly by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). For a successful proteomic analysis, several prerequisites must be fulfilled: (i) a protein should be brought and kept in solution during the whole isoelectrocofocusing separation process (ii) it should belong to the category of proteins, that can be separated by 2-D electrophoresis, i.e. it should have average pI (usually between pH 4 and 10) and molecular mass (between 10 and 120 kDa) values and should not be strongly hydrophobic and (iii) it should be usually present at a concentration, so that the corresponding spot(s) are visible in gels, which are stained with Coomassie blue, to can be identified by mass spectrometry (Fountoulakis, 2001; Fountoulakis and Takács, 2002). Many groups have tried to overcome these limitations of proteomics by using a large variety of solubilizing agents (Chevallet et al., 1998; Herbert at al., 1998; Rabilloud et al., 1999; Ferro et al., 2000; Fountoulakis and Takács, 2001) and variations in the 2-D electrophoresis technology (Hartinger et al., 1996).

Membrane proteins are of particular interest in proteomic studies. They exert important functions in signal transduction pathways, ion transport, cell interaction and other processes. Many of them are protein or drug receptors and consequently of pharmaceutical interest. There are numerous articles on the potential detection of hydrophobic and membrane proteins in 2-D gels. However, in most cases a limited number of real hydrophobic and membrane proteins have been detected. The efforts are summarized in several review articles (Herbert, 1999; Malloy, 2000; Santoni et al., 2000). Using current 2-D electrophoresis products, i.e. non-ionic or zwitterionic detergents like CHAPS and non-ionic, mild chaotropes like urea or thiourea, mainly the abundant, hydrophilic components of a protein mixture can be resolved and visualized (Fountoulakis, 2000; Fountoulakis and Takács, 2001).

Here we applied proteomics technologies to analyze a cell envelope preparation from *Escherichia coli*, a facultative anaerobic bacterium of medical interest. *E. coli* is widely used as an expression system for the production of mammalian proteins and as a microbial model to study approaches of drug discovery against infectious diseases by applying proteomics technologies. Several hundreds of unique *E. coli* proteins have been identified until now (Link et al., 1997; Tonella et al., 1998; Fountoulakis et al., 1999; Tonella et al., 2001). We analyzed membranes from *E. coli*, wild type and transformants producing various recombinant human cytochrome P450 (CYP) proteins and cytochrome P450 reductase as an electron carrier. We selected cytochrome P450s for our study because these proteins are strongly involved in drug metabolism, forming adducts and although abundant, to our knowledge, have not been efficiently separated in 2-D gels.

Materials and methods

Materials

Immobilized pH-gradient (IPG) strips were purchased from Amersham Biosciences (Uppsala, Sweden). Acrylamide was obtained from Serva (Heidelberg, Germany) and the other reagents for the polyacrylamide gel preparation were from Bio-Rad (Hercules, CA, USA). Ampholytes (Resolyte 3.5–10) were purchased from BDH Laboratory Supplies (Poole, UK). CHAPS and thiourea were from Sigma (St. Louis, MO, USA), urea, dithioerythritol and EDTA were obtained from Merck (Darmstadt, Germany), lithium dodecyl sulfate from Serva (Heidelberg, Germany), sodium cholate and sodium deoxycholate were from Fluka (Buchs, Switzerland).

Preparation of the E. coli membranes

E. coli strain JM109 and transformants co-expressing human cytochrome 450s, CYP3A4, CYP2D6 or CYP2E1, individually with human NADPH-P450 reductase, were prepared as described by Pritchard et al. (1998) and McGinnity et al. (1999). Single colonies from Luria-Bertani (LB)-agar plates, containing 50 μg/ml ampicillin, were used to inoculate 5–10 ml of LB-broth/ampicillin and the culture was shaken at 200 rpm, 37°C for 14 h. 1.25 ml were used for further inoculation. *E. coli* cells were grown in 125 ml cultures of modified Terrific broth in 1 l Erlenmeyer flasks, supplemented with ampicillin (50 μg/ml), 1 mM thiamin, 0.5 mM δ-aminolevulinic acid and trace elements. The cultures were shaken in a water bath at 200 rpm and 30°C. After 4 h, protein synthesis was induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside and incubation was continued at 30°C for another 24 h. All further operations were performed at 4°C, except where otherwise indicated. The fresh *E. coli* culture was divided into 50 ml polypropylene centrifuge tubes (Falcon) and chilled on ice for about 15 min. Cells were pelleted by centrifugation at 2,800 $\times$ *g* for 12 min and resuspended in 10 ml of ice-cold 50 mM Tris acetate, pH 7.6 buffer, containing 250 mM sucrose and 0.25 mM EDTA. Lysozyme was added to a final concentration of 0.25 mg/ml from a freshly prepared stock solution in water and the suspension was shaken gently for 60 min. After centrifugation at 2,800 $\times$ *g* for 12 min, the pellet was resuspended in 4 ml of ice-cold 100 mM potassium phosphate, pH 7.6, containing 6 mM magnesium acetate, 0.1 mM dithiothreitol and 20% (v/v) glycerol. Protease inhibitors antipain, aprotinin, leupeptin and pepstatin were added to final concentrations of 1 μg/ml and phenylmethylsulfonyl fluoride to 1 mM (from an 100 mM stock solution in isopropanol). The samples were cooled on ice and sonicated in 3–5 s bursts for a total of 60–80 s with a cell disrupter (W-375 sonicator, Ultrasonics). Unbroken cells and debris were pelleted at 12,000 $\times$ *g* for 30 min. The supernatant was centrifuged at 180,000 $\times$ *g* for 60 min. The membrane pellet was resuspended in ice-cold 50 mM Tris acetate, pH 7.6, containing 250 mM sucrose, 0.25 mM EDTA, divided into aliquots and stored at -70°C until use.

Two-dimensional gel electrophoresis

0.2 g of membranes were suspended in 1 ml of 7 M urea, 2 M thiourea, 4% CHAPS and 10 mM dithioerythritol by sonication for 30 s. The mixture was centrifuged at 150,000 $\times$ *g* for 30 min and the supernatant used for 2-D electrophoresis. Membrane proteins were also extracted with 1 ml of either 0.5% sodium cholate, or 1% sodium dodecyl sulfate (SDS), or 1% lithium dodecyl sulfate (LDS), by sonication for 30 s. After centrifugation at 150,000 $\times$ *g* for 30 min, the supernatant was applied onto a 0.4 ml Poros R column, equilibrated with 0.1% trifluoroacetic acid (TFA). The column was washed with 5 column volumes of 30% acetonitrile, containing 0.1% TFA. The proteins were eluted with 2 column volumes of 70% acetonitrile, containing 0.1% TFA. The solution was concentrated in a speedvac apparatus and the last 50 μl were diluted with 1 ml of 20 mM Tris, containing 7 M urea, 2 M thiourea, 4% CHAPS and 10 mM dithioerythritol and concentrated to about 50 μl by ultrafiltration in 10 kDa cut-off ultrafree membranes.

Two-dimensional gel electrophoresis was essentially performed as reported (Langen et al., 1997). Samples containing 0.5–1 mg of total protein were applied on immobilized pH 3–10 nonlinear gradient strips at their basic and acidic ends. Focusing started at 200 V and the voltage was gradually increased to 5,000 V at 3 V/min and kept constant for a further 24 h. The second-dimensional separation was performed in 11% SDS polyacrylamide gels (180 $\times$ 200 $\times$ 1.5 mm), run at 40 mA per gel, in an ISO-DALT apparatus (Amersham Biosciences). The cell envelope protein fraction was also separated using a discontinuous, two-detergent system (Hartinger et al., 1996). The first dimensional separation was performed in 7.5% polyacrylamide gels in the presence of 250 mM benzyldimethyl-*n*-hexadecylammonium chloride (16-BAC) and the second dimensional separation in 11% gels in the presence of 0.1% SDS, as reported (Langen et al., 2000).

After protein fixation for 12 h in 40% methanol, containing 5% phosphoric acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 24 h. Molecular masses were determined by running standard protein markers (Gibco, Basel, Switzerland), covering the range 10–200 kDa. pI values were used as given by the supplier of the IPG strips. Excess of dye was washed out from the gels with H_2O and the gels were scanned in an Agfa DUOSCAN densitometer (resolution 200). Electronic images of the gels were recorded using Photoshop (Adobe) and PowerPoint (Microsoft) software. The images were stored as both tiff (about 5 Mbytes/file) and jpeg (about 50 Kbytes/file) formats.

Matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS)

The MS analysis was performed as previously described (Fountoulakis and Langen, 1997) with certain modifications

(Fountoulakis et al., 2002). Protein spots were excised from 2-D gels with a spot picker and placed into 96-well microtiter plates. Each spot was destained with 100 μl of 30% acetonitrile in 50 mM ammonium bicarbonate and dried in a speedvac evaporator. Each dried gel piece was rehydrated with 4 μl of 3 mM Tris-HCl, pH 9.0, containing 50 ng trypsin (Promega, Madison, WI, USA). After 16 h at room temperature, 7 μl of H_2O were added to each gel piece and the samples were shaken for about 10 min. Four μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid and the standard peptides des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotropic hormone fragment 18–39 (Sigma, 2,465.1989 Da) were added to each gel piece and shaken as above. The application of the samples was performed with a SymBiot I sample processor (PE Biosystems, Framingham, MA, USA). 1.5 μl of the peptide mixture was simultaneously applied with 1 μl of matrix, consisting of a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma) in 50% acetonitrile, containing 0.1% trifluoroacetic acid. Samples were analyzed in a time-of-flight mass spectrometer (Reflex 3, Bruker Daltonics, Bremen, Germany). An accelerating voltage of 20 kV was used. Peptide matching and protein searches were performed automatically with the use of in-house developed software (Berndt et al., 1999). The peptide masses were compared to the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used with a mass tolerance of 0.0025%. Four matching peptides were required for an identity assignment. Unmatched peptides or miscleavage sites were not considered. The automatically identified proteins were checked individually and only *E. coli* proteins with pI and Mr values close to the theoretical were considered (a deviation of about 20% was allowed).

Results

Analysis of the E. coli membrane proteins

The proteins of the *E. coli* cell envelope fraction were separated by 2-D electrophoresis using pH 3–10 IPG strips. Figure 1 shows the two-dimensional gel analysis of the membrane proteins of the wild type and Fig. 2 the analysis of the membrane proteins of a transformant producing cytochrome P450 2E1 and cytochrome P450 reductase. In both cases, 1 mg of total proteins were applied. In both gels, mainly the same proteins were detected, but certain quantitative and qualitative differences were also found which are due to changes introduced in the transformed expression system and partially due to artifacts of the 2-D electrophoresis technology.

394 different gene products were identified in the membrane fraction using MALDI-MS, based on peptide mass fingerprinting (Henzel et al., 1993). The identity assignment relied on a minimal number of 4 matching peptides. In most cases, 5 to 9 matches were found. Theoretical estimation for the probability of a random identity assignment was usually lower than 10^{-5} and was related to the number of peptide matches and consequently to the protein mass. The probability of a wrong identification was practically zero when the assignment was based on seven or more matching peptides, which were usually generated from proteins with a mass of about 50 kDa or higher (Table 1).

About 66% of the proteins are enzyme subunits with a broad spectrum of catalytic activities. The list includes several classes of membrane proteins, like outer membrane, iron-binding, transport, cell division proteins and others. The outer membrane protein 3A (OMPA) is one of the most abundant components (Fig. 1 and 2). Several membrane ATP-binding components were found, like ATP-binding protein (OPPF) and the ATP-binding transport components, phosphate-specific (PSTB), glutamine-transport (GLNQ), maltose-transport system (MALK) and hypothetical components of transport systems (YECC, YHBG). Also several iron-binding proteins (B1451, FDOH, FERA, FHUA), lipoproteins (NLPB, PAL, YAEC) and receptors (vitamin B12 receptor (BTUB), cocilin I receptor (CIRA), cyclic AMP receptor (CRP), ferrichrome-iron receptor (FHUA), iron transport receptor (B1451), maltose high affinity receptor (LAMB), bacteriophage N4 receptor (NFRA), bacteriophage T2 receptor (FADL) and necleoside channel (TSX)), were detected. Table 1 also includes many hypothetical proteins and some of them, like YAET and YEAF, are potential outer membrane proteins.

When the cell envelope fractions of the transformants were solubilized with the IEF-compatible agents CHAPS, urea and thiourea, cytochrome P450 was not detected in the 2-D gels. Only a weak spot representing cytochrome P450 reductase (NCPR) was found (Fig. 2). Cytochrome P450 was probably not dissolved in the mild detergents and chaotropes and therefore we used strong, ionic detergents, i.e. sodium cholate, sodium deoxycholate, SDS and LDS. For the 2-D electrophoretic analysis, the ionic detergents were exchanged against the IEF-compatible detergents CHAPS or Triton $\times$100 (Fig. 3A and 3B, respectively). When sodium cholate was used as a solubilizing agent, cytochrome P450 2D6 (CPD6) was represented by two strong spots, which were found at the sample application positions. Cytochrome P450 reductase (NCPR) was also represented by spots detected at the basic sample application position. Except for the spots representing cytochrome P450 and cytochrome P450 reductase, in general, less and weaker spots representing *E. coli* proteins were found in the cholate extract (Fig. 3A and B) in comparison

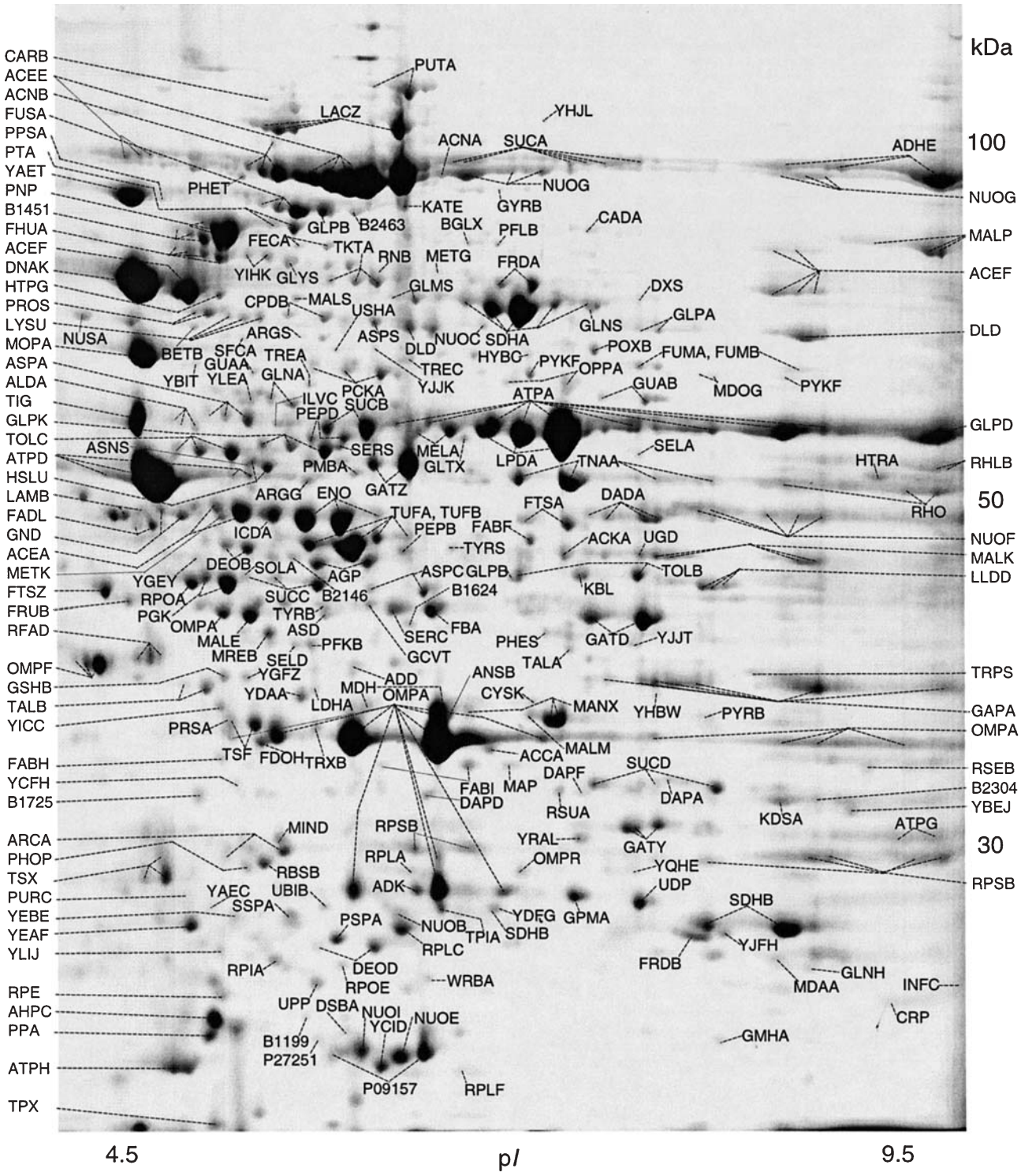

Fig. 1. Two-dimensional electrophoretic analysis of the cell envelope fraction of the *E. coli* proteins. The membrane proteins of the wild type strain were solubilized with 7 M urea, 2 M thiourea and 4% CHAPS and separated in a pH 3–10 nonlinear IPG strip, followed by an 11% SDS-polyacrylamide gel, as stated under Materials and methods. The gel was stained with Coomassie blue. The proteins were identified by MALDI-MS and are designated with their ECOGP names or the accession numbers of other databases. The names of the proteins together with analytical data are listed in Table 1

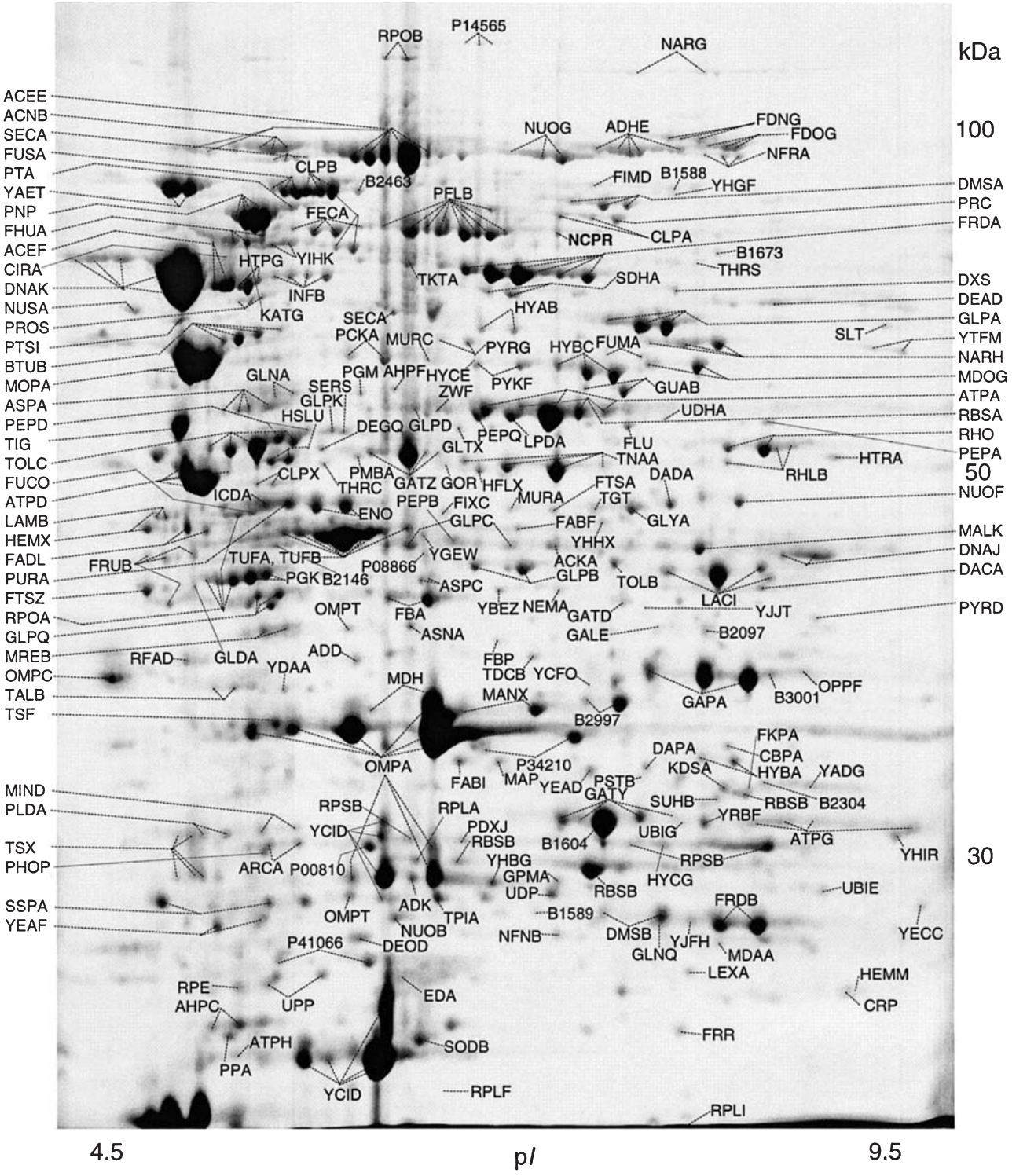

Fig. 2. Two-dimensional electrophoretic analysis of the cell envelope fraction of the *E. coli* proteins. The membrane proteins of the *E. coli* transformant producing recombinant human cytochrome P450 2E1 and cytochrome P450 reductase were analyzed as stated under legend to Fig. 1. The gel was stained with Coomassie blue. The identities assigned are listed in Table 1

Table 1. *Escherichia coli* cell envelope proteins

Name	Protein	Number	Full Name	pI	MM	Matches	Probability	Location	GRAVY	TM	Figure
ACCA	ECOGP:ACCA	P30867	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	5.89	35,333	7	4.70E-09		−0.23	0	1, 3A, 5A
ACCC	ECOGP:ACCC	P24182	Biotin carboxylase	7.10	49,745	7	1.00E-08		−0.17	0	3B
ACCD	ECOGP:ACCD	P08193	AcetylCoA carboxylase, carboxytransferase component, beta subunit	7.65	33,642	5	1.00E-05		−0.13	0	3A
ACEA	ECOGP:ACEA	P05313	Isocitrate lyase	5.60	47,776	8	1.75E-11	C	−0.22	0	1
ACEE	ECOGP:ACEE	P06958	Pyruvate dehydrogenase E1 component	5.50	99,948	15	1.49E-20		−0.43	0	1, 2, 5A, 5B
ACEF	ECOGP:ACEF	P06959	Dihydrolipoamide acetyltransferase comp. (E2) of pyruvate dehydrogenase	4.93	66,111	10	2.05E-09		0	1	1, 2, 5A
ACKA	ECOGP:ACKA	P15046	Acetate kinase	6.25	43,605	10	5.52E-06	C	−0.05	1	1, 2, 3A
ACNA	ECOGP:ACNA	P25516	Aconitate hydrase 1	5.73	98,014	11	7.46E-17		−0.23	1	1, 2
ACNB	ECOGP:ACNB	P36683	Aconitate hydratase 2 (citrate hydro-lyase 2)	5.14	94,009	15	2.57E-24		−0.11	2	1
ACRA	ECOGP:ACRA	P31223	Acridine efflux pump	8.35	42,228	8	2.27E-11	attached IM	−0.25	1	5B
ADD	ECOGP:ADD	P28333	Adenosine deaminase	5.45	36,602	6	8.68E-06		−0.02	0	1, 2
ADHE	ECOGP:ADHE	P17547	Alcohol/acetaldehyde dehydrogenase	6.77	96,579	14	4.75E-20		−0.06	2	1, 2, 3A, 3B
ADK	ECOGP:ADK	P05082	Adenylate kinase	5.54	23,628	8	8.26E-08	C	−0.38	0	1, 2
AGP	ECOGP:AGP	P19926	Glucose-1-phosphatase precursor (G1Pase)	5.53	45,995	10	7.69E-06	P	−0.32	1	1
AHPC	ECOGP:AHPC	P26427	Alkyl hydroperoxide reductase, C22 subunit	4.90	20,862	5	2.10E-07		−0.27	0	1, 2
AHPF	ECOGP:AHPF	P35340	Alkyl hydroperoxide reductase F52A protein	5.49	57,707	7	3.42E-08		−0.12	0	2
ALDA	ECOGP:ALDA	P25553	Lactaldehyde dehydrogenase A	4.91	52,410	11	4.34E-19		−0.05	0	1
ANSB	ECOGP:ANSB	P00805	Periplasmic L-asparaginase II	6.30	36,941	6	2.00E-07	P	−0.11	1	1
ARCA	ECOGP:ARCA	P03026	Aerobic respiration control protein ArcA	5.09	27,388	6	1.19E-07	C	−0.48	0	1, 2
ARGG	ECOGP:ARGG	P22767	Argininosuccinate synthetase	5.14	50,037	9	7.69E-12		−0.37	0	1
ARGS	ECOGP:ARGS	P11875	Arginyl-tRNA synthetase	5.27	64,869	6	9.16E-09	C	−0.25	0	1
ASD	ECOGP:ASD	P00353	Aspartate-semialdehyde dehydrogenase	5.32	40,220	9	1.72E-06		−0.03	0	1
ASNA	ECOGP:ASNA	P00963	Asparagine synthetase A	5.61	36,741	7	5.98E-10	C	−0.29	0	2
ASNS	ECOGP:ASNS	P17242	Asparagine tRNA synthetase	5.05	52,765	7	2.69E-06	C	−0.25	0	1
ASPA	ECOGP:ASPA	P04422	Aspartate ammonia-lyase (aspartase)	5.60	54,713	8	6.08E-11		−0.05	0	1, 2
ASPC	ECOGP:ASPC	P00509	Aspartate aminotransferase	5.58	43,831	9	8.58E-14	C	−0.2	0	1, 2
ASPS	ECOGP:ASPS	P21889	Aspartyl-tRNA synthetase	5.45	66,156	8	1.44E-07	C	−0.26	0	1
ATPA	ECOGP:ATPA	P00822	ATP synthase F1 alpha subunit	6.02	55,415	10	5.62E-11		−0.04	0	1, 2, 3B, 5A, 5B
ATPD	ECOGP:ATPD	P00824	ATP synthase F1 beta subunit	4.74	50,350	13	9.18E-22		−0.11	0	1, 2, 5A, 5B
ATPG	ECOGP:ATPG	P00837	Membrane-bound ATP synthase, F1 sector, gamma-subunit	9.43	31,671	8	7.24E-13	M	−0.22	0	1, 2, 3B, 5A
ATPH	ECOGP:ATPH	P00831	Membrane-bound ATP synthase, F1 sector, delta-subunit	4.78	19,434	5	7.14E-06		0.06	0	1, 2
B1199	ECOGP:B1199	P45510	Putative dihydroxyacetone kinase (EC 2.7.1.2)	5.15	22,731	4	4.52E-05		−0.1	0	1
B1431	ECOGP:B1431	Q04205	Orf, hypothetical protein	8.61	24,525	5	1.00E-10	adherens junctions	−0.27	1	5A
B1451	ECOGP:B1451	P46359	Putative outer membrane receptor for iron transport	5.22	77,326	7	7.97E-08	OM	−0.55	1	1
B1588	ECOGP:B1588	P18775	Putative oxidoreductase, major subunit	7.00	90,802	6	9.61E-08	M	−0.44	1	2
B1589	ECOGP:B1589	P18776	Putative oxidoreductase, Fe-S subunit	6.15	23,648	4	1.00E-05		−0.41	0	2

B1604	ECOGP:B1604	P46477	Orf, hypothetical protein	10.11	33,882	8	6.42E-11		−0.32	1	2
B1624	ECOGP:B1624	P46853	Orf, hypothetical protein	5.92	39,712	5	1.71E-08		−0.26	0	1
B1673	ECOGP:B1673	P44875	Orf, hypothetical protein	6.74	78,681	8	2.71E-09		−0.33	0	2
B1725	ECOGP:B1725	P46381	Orf, hypothetical protein	4.87	32,666	9	1.86E-15		−0.31	0	1
B2097	ECOGP:B2097	P06177	Orf, hypothetical protein	8.51	41,108	8	1.94E-10		−0.15	0	2
B2146	ECOGP:B2146	P09832	Putative oxidoreductase	5.17	45,042	9	7.44E-12		−0.09	0	1, 2
B2304	ECOGP:B2304	P24550	Putative sugar nucleotide epimerase	7.26	32,906	7	1.69E-10		−0.06	0	1, 2, 3B
B2463	ECOGP:B2463	P43837	Putative multimodular enzyme	5.26	82,877	6	3.32E-08		0.05	2	1, 2
B2512	ECOGP:B2512	P42111	Putative dehydrogenase	4.57	41,918	6	2.01E-07		0	1	5B
B2595	ECOGP:B2595	P44553	Orf, hypothetical protein	6.56	27,868	6	1.10E-08	attached to M	−0.48	1	5A, 5B
B2817	ECOGP:B2817	P36548	Putative amidase	10.53	48,927	5	1.00E-07		−0.16	1	3B
B2989	ECOGP:B2989	P23202	Orf, hypothetical protein (URE2 protein)	7.09	34,232	6	1.00E-06		−0.37	0	3A
B2997	ECOGP:B2997	P21950	Putative hydrogenase subunit	6.74	40,368	7	4.83E-06	M	−0.19	2	2
B3001	ECOGP:B3001	P46336	Putative reductase (IOLS protein)	7.26	38,921	8	8.97E-09		−0.39	0	2, 3A
BETB	ECOGP:BETB	P17445	NAD+-dependent betaine aldehyde dehydrogenase	5.06	53,162	5	1.04E-07		−0.08	0	1
BGLX	ECOGP:BGLX	P33363	Periplasmic beta-glucosidase precursor	6.11	83,464	7	2.48E-06	P	−0.26	2	1
BTUB	ECOGP:BTUB	P06129	Vitamin B12 receptor precursor	5.18	68,422	15	1.11E-26	OM	−0.53	0	2, 5B
CADA	ECOGP:CADA	P23892	Lysine decarboxylase 1	6.32	81,606	5	7.59E-07	C	−0.26	0	1
CARB	ECOGP:CARB	P00968	Carbamoyl-phosphate synthase large chain	5.09	118,565	10	1.20E-09		−0.16	1	1
CBPA	ECOGP:CBPA	P36659	Curved DNA-binding protein; functions closely related to DnaJ	6.82	34,433	4	1.05E-04		−0.59	0	2
CDD	ECOGP:CDD	P13652	Cytidine/deoxycytidine deaminase	5.58	31,805	7	5.56E-09		0.001	0	3A
CIRA	ECOGP:CIRA	P17315	Colicin I receptor precursor	4.98	74,078	6	1.00E-07	OM	−0.6	1	2, 3B
CLPA	ECOGP:CLPA	P15716	ATP-dependent Clp protease ATP-binding subunit ClpA	6.27	84,325	13	5.17E-19		−0.3	0	2, 3A
CLPB	ECOGP:CLPB	P03815	ClpB protein (heat shock protein f84.1)	5.30	95,697	15	3.02E-23		−0.357	0	2
CLPX	ECOGP:CLPX	P33138	ATP-dependent clp protease ATP-binding subunit ClpX	5.10	46,726	8	1.69E-10		−0.238	1	2
CPDB	ECOGP:CPDB	P08331	2′,3′-Cyclic-nucleotide 2′-phosphodiesterase	5.48	70,901	8	2.16E-09	P	−0.285	1	1
CRP	ECOGP:CRP		Cyclic AMP receptor protein	8.37	23,796	6	2.12E-07		−0.21	0	1, 2, 3A
CYSK	ECOGP:CYSK	P11096	Cysteine synthase A	5.95	34,525	7	1.40E-10		−0.078	1	1
DACA	ECOGP:DACA	P04287	D-alanine carboxypeptidase (EC 3.4.16.4)	8.60	44,529	11	4.73E-17	IM associated	−0.139	0	2, 3A, 3B, 5A
DACB	ECOGP:DACB	P24228	D-alanyl-D-alanine carboxypeptidase, fraction B; penicillin-binding prot 4	9.22	52,050	6	5.00E-07	P	−0.05	0	3B
DACC	ECOGP:DACC	P08506	D-alanyl-D-alanine carboxypeptidase; penicillin-binding protein 6	8.33	43,638	6	1.00E-07	IM associated	−0.078	1	3A, 3B
DADA	ECOGP:DADA	P29011	D-amino acid dehydrogenase subunit	6.61	47,919	10	4.40E-15	IM bound	−0.215	1	1, 2
DAPA	ECOGP:DAPA	P05640	Dihydrodipicolinate synthase	6.42	31,535	7	7.95E-10	C	0.066	0	1, 2
DAPD	ECOGP:DAPD	P03948	2,3,4,5-Tetrahydropyridine-2-carboxylate N-succinyltransferase	5.53	30,044	8	3.67E-09	C	−0.055	0	1
DAPF	ECOGP:DAPF	P08885	Diaminopimelate epimerase	6.25	30,662	4	1.20E-04	C	−0.082	0	1
DEAD	ECOGP:DEAD		Inducible ATP-independent RNA helicase	9.40	72,725	13	5.01E-15		−0.571	0	2, 3A, 3B, 5B
DEGQ	ECOGP:DEGQ	P39099	Serine endoprotease	5.69	47,176	8	5.79E-10	P	0.055	1	2
DEOB	ECOGP:DEOB	P07651	Phosphopentomutase	5.02	44,684	11	2.36E-19		−0.27	0	1
DEOC	ECOGP:DEOC	P00882	Deoxyribose-phosphate aldolase	5.50	27,944	10	3.94E-07	C	−0.013	0	3A
DEOD	ECOGP:DEOD	P09743	Purine-nucleoside phosphorylase	5.43	26,161	8	1.65E-07		0.07	0	1, 2
DLD	ECOGP:DLD	P06149	D-lactate dehydrogenase, FAD protein, NADH independent	6.66	64,913	9	4.34E-12	membrane bound	−0.445	1	1
DMSA	ECOGP:DMSA	P18775	Anaerobic dimethyl sulfoxide reductase subunit A	6.56	88,248	13	1.50E-18	M	−0.464	0	2
DMSB	ECOGP:DMSB	P18776	Anaerobic dimethyl sulfoxide reductase subunit B	6.58	23,766	5	9.99E-06	M	−0.484	0	2

Table 1. *Continued*

Name	Protein	Number	Full Name	pI	MM	Matches	Probability	Location	GRAVY	TM	Figure
DNAJ	ECOGP:DNAJ	P08622	DnaJ protein	7.79	41,644	7	1.00E-10	C	−0.634	0	2, 3B
DNAK	ECOGP:DNAK	P04475	DnaK protein (heat shock protein 70)	4.67	69,129	12	2.46E-18		−0.408	0	1, 2, 5A, 5B
DPS	ECOGP:DPS	P27430	DNA protection during starvation protein	6.03	18,683	7	7.30E-09		−0.214	0	5A, 5B
DSBA	ECOGP:DSBA	P24991	DsbA	6.29	23,203	5	1.69E-07	P	−0.13	1	1
DXS	ECOGP:DXS	P45205	1-Deoxyxylulose-5-phosphate synthase; flavoprotein	6.61	67,915	8	7.32E-10		−0.08	0	1, 2
EDA	ECOGP:EDA	P10177	2-Keto-3-deoxygluconate 6-P aldolase; 2-keto-4-hydroxyglutarate aldolase	5.47	22,440	4	7.03E-06	C	0.322	0	2
ENO	ECOGP:ENO	P08324	Enolase	5.24	45,683	12	1.14E-20	C	−0.157	1	1, 2
FABF	ECOGP:FABF	P39435	3-oxoacyl-[acyl-carrier-protein] synthase II	6.03	43,246	8	3.11E-06		0.007	1	1, 2
FABH	ECOGP:FABH	P24249	3-oxoacyl-[acyl-carrier-protein] synthase III	4.98	33,779	7	2.52E-10		0.143	1	1
FABI	ECOGP:FABI	P29132	Enoyl-[acyl-carrier-protein] reductase (NADH)	5.72	28,074	8	5.61E-13	IM associated	0.163	0	1, 2
FADL	ECOGP:FADL	P10384	Transport of long-chain fatty acids; sensitivity to phage T2	4.97	48,741	6	1.34E-06	integral M protein	−0.299	0	1, 2
FBA	ECOGP:FBA	P11604	Fructose 1,6-bisphosphate aldolase	5.76	39,350	7	1.39E-07		−0.233	0	1, 2, 3A
FBP	ECOGP:FBP	P09200	Fructose-bisphosphatase	5.89	37,152	5	5.08E-08	C	−0.221	0	2
FDNG	ECOGP:FDNG		Formate dehydrogenase-N, nitrate-inducible, alpha subunit	6.98	113,483	14	1.16E-20		−0.421	0	2
FDOG	ECOGP:FDOG		Formate dehydrogenase-O, major subunit	7.26	113,236	15	4.52E-21		−0.346	0	2, 3A
FDOH	ECOGP:FDOH	P32175	Formate dehydrogenase-O, iron-sulfur subunit	5.07	33,391	5	2.27E-06	integral M protein	−0.36	0	1
FECA	ECOGP:FECA	P13036	Iron(III) dicitrate transport protein FecA precursor	5.79	85,268	15	1.62E-26	OM	−0.516	0	1, 2, 5B
FEPA	ECOGP:FEPA	P05825	Ferrienterobactin receptor precursor	5.34	82,170	5	1.21E-04	integral M protein, OM	−0.581	0	5A
FHUA	ECOGP:FHUA	P06971	Ferrichrome-iron receptor precursor	5.43	82,359	11	2.01E-14	integral M protein, OM	−0.481	1	1, 2
FIMD	ECOGP:FIMD	P30130	Outer membrane protein; export and assembly of type 1 fimbriae	7.12	96,764	7	9.06E-09	OM	−0.415	1	2
FIXC	ECOGP:FIXC	P31575	Flavoprotein; electron transport	5.88	45,901	5	1.53E-06		0.055	1	2
FKPA	ECOGP:FKPA	P45523	FKBP-type peptidyl-prolyl cis-trans isomerase (rotamase)	9.25	28,864	7	1.00E-09	P	−0.458	1	2
FLU	ECOGP:FLU	Q03155	Outer membrane fluffing protein, similar to adhesin	6.49	112,823	11	2.08E-11	OM	−0.287	1	2
FOLE	ECOGP:FOLE	P27511	GTP cyclohydrolase I	7.35	24,928	5	1.00E-04		−0.121	0	3A
FRDA	ECOGP:FRDA	P00363	Fumarate reductase, flavoprotein subunit	6.25	66,499	11	5.85E-13		−0.361	1	1, 2, 3A, 3B
FRDB	ECOGP:FRDB	P00364	Fumarate reductase, anaerobic, iron-sulfur protein subunit	6.49	27,732	6	2.95E-10		−0.223	0	1, 2
FRR	ECOGP:FRR	P16174	Ribosome releasing factor	6.86	20,650	4	6.95E-06	C	−0.521	0	2, 3A
FRUB	ECOGP:FRUB	P24217	PTS system, fructose-specific IIA/fpr component	4.63	39,623	5	3.16E-05	C	−0.028	0	1, 2
FRUR	ECOGP:FRUR	P21168	Transcriptional repressor of fru operon and others	6.98	38,114	6	1.00E-08		−0.339	0	3A
FTSA	ECOGP:FTSA	P06137	ATP-binding cell division protein, septation process, compl. with FtsZ	6.21	45,814	8	2.30E-08		−0.024	1	1, 2
FTSE	ECOGP:FTSE	P10115	ATP-binding component of membrane-associated complex in cell division	10.11	24,480	4	1.00E-04		−0.077	0	3B

FTSZ	ECOGP:FTSZ	P06138	Cell division protein FtsZ	4.47	40,298	6	9.35E-08	inner surface cyt. M	−0.005	1	1, 2
FUCO	ECOGP:FUCO	P11549	L-1,2-propanediol oxidoreductase	4.99	40,846	6	1.72E-05		0.086	0	2
FUMA	ECOGP:FUMA	P00923	Fumarate hydratase class I	6.52	60,773	10	1.75E-14		−0.368	0	1, 2
FUMB	ECOGP:FUMB	P14407	Fumarase B = fumarate hydratase Class I; anaerobic isozyme	6.26	60,580	6	1.02E-07		−0.315	0	1
FUSA	ECOGP:FUSA	P02996	FusA	5.14	77,703	12	1.34E-11	C	−0.285	0	1, 2
GALE	ECOGP:GALE	P09147	UDP-galactose-4-epimerase	6.33	37,412	6	1.00E-06		−0.311	0	2
GAPA	ECOGP:GAPA	P06977	Glyceraldehyde 3-phosphate dehydrogenase A	7.11	35,681	10	1.43E-10	C	−0.133	0	1, 2, 3A
GATD	ECOGP:GATD	P37190	Galactitol-1-phosphate dehydrogenase	6.35	37,822	6	5.10E-05		0.139	2	1, 2
GATY	ECOGP:GATY	P37192	Tagatose-bisphosphate aldolase gaty	6.45	31,279	7	2.44E-10		−0.097	0	1, 2, 3A
GATZ	ECOGP:GATZ	P37191	Putative tagatose 6-phosphate kinase 1	5.64	47,534	10	2.29E-18		−0.12	1	1, 2
GCVT	ECOGP:GCVT	P27248	Aminomethyltransferase (T protein; tetrahydrofol-dep.) of glycine cleavage	5.36	40,235	4	3.76E-05		−0.157	0	1
GLDA	ECOGP:GLDA	P32665	Glycerol dehydrogenase	4.75	40,431	4	1.00E-04		0.206	1	2
GLMS	ECOGP:GLMS	P17169	Glutamine amidotransferase; glucosamine-fruct-6-phosph. aminotransferase	5.79	67,080	9	2.70E-14	C	−0.101	1	1
GLNA	ECOGP:GLNA	P06711	Glutamine synthetase	5.24	52,098	9	5.11E-08	C	−0.292	0	1, 2
GLNH	ECOGP:GLNH	P10344	Glutamine-binding periplasmic protein precursor	9.31	27,173	4	1.77E-05	P	−0.233	1	1
GLNQ	ECOGP:GLNQ	P10346	Glutamine transport ATP-binding protein GlnQ	5.53	26,771	5	1.00E-04	IM associated	−0.128	0	2
GLNS	ECOGP:GLNS	P00962	Glutaminyl-tRNA synthetase	6.23	64,008	5	2.49E-06	C	−0.517	0	1
GLPA	ECOGP:GLPA	P13032	Sn-glycerol-3-phosphate dehydrogenase (anaerobic), large subunit	6.62	59,719	19	4.62E-29	bound cytosolic M	−0.133	0	1, 2, 3A, 3B
GLPB	ECOGP:GLPB	P13033	Sn-glycerol-3-phosphate dehydrogenase, membrane anchor subunit	6.07	45,898	9	4.75E-08		0.062	2	1, 2, 5B
GLPC	ECOGP:GLPC	P13034	Sn-glycerol-3-phosphate dehydrogenase (anaerobic), K-small subunit	8.36	45,105	12	5.95E-20		−0.214	0	2, 3B
GLPD	ECOGP:GLPD	P13035	Sn-glycerol-3-phosphate dehydrogenase (aerobic)	7.47	56,886	12	1.03E-09	C	−0.384	0	1, 2, 3A, 3B
GLPK	ECOGP:GLPK	P08859	Glycerol kinase	5.29	56,480	12	2.82E-20		−0.291	1	1, 2
GLPQ	ECOGP:GLPQ	P09394	Glycerophosphoryl diester phosphodiesterase periplasmic precursor	5.37	40,874	8	5.18E-05	P	−0.552	0	2
GLTX	ECOGP:GLTX	P04805	Glutamyl-tRNA synthetase (EC 6.1.1.17)	5.84	54,180	6	6.42E-08	C	−0.527	0	1, 2
GLYA	ECOGP:GLYA	P00477	Serine hydroxymethyltransferase	6.46	45,459	6	3.75E-08	C	−0.233	1	2
GLYS	ECOGP:GLYS	P00961	Glycine-tRNA synthetase, beta subunit	5.18	76,936	11	5.58E-09	C	−0.219	1	1
GMHA	ECOGP:GMHA	P51001	Phosphoheptose isomerase	6.40	20,972	5	7.61E-05	C	−0.061	0	1
GND	ECOGP:GND	P00350	6-phosphogluconate dehydrogenase, decarboxylating	4.89	51,563	12	2.61E-20		−0.199	0	1
GOR	ECOGP:GOR	P06715	Glutathione oxidoreductase	5.91	49,083	6	1.04E-07	C	−0.112	1	2
GPMA	ECOGP:GPMA	P31217	Phosphoglycerate mutase 1	6.11	28,538	9	5.06E-16		−0.568	0	1, 2, 5A
GSHB	ECOGP:GSHB	P04425	Glutathione synthetase	4.96	35,766	9	4.88E-14		−0.261	0	1
GUAA	ECOGP:GUAA	P04079	GMP synthase (glutamine-hydrolyzing)	5.16	59,041	6	7.85E-07		−0.096	0	1
GUAB	ECOGP:GUAB	P06981	Inosine-5′-monophosphate dehydrogenase	6.38	52,275	12	1.07E-18		−0.09	1	1, 2, 3A
GYRB	ECOGP:GYRB	P06982	DNA gyrase, subunit B	5.97	90,205	1	1.74E-11		−0.458	0	1
HEMM	ECOGP:HEMM	P24208	Enzyme in synthesis of 5-aminolevulinate (glutamyl-tRNA dehydrogenase)	9.59	23,593	6	1.00E-07	OM	−0.643	1	2, 3B
HEMX	ECOGP:HEMX	P09127	Uroporphyrinogen III methylase	4.49	42,936	8	8.10E-10		−0.434	1	2
HFLB	ECOGP:HFLB	P28691	Degrades sigma 32, integral membrane peptidase, cell division	6.16	70,777	11	1.00E-15	integral M protein	−0.263	2	3B, 5B

Table 1. *Continued*

Name	Protein	Number	Full Name	pI	MM	Matches	Probability	Location	GRAVY	TM	Figure
HFLC	ECOGP:HFLC	P25661	Protease specific for phage lambda cII repressor	6.64	37,626	7	1.06E-05		−0.364	1	5B
HFLX	ECOGP:HFLX	P25519	GTP-binding subunit of protease spec. for phage lambda cII repressor	5.89	48,450	6	5.77E-07		−0.328	0	2
HRPA	ECOGP:HRPA	P43329	Helicase, ATP-dependent	7.94	147,216	8	1.00E-08		−0.439	0	3A
HSLU	ECOGP:HSLU	P32168	Heat shock protein HslU	5.11	49,676	6	2.75E-08	C	−0.363	0	1, 2
HTPG	ECOGP:HTPG	P10413	Heat shock protein HtpG	4.95	71,378	13	2.34E-21		−0.523	0	1, 2
HTRA	ECOGP:HTRA	P09376	Protease DO precursor; heat shock protein HtrA	9.05	49,437	7	2.93E-10	P	−0.067	1	1, 2
HYAB	ECOGP:HYAB	P19927	Hydrogenase-1 large subunit	5.88	66,895	15	2.87E-25	membrane bound	−0.21	1	2
HYBA	ECOGP:HYBA	P37179	Hydrogenase-2 small subunit	7.29	36,916	9	6.61E-13	P	−0.444	1	2, 3B
HYBC	ECOGP:HYBC	P37181	Probable large subunit, hydrogenase-2	6.24	62,907	12	2.33E-13	membrane bound	−0.14	0	1, 2, 3A
HYCE	ECOGP:HYCE	P16431	Large subunit of hydrogenase 3 (part of FHL complex)	6.58	65,394	9	1.04E-07		−0.354	0	2
HYCG	ECOGP:HYCG	P16433	Hydrogenase activity	6.51	28,380	5	1.19E-05		−0.149	0	2
ICDA	ECOGP:ICDA	P08200	Isocitrate dehydrogenase	5.01	46,069	6	5.40E-05		−0.153	0	1, 2
ILVC	ECOGP:ILVC	P05793	Ketol-acid reductoisomerase	5.07	54,376	8	6.98E-07		−0.204	0	1
IMP	ECOGP:IMP	P31554	Organic solvent tolerance	4.80	89,843	11	1.00E-11	OM	−0.657	0	5B
INFB	ECOGP:INFB	P02995	Protein chain initiation factor 2	5.96	97,461	11	5.97E-15	C	−0.533	0	2
INFC	ECOGP:INFC	P02999	Protein chain initiation factor IF-3	10.33	20,590	4	6.67E-05	C	−0.656	0	1
KATE	ECOGP:KATE	P21179	Catalase HPII	5.76	84,258	11	5.57E-14	C	−0.498	0	1
KATG	ECOGP:KATG	P13029	Catalase; hydroperoxidase HPI(I)	5.02	80,031	8	6.73E-10		−0.372	1	2
KBL	ECOGP:KBL		2-Amino-3-ketobutyrate CoA ligase (glycine acetyltransferase)	5.87	43,431	10	1.05E-16		−0.049	1	1
KDSA	ECOGP:KDSA	P17579	2-Dehydro-3-deoxyphosphooctulonate aldolase	6.78	31,040	5	1.13E-06	C	−0.027	0	1, 2, 3B
LACI	ECOGP:LACI	P03023	Transcriptional repressor of the lac operon	6.88	38,705	10	6.53E-17		0.038	0	2, 3A
LACZ	ECOGP:LACZ	P00722	Beta-D-galactosidase	5.28	117,321	18	1.48E-30		−0.432	0	1
LAMB	ECOGP:LAMB	P02943	Phage lambda receptor protein; maltose high-affinity receptor	4.66	49,994	9	2.89E-13	integral M protein, OM	−0.515	1	1, 2, 5A, 5B
LDHA	ECOGP:LDHA	P52643	Fermentative D-lactate dehydrogenase, NAD-dependent	5.22	36,853	6	2.28E-06		−0.1	0	1
LEXA	ECOGP:LEXA		Regulator for SOS(lexA) regulon	6.70	22,343	7	1.35E-11		−0.241	0	2
LLDD	ECOGP:LLDD		L-lactate dehydrogenase	6.80	42,872	11	1.40E-17		0.036	0	1, 3B
LON	ECOGP:LON	P08177	Lon protease	6.34	87,724	6	1.00E-09	C	−0.312	0	3A
LPDA	ECOGP:LPDA	P00391	Dihydrolipoamide dehydrogenase	6.10	50,941	9	2.10E-11	C	−0.01	0	1, 2, 3A, 5A
LYSU	ECOGP:LYSU	P14825	Lysyl-tRNA synthetase	4.97	57,847	11	8.48E-16	C	−0.399	0	1
MALE	ECOGP:MALE		Periplasmic maltose-binding protein	5.46	43,360	6	3.81E-08	P	−0.251	1	1
MALK	ECOGP:MALK	P02914	ATP-binding component of transport system for maltose	6.69	41,135	7	4.15E-07	IM associated	−0.029	0	1, 2, 3B, 5A
MALM	ECOGP:MALM		Periplasmic protein of mal regulon	8.37	31,980	5	6.63E-05	P	−0.021	1	1
MALP	ECOGP:MALP	P00490	Maltodextrin phosphorylase	7.53	90,806	16	4.46E-27		−0.343	1	1, 3A
MALS	ECOGP:MALS	P25718	Alpha-amylase	5.40	75,950	7	2.22E-06	P	−0.468	1	1
MANX	ECOGP:MANX	P08186	Phosphotransferase enzyme II, AB component	5.87	35,025	8	6.82E-09	C	−0.051	0	1, 2, 3B, 5A, 5B
MAP	ECOGP:MAP	P07906	Methionine aminopeptidase	5.87	29,711	6	6.99E-09		−0.162	0	1, 2
MDAA	ECOGP:MDAA	P17117	Modulator of drug activity A	6.93	27,068	6	8.85E-09		−0.154	0	1, 2

MDH	ECOGP:MDH	P06994	Malate dehydrogenase	5.56	32,488	7	3.10E-08		0.193	1	1, 2, 3A
MDOG	ECOGP:MDOG	P33136	Periplasmic glucans biosynthesis protein MdoG precursor	7.27	57,876	10	1.25E-14	P	−0.541	0	1, 2, 3A
MELA	ECOGP:MELA	P06720	Alpha-galactosidase	5.73	51,308	8	9.84E-08		−0.181	0	1
METG	ECOGP:METG	P00959	Methionyl-tRNA synthetase	5.71	76,662	6	9.60E-08	C	−0.303	0	1
METK	ECOGP:METK	P30869	S-adenosylmethionine synthetase	4.98	42,153	12	4.26E-16	C	−0.189	0	1
MIND	ECOGP:MIND	P18197	Cell division inhibitor MinD	5.09	29,709	6	1.53E-07	IM associated	−0.118	0	1, 2
MLTA	ECOGP:MLTA	P46885	Membrane-bound lytic murein transglycosylase A	9.85	40,442	5	2.20E-07	attached OM	−0.409	1	5A, 5B
MOPA	ECOGP:MOPA		GroEL protein	4.67	57,463	12	1.05E-18		−0.008	0	1, 2
MREB	ECOGP:MREB	P13519	Rod shape-determining protein mreb	5.14	39,119	8	1.90E-11		0.091	0	1, 2
MURA	ECOGP:MURA	P28909	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	6.10	45,131	7	1.00E-08	C	0.07	0	2
MURC	ECOGP:MURC	P17952	L-alanine adding enzyme, UDP-N-acetyl-muramate:alanine ligase	5.73	53,706	8	3.82E-12	C	−0.16	0	2
NARG	ECOGP:NARG	P09152	Nitrate reductase 1, alpha subunit	6.48	141,084	9	1.00E-08	M associated	−0.511	0	2
NARH	ECOGP:NARH	P11349	Nitrate reductase 1, beta subunit	6.74	58,998	6	2.47E-08	M associated	−0.476	0	2
NEMA	ECOGP:NEMA	P41816	N-ethylmaleimide reductase	6.16	39,492	6	1.00E-07		−0.311	0	2
NFNB	ECOGP:NFNB	P38489	Oxygen-insensitive NAD(P)H nitroreductase	6.18	23,947	8	3.33E-13		−0.21	1	2
NFRA	ECOGP:NFRA	P31600	Bacteriophage N4 receptor, outer membrane protein	7.00	111,467	14	2.46E-20	OM	−0.454	0	2
NLPB	ECOGP:NLPB	P21167	Lipoprotein-34	5.24	36,989	7	3.15E-05	attached OM by LA	−0.253	1	5A, 5B
NUOB	ECOGP:NUOB	P33598	NADH dehydrogenase I chain B	5.50	25,324	5	5.23E-05		−0.32	0	1, 2
NUOC	ECOGP:NUOC	P33600	NADH dehydrogenase I chain C, D	6.40	68,878	6	1.03E-06		−0.307	0	1, 5B
NUOE	ECOGP:NUOE	P33601	NADH dehydrogenase I chain E	6.84	49,774	5	9.71E-13		−0.391	0	1, 5A
NUOF	ECOGP:NUOF	P31979	NADH dehydrogenase I chain F	6.84	49,774	12	2.10E-14		−0.318	0	1, 2, 3A
NUOG	ECOGP:NUOG	P33602	NADH dehydrogenase I chain G	6.21	101,392	15	1.23E-25		−0.318	0	1, 2, 3B
NUOI	ECOGP:NUOI	P33604	NADH dehydrogenase I chain I	5.25	21,037	4	1.22E-05		−0.327	1	1
NUSA	ECOGP:NUSA	P03003	L factor	4.36	55,008	8	5.43E-12		−0.277	0	1, 2
OMPA	ECOGP:OMPA	P02934	Outer membrane protein 3a (II*;G;d)	6.38	37,291	10	1.22E-14	integral M protein, OM	−0.338	1	1, 2, 3A-B, 5A-B
OMPC	ECOGP:OMPC	P06996	Outer membrane protein 1b (Ib;c)	4.43	40,343	9	4.82E-15	integral M protein, OM	−0.533	1	2, 5B
OMPF	ECOGP:OMPF	P02931	Outer membrane protein 1a (Ia;b;F)	4.60	39,309	8	7.39E-09	integral M protein, OM	−0.398	1	1, 5A
OMPR	ECOGP:OMPR	P03025	Response regulator (sensor, EnvZ) affecting transcription of ompC and ompF	6.34	27,339	4	1.89E-04	C	−0.345	0	1
OMPT	ECOGP:OMPT	P09169	Protease VII precursor	5.89	35,540	11	1.51E-19	OM	−0.644	1	2
OPPA	ECOGP:OPPA	P23843	Periplasmic oligopeptide-binding protein precursor	6.46	60,974	10	9.47E-11	P	−0.436	1	1
OPPF	ECOGP:OPPF	P08007	Homolog of Salmonella ATP-binding protein of ABC transport system	7.72	37,572	7	8.07E-07	IM associated	−0.04	0	2
PAL	ECOGP:PAL	P07176	Peptidoglycan-associated lipoprotein	6.80	18,869	5	1.00E-08	attached OM by LA	−0.453	1	5B
PCKA	ECOGP:PCKA	P22259	Phosphoenolpyruvate carboxykinase	5.47	59,891	14	3.87E-25		−0.315	0	1, 2
PDXB	ECOGP:PDXB	P05459	Erythronate-4-phosphate dehyrogenase	6.68	41,683	8	1.00E-11		−0.03	1	3B
PDXH	ECOGP:PDXH	P28225	Pyridoxinephosphate oxidase	9.94	25,586	5	1.00E-05		−0.621	0	3B
PDXJ	ECOGP:PDXJ	P24223	Pyridoxine biosynthesis	5.86	26,595	4	1.46E-05	C	−0.091	0	2
PEPA	ECOGP:PEPA	P11648	Aminopeptidase A/I	7.21	55,358	5	1.03E-07		−0.217	1	2
PEPB	ECOGP:PEPB		Putative peptidase	6.52	49,750	12	2.68E-15		−0.219	0	1, 2, 3B

Table 1. *Continued*

Name	Protein	Number	Full Name	pI	MM	Matches	Probability	Location	GRAVY	TM	Figure
PEPD	ECOGP:PEPD	P15288	Aminoacyl-histidine dipeptidase precursor	5.15	53,110	10	2.10E-13		−0.14	0	1, 2
PEPQ	ECOGP:PEPQ	P21165	Proline dipeptidase	5.91	50,315	9	2.68E-14		−0.24	0	2, 3A
PFKB	ECOGP:PFKB	P06999	6-phosphofructokinase isozyme	5.15	32,663	5	2.09E-05		0.011	0	1
PFLB	ECOGP:PFLB	P09373	Formate acetyltransferase 1	5.88	85,587	13	3.60E-14	C	−0.38	0	1, 2, 3A
PGK	ECOGP:PGK	P11665	Phosphoglycerate kinase	4.93	41,263	9	5.10E-14	C	0.071	0	1, 2
PGM	ECOGP:PGM	P36938	Phosphoglucomutase	5.52	58,609	4	2.42E-05		−0.167	1	2
PHES	ECOGP:PHES	P08312	Phenylalanine tRNA synthetase, alpha-subunit	6.16	36,865	9	1.88E-08	C	−0.324	0	1
PHET	ECOGP:PHET	P07395	Phenylalanyl-tRNA synthetase beta chain	5.05	88,064	13	8.08E-16	C	−0.097	0	1
PHOP	ECOGP:PHOP	P23836	Transcriptional regulatory protein	4.99	25,519	6	1.27E-07	C	−0.256	0	1, 2
PLDA	ECOGP:PLDA	P00631	Outer membrane phospholipase A	5.05	33,142	8	1.49E-12	OM	−0.445	0	2
PMBA	ECOGP:PMBA	P24231	Pmba protein	5.41	48,624	9	9.96E-12	C	−0.16	0	1, 2
PNP	ECOGP:PNP	P05055	Polynucleotide phosphorylase	5.30	79,910	12	2.05E-10	C	−0.169	3	1, 2, 5A, 5B
POXB	ECOGP:POXB	P07003	Pyruvate oxidase	6.25	62,541	6	1.14E-06	M associated	0.007	0	1
PPA	ECOGP:PPA	P17288	Inorganic pyrophosphatase (pyrophosphate phospho.)	4.90	19,806	5	8.44E-06	C	−0.271	0	1, 2
PPSA	ECOGP:PPSA		Phosphoenolpyruvate synthase	4.78	87,835	8	1.16E-07		−0.242	0	1
PRC	ECOGP:PRC	P23865	Tail-specific protease precursor	6.48	76,615	11	1.39E-16	associated cytopl. M	−0.481	1	2
PROS	ECOGP:PROS	P16659	Prolyl-tRNA synthetase	4.98	63,709	11	4.96E-10	C	−0.242	0	1, 2
PRSA	ECOGP:PRSA	P08330	Ribose-phosphate pyrophosphokinase	5.13	34,424	6	4.06E-06		0.103	0	1
PSPA	ECOGP:PSPA	P23853	Phage shock protein, inner membrane protein	5.27	25,477	6	2.62E-09	IM associated	−0.222	1	1
PSSA	ECOGP:PSSA	P23830	Phosphatidylserine synthase; phospholipid synthesis	9.71	53,095	8	1.00E-12	inner membrane	−0.419	0	3B
PSTB	ECOGP:PSTB	P07655	ATP-binding comp. of high-affinity phosphate-specific transport system	6,53	29,236	5	1.00E-04	IM associated	−0.27	0	2
PTA	ECOGP:PTA	P39184	Phosphate acetyltransferase	5.16	77,466	9	1.66E-07	C	0.077	1	1, 2, 3B
PTSI	ECOGP:PTSI	P08839	Phosphoenolpyruvate-protein phosphotransferase	4.61	63,749	9	1.00E-11	C	−0.12	0	2
PURA	ECOGP:PURA	P12283	Adenylosuccinate synthetase	5.22	47,543	5	1.06E-06		−0.199	0	2
PURC	ECOGP:PURC	P21155	Phosphoribosylaminoimidazole-succinocarboxamide synthase	4.89	27,148	6	1.00E-06		−0.369	0	1
PUTA	ECOGP:PUTA	P09546	Proline dehydrogenase, P5C dehydrogenase	5.88	144,466	11	2.11E-13		−0.131	1	1, 5A
PYKA	ECOGP:PYKA	P21599	Pyruvate kinase A	6.65	51,552	8	1.00E-11		−0.024	0	3A
PYKF	ECOGP:PYKF	P14178	Pyruvate kinase	5.98	51,039	9	1.54E-07		−0.081	0	1, 2
PYRB	ECOGP:PYRB	P00479	Aspartate carbomoyltransferase catalytic subunit	6.60	34,462	8	6.61E-11		−0.104	0	1
PYRD	ECOGP:PYRD	P05021	Dihydroorotate dehydrogenase	7.86	36,979	8	4.67E-11	inner side of M	−0.11	0	2
PYRG	ECOGP:PYRG	P08398	CTP synthetase	5.83	60,792	6	7.65E-10		−0.138	1	2, 3A
RBSA	ECOGP:RBSA	P04983	High affinity ribose transport protein	6.04	55,120	9	1.60E-11	IM associated	−0.088	0	2, 3A
RBSB	ECOGP:RBSB	P02925	Periplasmic ribose-binding protein precursor	7.76	30,931	10	2.37E-13	P	−0.03	0	1, 2, 3A
RFAD	ECOGP:RFAD	P17963	ADP-L-Glycero-D-mannoheptose-6-epimerase	4.64	34,985	14	1.33E-26		−0.283	0	1, 2, 5A, 5B
RFAF	ECOGP:RFAF	P37692	ADP-heptose-lps heptosyltransferase II;. lipopolysacchar. core biosynth	7.55	39,302	8	1.00E-09		−0.185	0	3A

RHLB	ECOGP:RHLB	P24229	Putative ATP-dependent RNA helicase	7.71	47,324	10	5.27E-15		−0.346	0	1, 2, 3A, 3B
RHO	ECOGP:RHO	P03002	Transcription termination factor rho	7.30	43,031	9	1.50E-12		−0.297	0	1, 2, 3A, 3B
RNB	ECOGP:RNB	P30850	RNase II, mRNA degradation	5.39	72,844	9	1.00E-07	C	−0.276	0	1
RNC	ECOGP:RNC	P05797	RNase III, ds RNA	6.89	25,648	4	1.00E-04	C	−0.448	0	3A, 3B
RNE	ECOGP:RNE	P21513	RNase E, membrane attachment, mRNA turnover, maturation 5S RNA	5.45	118,352	13	1.00E-20	C	−0.644	1	3A, 3B
RPE	ECOGP:RPE	P32661	D-ribulose-5-phosphate 3-epimerase	5.01	24,595	4	1.16E-04		0.066	0	1, 2
RPIA	ECOGP:RPIA	P27252	Ribosephosphate isomerase, constitutive	5.12	22,903	4	2.01E-04		0.23	0	1
RPLA	ECOGP:RPLA	P02384	50S ribosomal subunit protein L1	10.45	24,714	7	7.66E-08		−0.108	0	1, 2, 3A-B, 5A-B
RPLB	ECOGP:RPLB	P02387	50S ribosomal subunit protein L2	11.62	29,956	11	3.26E-15		−0.699	0	3A, 5A, 5B
RPLC	ECOGP:RPLC	P02386	50S ribosomal subunit protein L3	10.70	22,229	7	2.76E-06		−0.234	0	1, 5A, 5B
RPLD	ECOGP:RPLD	P02388	50S ribosomal subunit protein L4	0.53	22,072	4	1.44E-05		−0.235	0	5A
RPLE	ECOGP:RPLE	P02389	50S ribosomal subunit protein L5	10.30	20,345	6	1.00E-06		−0.282	0	1, 3B, 5A
RPLF	ECOGP:RPLF	AAC76330	50S ribosomal subunit protein L6	10.52	18,949	6	8.67E-05		−0.227	0	2, 5A, 5B
RPLI	ECOGP:RPLI	AAC77160	50S ribosomal subunit protein L9	6.57	15,759	6	1.86E-05		0.09	0	2, 5A, 5B
RPLM	ECOGP:RPLM	P02410	50S ribosomal subunit protein L13	10.74	16,008	6	1.00E-08		−0.54	0	5A, 5B
RPOA	ECOGP:RPOA	P00574	RpoA	4.82	36,717	10	2.21E-16		−0.227	0	1, 2
RPOB	ECOGP:RPOB	P00575	DNA-directed RNA polymerase, beta-subunit	5.00	150,937	13	2.36E-15		−0.394	0	2
RPOC	ECOGP:RPOC	P00577	RNA polymerase, beta prime subunit	7.05	155,886	6	1.00E-07		−0.238	0	3B
RPOE	ECOGP:RPOE	P34086	RNA polymerase, sigma-E factor; heat shock and oxidative stress	5.24	21,739	6	6.89E-07		−0.254	0	1
RPSA	ECOGP:RPSA	P02349	30S ribosomal protein S1	4.72	61,234	8	1.14E-07		−0.3	1	5B
RPSB	ECOGP:RPSB	P02351	30S ribosomal protein S2	7.16	26,783	8	6.91E-13		−0.267	0	1, 2, 3A-B, 5A-B
RPSC	ECOGP:RPSC	P02352	30S ribosomal subunit protein S3	11.07	25,967	6	2.85E-09		−0.421	0	5B
RPSD	ECOGP:RPSD	P02354	30S ribosomal subunit protein S4	10.87	23,511	6	4.88E-06		−0.664	0	5
RPSE	ECOGP:RPSE	AAC76328	30S ribosomal subunit protein S5	10.91	17,592	5	3.36E-05		−0.1	0	5A, 5B
RPSG	ECOGP:RPSG	P02359	30S ribosomal subunit protein S7, initiates assembly	11.15	20,005	4	1.00E-05		−0.459	0	5A
RSEB	ECOGP:RSEB	P46186	Regulates activity of sigma-E factor	9.13	35,784	6	1.12E-06	P	−0.257	0	1
RSUA	ECOGP:RSUA	AAC75244	16S pseudouridylate 516 synthase	6.14	25,963	6	8.50E-06		−0.362	0	1
SAPA	ECOGP:SAPA	P36634	Homolog of Salmonella peptide transport periplasmic protein	7.33	61,554	5	1.00E-06	P	−0.321	1	3A
SDHA	ECOGP:SDHA	P10444	Succinate dehydrogenase, flavoprotein subunit	6.22	65,008	11	2.35E-17		−0.324	1	1, 2, 3A
SDHB	ECOGP:SDHB	P07014	Succinate dehydrogenase, iron sulfur protein	6.68	27,379	8	3.22E-10		−0.299	0	1, 5A
SECA	ECOGP:SECA	P10408	Preprotein translocase; secretion protein	5.41	102,187	11	1.98E-15	plasma membrane	−0.467	0	2
SELA	ECOGP:SELA	P23328	Selenocysteine synthase: L-seryl-tRNA (Ser) selenium transferase	6.45	50,917	6	4.60E-07		−0.054	1	1
SELB	ECOGP:SELB	AAC76614	Selenocysteinyl-tRNA-specific translation factor	6.56	68,995	10	1.00E-13		−0.353	1	3A
SELD	ECOGP:SELD	P16456	Selenophosphate synthase, H(2)Se added to acrylyl-Trna	5.22	37,062	7	5.22E-10		0.068	0	1
SERC	ECOGP:SERC	P23721	Phosphoserine aminotransferase	5.34	39,986	7	2.84E-09	C	−0.112	0	1
SERS	ECOGP:SERS	P09156	Seryl-tRNA synthetase	5.28	48,668	5	3.99E-08	C	−0.447	0	1, 2
SFCA	ECOGP:SFCA	P26616	NAD-linked malic enzyme; malate oxidoreductase	5.06	64,571	9	1.06E-08		−0.192	2	1
SLT	ECOGP:SLT	P03810	Soluble lytic murein transglycosylase	9.00	74,531	12	1.99E-16	P	−0.486	1	2
SODB	ECOGP:SODB	P09157	Superoxide dismutase	5.88	21,309	5	1.35E-04		−0.168	0	2, 3A
SOLA	ECOGP:SOLA	P40859	Sarcosine oxidase-like protein	5.09	41,218	6	1.00E-07	C	−0.233	0	1
SSPA	ECOGP:SSPA	P05838	Stringent starvation protein	5.11	24,346	7	3.72E-10		−0.265	0	1, 2

Table 1. *Continued*

Name	Protein	Number	Full Name	pI	MM	Matches	Probability	Location	GRAVY	TM	Figure
SUCA	ECOGP:SUCA	P07015	2-oxoglutarate dehydrogenase E1 component	6.47	105,565	14	9.02E-09		−0.441	0	1
SUCB	ECOGP:SUCB	P07016	Dihydrolipoamide succinyltransferase component (E2)	5.63	43,984	7	1.11E-06		−0.216	0	1
SUCC	ECOGP:SUCC	P07460	Succinyl-CoA synthetase beta chain	5.28	41,651	8	3.62E-10		0.023	3	1
SUCD	ECOGP:SUCD	P07459	Succinyl-CoA synthetase alpha chain	6.78	30,043	5	4.32E-08		0.161	0	1
SUHB	ECOGP:SUHB	P22783	Enhances synthesis of sigma32 in mutant; extragenic suppressor	6.97	29,211	7	3.54E-08		−0.171	0	2
TALA	ECOGP:TALA	P30148	Transaldolase A	6.21	35,864	9	9.10E-14	C	−0.314	0	1
TALB	ECOGP:TALB	P30148	Transaldolase B	4.95	35,368	9	4.54E-13	C	−0.209	0	1, 2
TDCB	ECOGP:TDCB	P05792	Threonine dehydratase, catabolic	6.01	35,552	7	5.09E-10		0.042	1	2, 3B
TGT	ECOGP:TGT	P19675	TRNA-guanine transglycosylase	6.36	43,022	6	1.67E-07		−0.412	0	2
THRC	ECOGP:THRC	P00934	Threonine synthase	5.17	47,312	7	2.77E-09		−0.096	0	2
THRS	ECOGP:THRS	P00955	Threonyl-tRNA synthetase	6.14	74,707	6	1.00E-06	C	−0.493	0	2
TIG	ECOGP:TIG	P22257	Trigger factor	4.65	48,162	12	1.03E-19		−0.427	0	1, 2, 5A, 5B
TKTA	ECOGP:TKTA	P27302	Transketolase	5.49	72,441	10	1.44E-09		−0.226	0	1, 2
TNAA	ECOGP:TNAA	P00913	Tryptophanase	6.16	53,775	13	8.42E-19		−0.219	0	1, 2, 3A, 3B
TOLB	ECOGP:TOLB	P19935	TolB protein precursor; 21 aa signal peptide	7.89	45,927	9	3.02E-12	P	−0.215	1	1, 2
TOLC	ECOGP:TOLC	P02930	Outer membrane channel; specific tolerance to colicin E1	5.39	53,967	10	2.84E-14	OM	−0.428	1	1, 2, 3B, 5A, 5B
TPIA	ECOGP:TPIA	P04790	Triosephosphate isomerase	5.89	27,125	5	1.48E-07		0.01	1	1, 2
TPX	ECOGP:TPX	AAC74406	Thiol peroxidase	4.59	17,995	4	1.51E-04		0.255	0	1
TREA	ECOGP:TREA	P13482	Trehalase, periplasmic	5.74	63,824	13	1.51E-16	P	−0.579	1	1
TREC	ECOGP:TREC	P28904	Trehalase 6-P hydrolase	5.72	64,082	7	9.88E-06	C	−0.559	0	1
TRPS	ECOGP:TRPS	P00954	Tryptophan tRNA synthetase	6.72	37,642	6	7.03E-09	C	−0.319	0	1
TRXB	ECOGP:TRXB	P09625	Thioredoxin reductase	5.29	34,829	6	1.00E-08	C	−0.193	0	1
TSF	ECOGP:TSF	P02997	Elongation factor Ts	5.07	30,517	8	3.34E-11	C	−0.118	0	1, 2
TSX	ECOGP:TSX	P22786	Nucleoside channel; receptor of phage T6 and colicin K	4.99	33,567	6	1.53E-07	intergal M protein, OM	−0.511	1	1, 2, 5B
TUFA	ECOGP:TUFA	P21694	TufA (elongation factor Tu)	5.24	43,427	11	1.40E-15	C	−0.19	0	1, 2, 3A-B, 5A-B
TUFB	ECOGP:TUFB	P02990	Elongation factor EF-Tu (duplicate gene)	5.24	43,457	11	7.25E-18	C and M associated	−0.196	0	1, 2, 3A-B, 5A-B
TYRB	ECOGP:TYRB	P04693	Tyrosine aminotransferase, tyrosine repressible	5.24	43,763	6	9.00E-09	C	0.015	0	1
TYRS	ECOGP:TYRS	P00951	Tyrosine tRNA synthetase	5.63	47,896	7	1.03E-06	C	−0.33	0	1
UBIB	ECOGP:UBIB	P23486	Ferrisiderophore reductase; flavin reductase	5.26	26,396	9	1.43E-15		−0.123	0	1
UBIE	ECOGP:UBIE	P27851	2-Octaprenyl-6-methoxy-1,4-benzoquinone	8.15	28,153	6	3.27E-05		−0.189	0	2, 3A, 3B
UBIG	ECOGP:UBIG	P17993	3-Demethylubiquinone-9 3-methyltransf. and 2-octaprenyl-6-hydroxy phen. meth.	6.59	26,709	5	3.30E-05		−0.225	0	1
UBIH	ECOGP:UBIH	P25534	2-Octaprenyl-6-methoxyphenol 2-octaprenyl-6-methoxy-1, 4-benzoquinone	5.26	26,396	6	1.00E-15		0.023	1	3A
UDHA	ECOGP:UDHA	P27306	Putative oxidoreductase	6.76	49,839	7	1.47E-10	C	−0.232	0	2
UDP	ECOGP:UDP	P12758	Uridine phosphorylase	6.18	27,312	6	1.00E-06		0.021	0	1, 2
UGD	ECOGP:UGD	P37791	UDP-glucose 6-dehydrogenase	6.41	43,743	8	3.45E-10		−0.206	0	1
UPP	ECOGP:UPP	P25532	Uracil phosphoribosyltransferase	6.17	23,601	7	3.32E-10		0.019	2	1, 2
USHA	ECOGP:USHA	P07024	UDP-sugar hydrolase (5′-nucleotidase)	5.49	60,899	9	3.42E-09	P	−0.41	0	1
UVRA	ECOGP:UVRA	P07671	Excision nuclease subunit A	6.62	104,600	14	1.00E-18	C	−0.288	1	3A

VACB	ECOGP:VACB	P21499	Putative enzyme	8.78	94,110	9	1.00E-10		−0.552	0	3A
VISC	ECOGP:VISC	P25535	Orf, hypothetical protein	7.27	44,444	6	1.00E-08		−0.131	1	3A, 3B
WRBA	ECOGP:WRBA	P30849	Trp repressor binding protein; affects assoc. of trp repressor and operator	5.74	20,832	5	6.76E-07		−0.081	0	1
YADG	ECOGP:YADG	P36879	Putative ATP-binding component of a transport system	9.00	34,682	6	2.03E-10		−0.237	0	2, 3B
YAEC	ECOGP:YAEC	P28635	Putative lipoprotein	4.98	29,470	5	1.00E-04	M attached by LA	−0.137	1	1
YAET	ECOGP:YAET	P46024	Orf, hypothetical protein	4.78	90,610	14	1.90E-20	OM	−0.482	0	1, 2, 5B
YBAU	ECOGP:YBAU	P44092	Putative protease maturation protein	4.78	68,017	8	2.92E-09	type II M protein	−0.405	1	5B
YBDQ	ECOGP:YBDQ	P45680	Orf, hypothetical protein	6.52	15,925	4	1.00E-04		−0.052	0	3A
YBEJ	ECOGP:YBEJ	P41408	Hypothetical protein in gltJ 5′region	9.02	33,513	7	1.49E-09	P	−0.472	0	1
YBEZ	ECOGP:YBEZ	P31544	Putative ATP-binding protein in pho regulon	6.66	40,743	10	8.57E-09	C	−0.37	0	2, 3A
YBIT	ECOGP:YBIT	P44808	Putative ATP-binding component of a transport system	4.82	59,845	5	1.72E-06		−0.376	0	1
YCFH	ECOGP:YCFH	P37346	Orf, hypothetical protein	5.15	30,075	5	3.16E-06		−0.211	0	1
YCFO	ECOGP:YCFO	P44955	Orf, hypothetical protein.	6.25	37,709	5	1.00E-04		−0.286	0	2
YCID	ECOGP:YCID	P21364	Putative outer membrane protein	6.52	22,913	5	1.44E-04	OM	−0.026	1	1, 2, 5B
YDAA	ECOGP:YDAA	P44195	Orf, hypothetical protein	5.09	35,798	8	1.98E-06		−0.207	1	1, 2
YDFG	ECOGP:YDFG	P39831	Putative oxidoreductase	5.92	27,345	5	9.00E-06		−0.174	0	1
YEAD	ECOGP:YEAD	P44160	Orf, hypothetical protein	6.45	33,748	7	2.01E-10		−0.239	0	2
YEAF	ECOGP:YEAF	P37681	Orf, hypothetical protein	5.66	27,781	5	3.65E-05	OM	−0.392	1	1, 2, 5B
YEBE	ECOGP:YEBE	P33218	Orf, hypothetical protein	5.22	23,729	4	1.66E-05		−0.236	1	1
YECC	ECOGP:YECC	P45022	Putative ATP-binding component of a transport system	9.71	27,659	5	3.17E-06	IM associated	−0.147	0	2
YFHO	ECOGP:YFHO		Putative aminotransferase	6.47	46,203	9	1.00E-11		−0.256	0	3A
YFIF	ECOGP:YFIF	P33635	Orf, hypothetical protein	9.20	37,989	5	1.00E-04		−0.681	0	3B
YGEW	ECOGP:YGEW	P21302	Putative carbamoyl transferase	5.14	40,681	8	1.00E-07	C	−0.303	0	2
YGEY	ECOGP:YGEY	P23908	Putative deacetylase	4.69	452,888	7	1.00E-07		−0.251	1	1
YGFZ	ECOGP:YGFZ	P44000	Orf, hypothetical protein	5.02	36,185	6	1.39E-08		−0.247	0	1
YGJO	ECOGP:YGJO	P42596	Putative enzyme	6.98	43,795	5	1.00E-08		−0.196	0	3A
YHBG	ECOGP:YHBG	P31220	Putative ATP-binding component of a transport system	5.98	26,841	8	1.00E-13		−0.124	0	2
YHBW	ECOGP:YHBW	P45529	Putative enzyme	6.46	37,162	5	3.93E-08		−0.166	0	1
YHGF	ECOGP:YHGF	P46837	YhgF	6.54	81,683	9	1.00E-08		−0.34	0	2
YHHX	ECOGP:YHHX	P46853	Hypothetical 38.8 kD protein in gntR-ggt intergenic region	6.52	38,911	7	1.00E-09		−0.346	0	2
YHIR	ECOGP:YHIR	P37634	Orf, hypothetical protein	9.48	31,921	11	4.23E-19		−0.361	0	2, 3A, 3B
YHJL	ECOGP:YHJL	P37650	Putative oxidoreductase subunit	6.27	128,658	13	1.52E-18		−0.596	0	1
YICC	ECOGP:YICC	P23839	Putative alpha helix protein	4.91	33,211	9	2.21E-13		−0.442	0	1
YIHK	ECOGP:YIHK	P32132	65.4 kDa GTP-binding protein in glnA-fdhE intergenic region	4.98	65,633	7	5.60E-09		−0.238	0	1, 2
YJFH	ECOGP:YJFH	P39290	Orf, hypothetical protein	6.63	26,767	5	3.30E-05		−0.033	0	1, 2
YJJK	ECOGP:YJJK	P37797	ABC transporter in nadR-slt intergenic region	5.39	62,518	8	3.48E-07		−0.473	0	1
YJJT	ECOGP:YJJT	P39406	Ribosomal RNA small subunit methyltransferase	6.43	37,829	8	6.90E-13		−0.111	1	1, 3A, 3B
YLEA	ECOGP:YLEA	P26168	Orf, hypothetical protein	5.08	53,971	5	8.55E-05		−0.372	0	1
YLEB	ECOGP:YLEB	P25535	Orf, hypothetical protein	7.15	43,097	5	1.00E-06		0.026	1	3A
YLIJ	ECOGP:YLIJ	AAC73925	Putative transferase	4.90	23,998	4	2.18E-04		−0.18	0	1
YQHE	ECOGP:YQHE	P15339	Orf, hypothetical protein	6.81	26,992	5	7.06E-06		−0.448	0	1

Table 1. *Continued*

Name	Protein	Number	Full Name	pI	MM	Matches	Probability	Location	GRAVY	TM	Figure
YRAL	ECOGP:YRAL	P45528	Orf, hypothetical protein	6.20	31,499	8	2.08E-15		−0.168	0	1
YRBF	ECOGP:YRBF	AAC76227	Putative ATP-binding component of a transport system	6.64	29,192	6	1.35E-08		0.133	0	2
YTFM	ECOGP:YTFM	P39320	Orf, hypothetical protein	9.27	64,895	6	2.66E-11		−0.411	1	2
ZWF	ECOGP:ZWF	P22992	Glucose-6-phosphate dehydrogenase	5.53	56,011	6	9.34E-07		−0.373	0	2
P00810	SW_BA:BLAT_ECOLI	P00810	Beta-lactamase (EC 3.5.2.6) (penicillinase)	5.95	31,666	5	1.11E-04				2
P08866	SW_BA:SOPA_ECOLI	P08866	Sopa protein (protein a)	5.68	43,917	6	3.78E-07				2
P08867	SW_BA:SOPB_ECOLI	P08867	Sopb protein (protein b)	7.95	35,521	5	1.00E-07				3A
P09157	SW_BA:SODF_ECOLI	P09157	Superoxide dismutase [fe] (EC 1.15.1.1)	5.88	21,178	5	1.08E-04				1, 5A
P10635	SW:CPD6_HUMAN	P10635	Cytochrome P450 2D6 (EC 1.14.14.1)	7.24	56,108	9	2.46E-06	M bound, ER	−0.283	1	5B
P13979	SW_BA:TRT2_ECOLI	P13979	Trat complement resistance protein precursor	9.92	26,058	5	8.15E-07	attached OM with LA	−0.175	1	5B
P14066	SW_BA:TRK1_ECOLI	P14066	Trak protein	7.31	25,668	8	2.89E-10				2
P14565	SW_BA:TRI1_ECOLI	P14565	Trai protein (dna helicase i) (EC 3.6.1.-) [contains: trai* protein]	5.94	191,897	9	4.98E-08				2
P15177	SW_BA:TRT4_ECOLI	P15177	Trat complement resistance protein precursor	9.92	25,914	5	8.15E-07	attached OM with LA	−0.171	1	5B
P16435	SW:NCPR_HUMAN	P16435	NADPH-cytochrome P450 reductase (EC 1.6.2.4)	5.38	76,966	12	1.00E-13	ER	−0.386	1	2
P27251	SW_BA:DEF_ECOLI	P27251	Polypeptide deformylase (EC 3.5.1.31) (formylmethionine deformylase)	5.12	19,299	5	2.40E-04				1
P32885	SW_BA:TRT1_ECOLI	P32885	Trat complement resistance protein precursor	9.92	25,971	5	8.15E-07	attached OM with LA	−0.171	1	5B
P34210	SW_BA:OMPP_ECOLI	P34210	Outer membrane protease ompp precursor (EC 3.4.21.-)	6.19	35,477	7	1.95E-10	OM	−0.632	0	2

Proteins from the cell envelope fraction of *E. coli* were analyzed as described under Materials and Methods. They were identified by MALDI-MS, following in-gel digestion with trypsin. The search in protein databases was performed with in house developed software (Berndt et al., 1999). At least 4 matching peptides were required for an identity assignment. The software usually assigned masses to 22 peptides of a mass spectrum. The spectra usually included a number of peptides higher than 22, which were not considered for protein search. The spots representing the identified proteins are indicated in Fig. 1–5 and are designated with their ECOGP names or the accession numbers of the other databases (column "Name"). In the column "Protein", the abbreviated name of the protein and the database used for protein search are indicated. In the column "Number", the corresponding accession number of the SWISS-PROT database is given. In the columns pI and MM, the theoretical pI and molecular mass values are listed. In the columns "Matches" and "Probability", the number of the matching peptides out of the 22 found and the probability of assignment of a wrong protein identity are given. In the column "Location", the annotated subcellular location for the corresponding protein in the SWISS-PROT database is given. In the column "GRAVY", the grand average hydrophobicity values according to Kyte-Doolittle (1982) and in the column "TM", the number of the predicted transmembrane regions according to Klein et al. (1985) are listed. Positive GRAVY values indicate hydrophobic and negative hydrophilic proteins. In the column "Figure", the figure number is given in which the spot(s) representing the corresponding protein can be seen. The data are sorted according to acceding accession names. *C*, cytoplasmic; *ER*, endoplasmic reticulum; *IM*, inner membrane; *LA*, lipid anchor; *M*, membrane; *OM*, outer membrane; *P*, periplasmic

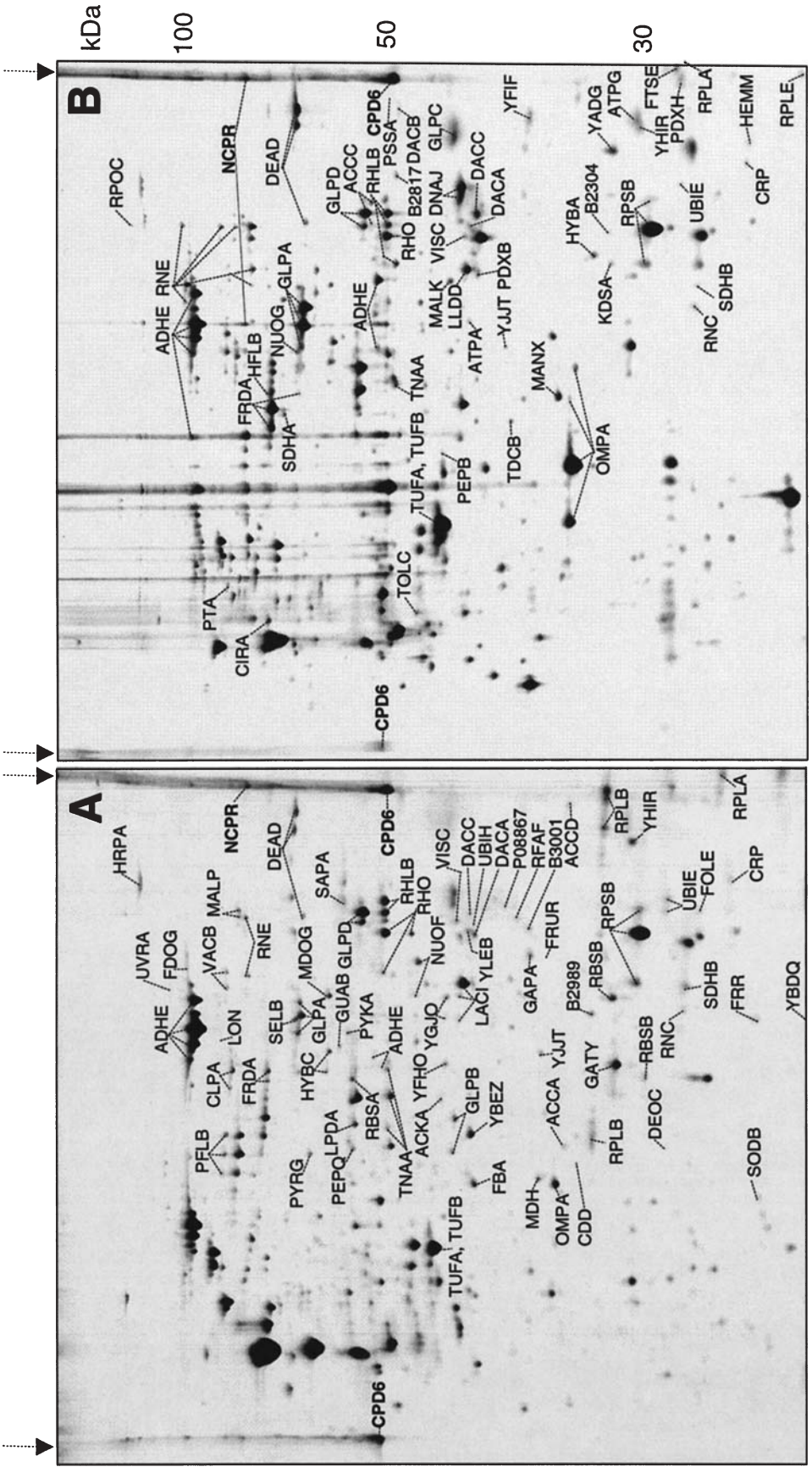

Fig. 3. Two-dimensional electrophoretic analysis of the cell envelope fraction of the *E. coli* proteins. The membrane proteins of *E. coli* producing human cytochrome P450 2D6 were solubilized with 0.5% sodium cholate. Cholate was exchanged against 7M urea, 2M thiourea and 4% CHAPS (**A**) or 7M urea, 2M thiourea and 1% Triton X-100 (**B**) prior to the first dimensional separation on pH 3–10 nonlinear IPG strips. The second dimensional separation was performed in both cases in 11% SDS-polyacrylamide gels. The gels were stained with Coomassie blue. Selected proteins were identified and are designated with their ECOGP names. The arrowheads indicate the sample application positions. The spots representing cytochrome P450 2D6 (CPD6) and cytochrome P450 reductase (NCPR) are indicated in boldface (at the sample application positions). NCPR is also represented by a weak spot within the gel (B). The full protein names are listed in Table 1

with the urea/CHAPS extracts (Fig. 1 and 2). This could be partially due to precipitation during the detergent exchange. Spots representing cytochrome P450 and cytochrome P450 reductase were also detected when sodium deoxycholate, SDS or LDS were employed for protein extraction. These spots were weaker and a lower number of total spots were detected in comparison with the cholate extraction (data not shown).

In the basic region of the Triton ×100 gel (Fig. 3B), spots representing certain proteins were stronger compared to the corresponding spots in the CHAPS gels (Fig. 1, 2 and 3A), for example the spot representing inducible ATP-independent RNA helicase (DEAD). Certain acidic or neutral membrane proteins expressed in large amounts did not completely enter the IPG strips, such as the outer membrane protein 1A (OMPF, theoretical pI 4.60) and the outer membrane protein 3A (OMPA, theoretical pI 6.38) (Fig. 4). The spots representing certain basic proteins, such as 50S ribosomal protein L1 (RPLA, theoretical pI value 10.45), 50S ribosomal protein L2 (RPLB, pI 11.62) and others were detected beyond the application position (Fig. 4).

In order to detect cytochrome P450 within the gels, we used a two-dimensional, two-detergent system. With this system, the proteins were separated in both dimensions in polyacrylamide gels, in the presence of two strong detergents, first of the cationic 16-BAC and then of the anionic detergent SDS (Hartinger et al., 1996). This system enhances the detection of proteins of low solubility. It is compromised by the fact that relatively few and often overlapping spots are detected. As a reference we used an *E. coli* preparation producing outer membrane protein 1A (OMPF) (Fig. 5A). Cytochrome P450 2D6 (P10635) was indeed detected in the gel and was represented by at least three spots, partially overlapping with the spots representing ATP synthase F1 beta subunit (ATPD) (Fig. 5B).

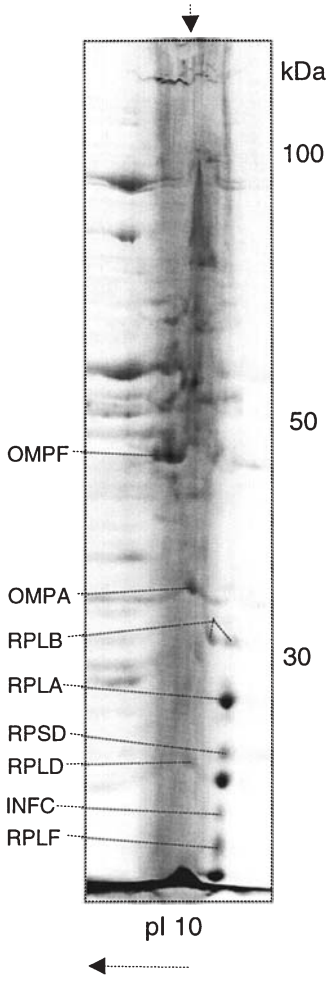

Fig. 4. Partial 2-D image showing the sample application position at the basic end of the IPG strip. The cell envelope fraction of *E. coli* wild type was solubilized with 7M urea, 2M thiourea and 4% CHAPS. Certain proteins did not enter the IPG strip and remained at the application position and certain migrated beyond the application position towards the basic region. The vertical arrowhead indicates the sample application position and the horizontal arrowhead indicates the direction of isoelectric focusing (toward lower pH values). The diffuse spots were generated on account of the CHAPS

Hydrophobicity

Table 1 lists the grand average hydrophobicity (GRAVY) values of the identified proteins, determined according to Kyte and Doolittle (1982), as well as the theoretical transmembrane (TM) regions, determined according to Klein et al. (1985). Positive GRAVY scores indicate hydrophobic and negative scores indicate hydrophilic proteins. The GRAVY values are usually varying in the range ±2. Figure 6A shows the proteins identified in this study sorted according to their GRAVY values. Most of the proteins are hydrophilic and only about 40 proteins have positive GRAVY scores. For comparison, the total *E. coli* proteins were sorted according to their GRAVY values (Fig. 6B). A larger percentage of total proteins have positive GRAVY scores, which indicates that hydrophobic proteins are underrepresented in our list (and they show lower GRAVY scores). Approximately 25% of the proteins of Table 1 carry

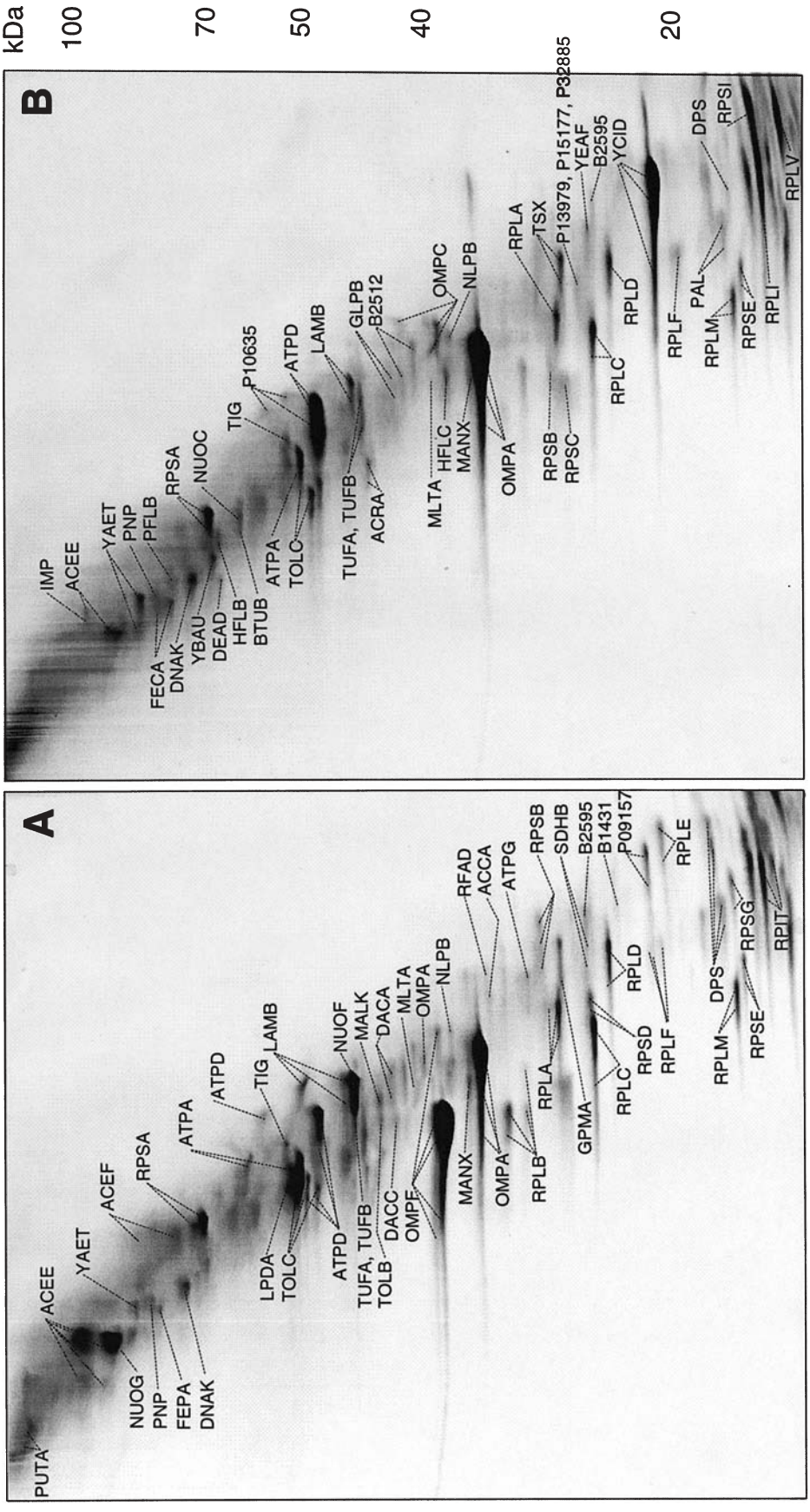

Fig. 5. Membrane proteins of *E. coli*, producing OMPF (**A**) and human cytochrome P450 2D6 (**B**), separated in a 2-D, two-detergent system. First dimensional (horizontal) separation was performed in 7.5% polyacrylamide gels in the presence of 250 mM 16-BAC and the second dimensional separation in 9–16% linear gradient gels in the presence of 0.1% SDS. The gels were stained with colloidal Coomassie blue. The identities assigned are listed in Table 1. Some spots represent more than one protein

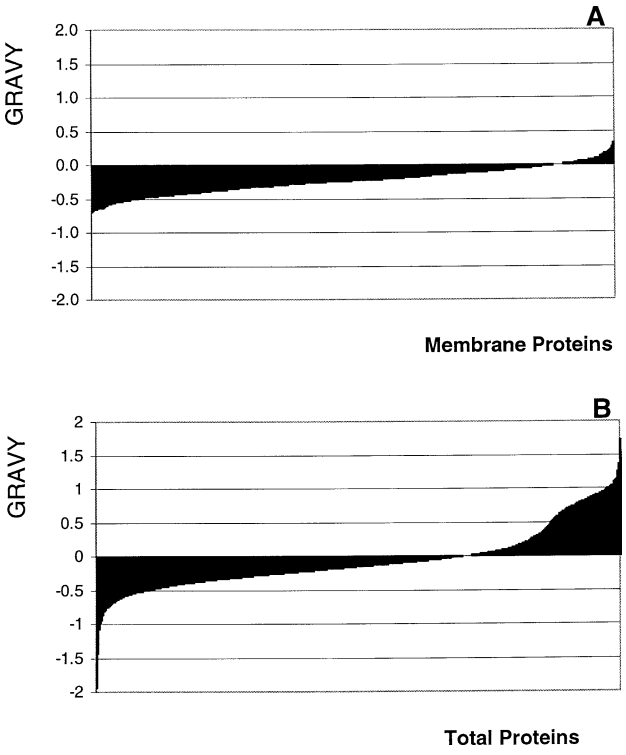

Fig. 6. Hydrophobicity profiles of the *E. coli* proteins identified in this study (**A**) and of all possible proteins of *E. coli* (**B**). The grand average hydrophobicity values (GRAVY) were calculated using the GCG PepPlot software, according to Kyte and Doolittle (1982). Negative GRAVY scores denote hydrophilic and positive scores hydrophobic proteins

transmembrane stretches, 100 proteins include one theoretical transmembrane region, ten proteins carry two and two proteins carry three predicted transmembrane domains. The 3-transmembrane-domain proteins, polynucleotide phosphorylase and succinyl-CoA synthetase beta chain, are really frequently detected proteins. They have been found in 11 *E. coli* samples out of the 21 analyzed by mass spectrometry in our laboratory. The two-transmembrane-domain proteins have been in average detected in 6 samples, the one-transmembrane-domain proteins in four and the proteins lacking a transmembrane domain in five samples.

Subcellular location

The proteins found in the *E. coli* cell envelope fraction (Table 1) should be theoretically membrane or membrane-associated proteins (cytosolic proteins could also be included as contaminants). Because a relatively small percentage of them were hydrophobic or were predicted to carry transmembrane domains, we examined the subcellular location of these proteins. Approximately 25% of the identified gene products had been annotated as membrane proteins in the SWISS-PROT database, 24 as periplasmic, 22 as outer membrane and 50 as membrane, membrane-associated, membrane-attached or integral membrane proteins (Table 1). 76 proteins had been annotated as cytosolic proteins. However, for 10 proteins, annotated as cytosolic, transmembrane domains have been predicted according to Klein et al. (1985), for nine proteins one transmembrane domain and for one protein (polynucleotide phosphorylase) three transmembrane domains. For 222 (56%) proteins, no annotation existed in the SWISS-PROT database concerning their subcellular location (stand May 2001). About 26% of the non-annotated proteins were predicted to be integral membrane proteins, 50 of them carrying one transmembrane domain, seven two domains and one protein three transmembrane domains (Table 1).

Approximately 80% of the annotated membrane or membrane-associated proteins were found in the CHAPS extract (many of them in the cholate and the two-detergent system as well). The use of the two-detergent system resulted in the detection of 15 additional proteins which are annotated as integral membrane proteins or as attached to the outer membrane with a lipid anchor, such as trat complement resistance proteins, which were not detected with the use of IPG strips. In the cholate extract, four membrane or membrane-associated proteins were detected which were not found in the CHAPS extract. For the other proteins exclusively detected in cholate extract, no annotation about their subcellular location existed in the SWISS-PROT database.

Discussion

Our goal was (i) to study the limitations of the proteomics technologies in detecting membrane proteins and (ii) to find out what proteins are really included in a membrane preparation used in biochemical laboratories. Our results could provide a reliable answer to these questions on the basis of a broad statistical analysis. Moreover, we compared experimental, annotated and predicted data concerning protein hydrophobicity and subcellular location. Here we consider as membrane proteins the integral membrane proteins, i.e. these carrying one or more

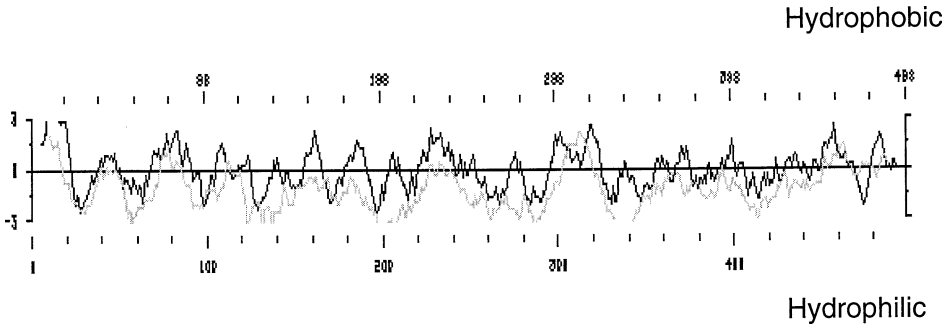

Fig. 7. Hydrophobicity profile of human cytochrome P450 2D6, according to Kyte and Doolittle (1982). The protein is really a hydrophilic with a GRAVY score of −0.238

(predicted) transmembrane domains and the others are designated as membrane-associated proteins.

The membrane fraction used in this study was prepared by centrifugation and pellet washing steps, and essentially included the cell envelopes, i.e. cell wall and cytoplasmic membrane proteins without further enrichment for membrane proteins, for example by carbonate incubation (Molloy et al., 2000). We further included human cytochrome P450s. Cytochrome P450 2D6 is a hydrophilic protein with a GRAVY score of −0.283 and one predicted transmembrane region (Fig. 7). This protein, which could be efficiently solubilized with strong detergents only, did not enter the IPG strips and could be only visualized in a separation system employing two strong detergents (but not an IEF step).

Approximately one fourth of the 396 proteins detected in our membrane fraction were predicted to be integral membrane proteins, carrying mainly one transmembrane domain. Similarly, about one fourth of these proteins have been annotated in the SWISS-PROT database as membrane proteins, however, not always the same species. About 60% of the predicted integral proteins have been annotated as membrane, membrane-associated or periplasmic proteins, about 10% as cytosolic and for the rest no information existed. Probably a significant percentage of the not annotated species of this study are in fact membrane proteins (68 out of the 222 proteins with no annotation include one predicted TM region). The GRAVY values provide an indication of the hydrophobicity of the proteins, however, they do not help predicting the subcellular location of the protein (about half of the 40 proteins with positive GRAVY values carry predicted transmembrane domains). Most of the proteins identified in this study, even those predicted to carry transmembrane domain(s), are hydrophilic (Table 1). Our results showed that only 10% of the proteins detected in the membrane fraction have positive GRAVY values, i.e. they are hydrophobic. Most likely, the local hydrophobicity in protein stretches is essential and determines whether the protein will enter the IPG strip or not. Strong hydrophobic stretches are probably responsible for lack of detection of cytochrome P450s in 2-D gels.

We found several discrepancies between annotated and predicted data concerning the protein subcellular location. For ten of the proteins annotated as cytosolic in the SWISS-PROT database, transmembrane regions have been predicted using the GCG PepPlot tool (Klein et al., 1985), for example for acetate kinase and enolase, one transmembrane region has been predicted. LAMB had been annotated as spanning the membrane 18 times, however, only one theoretical transamembrane region has been predicted. Similarly, the receptor of bacteriophage T2, which is involved in the transport of long-chain fatty acids across the outer membrane (FADL), is annotated as an integral membrane protein with several potential transmembrane domains. For this protein, a negative GRAVY value (−0.299) was calculated and no transmembrane stretch was predicted (Table 1). Therefore, results derived from predictions should be considered with caution. In our cell envelope fraction, proteins were detected which are annotated as cytosolic in the SWISS-PROT database. This may mean that our membrane preparation had not been subjected to sufficient washing steps, that certain proteins are possibly membrane-associated and that the original preparation included a higher number of proteins carrying hydrophophobic domains, which did not enter the IPG strips and consequently have not been detected.

The present database is one of the largest for *E. coli* proteins, including 72 membrane or membrane-

associated proteins and probably many additional membrane proteins not annotated yet. It includes the high molecular mass proteins YAET (91 kDa), FIMD (96 kDa), FLU (111 kDa), NFRA (111 kDa) and NARG (141 kDa), which are annotated as outer membrane or membrane-associated proteins and which often do not enter the IPG strips. About 110 proteins detected in our previous list of *E. coli* proteins bound to hydroxyapatite (Fountoulakis et al., 1999) are included in Table 1 and also about 100 proteins detected by Link et al. (1997) and 25 proteins found by Molloy et al. (2000). Application of strong solubilizing substances, such as cholate and 16-BAC, resulted in the efficient detection of at least 20 membrane proteins not detected employing the IEF-compatible agents. The detection of a large number of membrane proteins, potential and confirmed, brings us a step ahead in identifying low-abundance, membrane-spanning drug targets. In general, analysis of the proteins of a membrane fraction increases the likelihood of detecting membrane species by ten-fold compared to the total protein analysis (Fountoulakis and Takács, 2001).

In summary, we applied proteomics technologies to analyze a standard preparation of *E. coli* highly enriched in membrane proteins. In the cell envelope fraction, we identified 394 different gene products, including human cytochrome P450s and cytochrome P450 reductase, expressed in *E. coli* transformants. Approximately one third of the proteins were predicted to be integral membrane and membrane-associated, carrying one to three transmembrane domains. For 56% of the proteins, no annotation about their subcellular location existed in the SWISS-PROT database. About 25% of the annotated proteins of this study are membrane and 20% cytosolic proteins. Strong detergents were useful in the detection of membrane and membrane-associated proteins. The GRAVY scores appear insufficient for prediction of a subcellular location. For certain proteins there were discrepancies between predicted transmembrane domains and annotated subcellular location. Our results show that membrane preparations are usually contaminated with cytosolic proteins and that the currently existing knowledge about protein subcellular location is limited even in well-studied organisms.

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