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Differentiation of the Ribosomal Protein Compositions in the Genus *Escherichia* and Its Related Bacteria

SYOZO OSAWA, TAKUZI ITOH, AND EIKO OTAKA

Department of Biochemistry and Biophysics, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Kasumi-Cho, Hiroshima, Japan

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Compositions of the ribosomal proteins of 60 bacterial strains belonging to the genus *Escherichia* and its related genera were examined by use of a column of carboxymethyl cellulose. The ribosomes were classified into seven groups and were further differentiated into several types (subgroups) according to their protein compositions. It was shown that ribosomal protein composition is a useful characteristic for studies of bacterial taxonomy.

We have devised a technique of fractionating bacterial ribosomal proteins in which two different ribosomal samples, one ^{14}C-labeled and the other ^{3}H-labeled, are mixed and analyzed simultaneously by chromatography on a carboxymethyl cellulose (CMC) column. By use of this technique in conjunction with gel electrophoresis, the ribosomal proteins of *Escherichia coli* can be fractionated into about 40 components. The CMC chromatography also permits one to differentiate between ^{14}C- and ^{3}H-labeled components on the basis of their minor chemical differences. We have been able to show that two bacterial cultures which have a similar guanine plus cytosine (GC) content in their deoxyribonucleic acid (DNA), and which are genetically or taxonomically allied, exhibit a similarity in their ribosomal protein compositions, whereas the compositions among bacteria having different GC contents were very different from one another (7-9). These results immediately suggested a possible application of the ribosomal protein analyses to the phylogenic taxonomy of bacteria. We chose the genus *Escherichia* and its related enterobacteria for our analysis of ribosomal protein compositions. The results and significance of this study are described.

MATERIALS AND METHODS

The bacterial strains used in this study are shown in Table 1. The labeling of ribosomal proteins with ^{3}H- or ^{14}C-lysine, the preparation of labeled 30*S* and 50*S* ribosomal subunits by sucrose gradient centrifugation, and the comparative fractionation of two ribosomal protein samples, one ^{14}C-labeled and the other ^{3}H-labeled, by column chromatography on CMC (CM 52) were described in previous papers (7-9).

The nomenclature for each ribosomal protein component was essentially the same as already described (9; see also Fig. 1). The correspondence of the components in various bacterial strains to those of *E. coli* C was determined by considering their chromatographic positions, size, and tryptophan contents in comparison with those of *E. coli* C. A part of such a determination was described in a previous paper (2). Some of the component numbers might be changed in the future when a more detailed characterization of each component has been made. In addition to the component number, the symbol for ribosome groups or types (subgroups) is given in parentheses after the component number, such as 50-5(f-f), 30-3(Az), etc., by the following rule. No symbol is given for components which are chromatographically indistinguishable from those of *E. coli* C-type ribosomes. The specific symbol is given to the group- or type-"specific" component in the order of appearance in the text. For example, in *E. freundii f-f* type ribosomes, the symbol f-f is given to the component which is not common to *E. coli* C. In *E. freundii f-m*-type ribosomes, which appear just after the *f-f* type in the text, the f-m symbol is given to the component which is not common either to *E. coli C* or *f-f* type. The f-f symbol is applied to the component common to the *f-f* type but not to *E. coli* C (see Table 2).

Throughout this paper, we use the strain names as they are described in the original label without considering taxonomic nomenclature. Recently adopted nomenclature for them may be found in Table 1.

RESULTS

Ribosomal protein compositions in the genus Escherichia (including Citrobacter). In *Bergey's Manual of Determinative Bacteriology*, 7th ed., four species and four varieties were described

TABLE 1. *Strains used and classification of ribosomes according to their protein compositions*[a]

Ribosome group	Ribosome type (subgroup)	Bacterial strain	Remarks
Escherichia coli	*C*	*Escherichia coli* ATCC 11775	Neotype strain
		Escherichia coli C*	From N. Otsuji
		Escherichia coli IAM 1101*	Originally from IID
		Escherichia coli IAM 1137*	Originally from Hattori
		Escherichia coli IAM 1159*	Originally from IID
		Escherichia coli IAM 1204*	Originally from Pasteur Institute
		Escherichia coli IAM 1222*	Originally from Pasteur Institute
		Escherichia coli IAM 1239*	= ATCC 11246
		Escherichia coli IAM 1253*	= ATCC 3655
		Escherichia coli IFO 12433	From S. Taniguchi
		Escherichia coli MRE 600	From B. Jarry
		Escherichia coli subsp. *acidilactici* ATCC 128	
		Escherichia coli subsp. *neapolitana* ATCC 133	
		Escherichia coli subsp. *communior* IAM 1518*	= ATCC 745
		Escherichia coli subsp. *communior* IAM 1519*	= ATCC 206
		Escherichia coli subsp. *communior* IAM 1272*	= ATCC 7009
		Escherichia aurescens ATCC 12814	L. W. Parr T6
		Escherichia anaerogenes ATCC 6878	D. Nabarro 2680
		Escherichia sp. ATCC 9492	Originally from Golden State Co. Ltd.
		Paracolobactrum coliforme[b] 57879	From CSDH
		Paracolobactrum coliforme[b] 59386	From CSDH
		Shigella alkalescens ATCC 19413	Reference strain
	B	*Escherichia coli* ATCC 11303*	Luria strain B
		Escherichia coli B (H)*	From Y. Takagi
	K	*Escherichia coli* ATCC 10798*	Clifton K-12
		Escherichia coli K-12 W1895*	From Lederberg
		Escherichia coli Q13*	From Gilbert
		Escherichia coli JC 411	From A. J. Clark
		Escherichia coli AB 313	From M. Ohki
	KW	*Escherichia coli* K-12 W3637*	From Lederberg
	D	*Paracolobactrum coliforme* 48859	From CSDH
Escherichia freundii	*f-f*	*Citrobacter freundii* ATCC 8090	Neotype strain
		Escherichia anindolica ATCC 6879	Originally from Sarles and Hammer
	f-m	*Citrobacter freundii* ATCC 6750	Original strain of *E. intermedia*
		Escherichia freundii AHU 1533	Originally from H. Sakasaki, from grass silage
		Escherichia freundii AHU 1534	Originally from H. Sakasaki, from grass silage
		Citrobacter freundii ATCC 10787	Originally from IID, strain Shoku No. 2
		Escherichia coli IAM 1132*	

(*continued*)

under the genus *Escherichia*. They are *E. coli* plus its four varieties (var. *communis*, *acidilactici*, *neapolitana*, and *communior*), *E. aurescens*, *E. freundii*, and *E. intermedia*. In addition to them, two species designated under the names of *Paracolobactrum coliforme* and *P. intermedium* were described as closely allied to *E. coli* and *E. freundii*, respectively. In the American Type Culture

TABLE 1—*continued*

Ribosome group	Ribosome type (subgroup)	Bacterial strain	Remarks
		Escherichia coli IAM 1182*	Antibiotic test strain
		Paracolobactrum intermedium[c] 4920	= ATCC 11606, neotype strain
		Paracolobactrum intermedium[d] 54207	From CSDH
		Paracolobactrum intermedium[d] 54208	From CSDH
		Paracolobactrum intermedium[d] 54672	From CSDH
		Paracolobactrum intermedium[d] 57191	From CSDH
	f-i	*Paracolobactrum intermedium*[d] 49761	From CSDH
Escherichia paraintermedia	*p*	*Escherichia intermedia* AHU 1412	Originally from Y. Suzuki, from "Vita-milk"
	j	*Escherichia intermedia* ATCC 21073	Originally from Hayashibara Co., Ltd., from soil
Escherichia adecarboxylata	*a*	*Escherichia adecarboxylata* ATCC 23216	Originally from H. Leclerc (1783), from drinking water (type strain)
Shigella	—	*Shigella dysenteriae* ATCC 13313	Neotype strain
Salmonella	—	*Salmonella typhimurium* LT7	From T. Miyake
		Salmonella typhimurium L$^+$ 15 – 11	From T. Iino
		Salmonella abony[e] SW 803	From T. Iino
		Salmonella heidelberg[f] SW 1092	From T. Iino
		Paracolobactrum arizonae[g] LYO 10250	From CSDH
		Paracolobactrum arizonae[g] 33886	From CSDH
		Paracolobactrum arizonae[g] 47036	From CSDH
Arizona	—	*Arizona arizonae*[g] ATCC 13314	
		Paracolobactrum arizonae[g] LYO 10313	Neotype strain
—	—	*Proteus morganii* IFO 3848	
—	—	*Paracolobactrum aerogenoides* LYO 3565	From CSDH

[a] Abbreviations used: ATCC, American Type Culture Collection; IAM, Institute of Applied Microbiology, University of Tokyo; CSDH, Connecticut State Department of Health; AHU, Faculty of Agriculture, Hokkaido University, Sapporo; IFO, Institute for Fermentation, Osaka; IID, Institute for Infectious Diseases, University of Tokyo, Tokyo; Ribosomal protein compositions of the strains marked with an asterisk were reported in a previous paper (9), and are included here for convenience.

[b] Recent nomenclature according to ATCC Catalogue, Edwards and Ewing (3), and Ewing (4): *Escherichia coli*.

[c] Recent nomencalture (above sources): *Citrobacter freundii*.

[d] Recent nomenclature (above sources): *Citrobacter* sp.

[e] Recent nomenclature (above sources): *Salmonella* sp. (serotype *abony*).

[f] Recent nomenclature (above sources): *Salmonella* sp. (serotype *heidelberg*).

[g] Recent nomenclature (above sources): *Arizona hinshawii*.

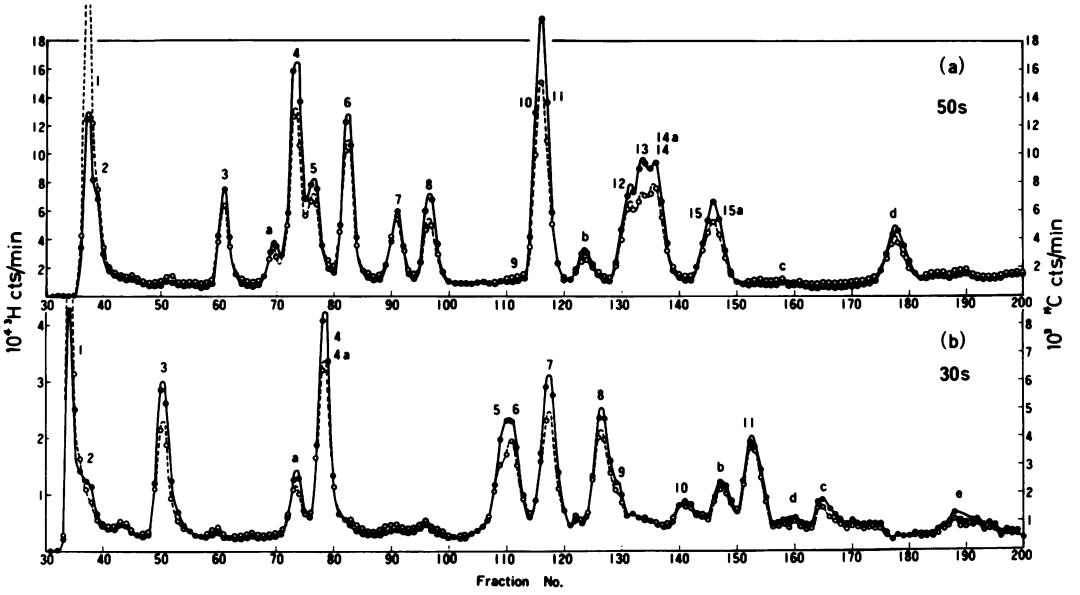

FIG. 1. *Chromatography on CMC columns of ribosomal proteins from ATCC 11775 (E. coli C) and E. aurescens ATCC 12814.* (○) ^{14}*C-lysine-labeled E. coli C protein;* (●) ^{3}*H-lysine-labeled ATCC 12814 protein; (a) 50S; (b) 30S. Throughout all the figure legends, the name in parentheses after the strain name represents the ribosome group or type to which the ribosomes of the strain belong.*

Collection Catalogues of Strains, 9th ed., three more species, *E. adecarboxylata*, *E. anaerogenes*, and *E. anindolica*, were included. (*E. freundii* was transferred to the genus *Citrobacter*, but in this paper we adopt *Escherichia freundii* for the sake of convenience.) In addition, *E. intermedia* and *P. intermedium* were made synonymous with *E. freundii*, and *P. coliforme* was considered synonymous with *E. coli*. Furthermore, *Shigella alkalescens* and *S. dispar* have sometimes been treated as special types of *E. coli* (3).

We have collected 48 cultures [(including 19 previously reported strains (8)] which had been identified as the above enumerated species (except *Shigella dispar*) and have analyzed their ribosomal protein compositions.

For convenience in presenting the results, the ribosomes whose protein compositions revealed more than 90% similarity are classified into a group and further differentiated into types (or subgroups) to distinguish ribosomes with small differences in their protein compositions.

E. coli group ribosomes. In a previous paper (8), we examined the ribosomal protein compositions of several cultures labeled as *E. coli* and *E. coli* subsp. *communior*, and classified them into *C*, *B*, *K*, *KW 3637*, and *R* types. Additional information on the *E. coli* group ribosomes is given below.

E. coli type C. The ribosomal protein compositions of the following 11 strains were indistinguishable from that of *E. coli C* type: three *E. coli* strains, two "subspecies" of *E. coli*, two strains of *P. coliforme*, *E. aurescens* ATCC 12814, *E. anaerogenes* ATCC 6878, *Escherichia* sp. ATCC 9492, and *S. alkalescens* ATCC 19413 (Table 1). Figure 1 shows a simultaneous analysis of 50*S* (or 30*S*) ribosomal proteins from *E. coli* ATCC 11775 (^{14}C) and *E. aurescens* ATCC 12814 (^{3}H). Adding the 11 strains previously reported to the above, we now have 22 strains which carry the *E. coli C*-type ribosomes.

E. coli type B. It was reported that ribosomes from *E. coli* ATCC 11303 and *E. coli* B were different from the *E. coli* type *C* ribosomes in having a specific 30*S* ribosomal protein component, 30-4(B), in place of 30-4 (8). In the present study, no strains with *B*-type ribosomes were found.

E. coli type K. It was reported that ribosomal protein compositions of *E. coli* ATCC 10798, K-12 W1895, and Q13 may be distinguished from the *C* type by the presence of a specific 30*S* component 30-7(K), in place of 30-7 (9; American Type Culture Collection Catalogues of Strains). It has now been found that two laboratory strains of *E. coli* carry ribosomes of the *K* type (Table 1).

E. coli type KW. The type *KW* (=*KW 3637* type in reference 8) is distinguished from the *K* type in having a specific 30*S* component, 30-9 (KW), instead of 30-9 (8, 11). No strains having the *KW* type have been found in the present study.

E. coli type D. A hitherto unreported ribosome type, designated here as type *D*, was found in a strain of *P. coliforme*, 48859. Chromatographic comparisons of ribosomal proteins were made between strain 48859 and *E. coli* C. No differ-

TABLE 2. *Differentiation of the protein components in various ribosome groups or types*[a]

Ribosome group or type	Component no.																											
	50S															30S												
	2	3	a	4	5	6	7	8	10	11	12	13	14	15	15a	d	3	a	4	4a	5	6	7	8	9	10	b	11	e
E. coli C																													
E. coli B																			B										
E. coli K																							K						
E. coli KW																							K		KW				
E. coli D							D						D																
E. freundii f-f			f-f	f-f	f-f	f-f	f-f	f-f			f-f	f-f						f-f	f-f		f-f					f-f			
E. freundii f-m			f-f	f-m	f-f	f-f	f-f	f-f			f-f	f-f						f-f	f-f		f-f					f-f			
E. freundii f-i			f-f	f-i	f-f	f-f	f-f	f-f			f-f	f-f						f-f	f-f		f-f					f-f			
E. paraintermedia p		p	f-f	f-m			p				f-f	f-f			p?	p		f-f	p			p	?	p		p			
E. paraintermedia j		p	f-f	j	j		j				f-f	f-f			p?	p		f-f	p			p	?	p		p			
E. adecarboxylata	a	p	f-f	a	f-f	a	f-f	f-f				f-f	?			p	a	f-f	p			a				p			a
Shigella									Sg		Sg																		
Salmonella			f-f	S		f-f	f-f						S		?			f-f			f-f					f-f			
Arizona				S		f-f	Az					f-f	S		?		Az	f-f			f-f					f-f			

[a] Components with no symbol indicate those indistinguishable from *E. coli C*.

ence in the 30*S* proteins of the two strains was found. In the 50*S* proteins, the 50-7 component of 48859, i.e., 50-7(D), was positioned between the 50-6 and 50-7 components of the *C*-type, and the 50-14 component of 48859, i.e. 50-14(D), was found a little after the 50-14 component of the *C* type. Since the positions of the 50-7(D) and 50-14(D) appear to be close to those of 50-7(f-f) and 50-14(S) from *Salmonella*, respectively (see below), it was confirmed that the 50-7(D) component is situated between 50-7(f-f) and 50-7, and the 50-14(D) component is between 50-14 and 50-14(S).

It was found that "*E. coli*" strains having *R*-type ribosomes belong to *E. freundii* and not to *E. coli* (see below). Thus, the *R* type was removed from the *E. coli* ribosome group.

Kaltschmidt et al. (5, 6) recently reported that strains K and B of *E. coli* differ in two ribosomal proteins and strain C differs from strain K and strain B in only one protein, respectively, based on two-dimensional electrophoresis of ribosomal proteins. They also reported that the ribosomal proteins of strain MRE 600 are indistinguishable from those of strain C. These results agree well with ours.

E. freundii group ribosomes. A group of ribosomes here classified as the *E. freundii* group were found mainly in strains previously identified as *E.* (*Citrobacter*) *freundii*, *E. intermedia*, or *P. intermedium*. Ribosomes in this group may be divided into three types.

E. freundii type f-f. The ribosomal protein compositions of *E.* (*Citrobacter*) *freundii* ATCC 8090, the neotype strain of *E. freundii*, were chromatographically compared with those of *E. coli* C. It was found that at least seven 50*S* and four 30*S* protein components from strain 8090 are different from the corresponding components of *E. coli* type *C*. We call the ribosomes found in strain 8090 the *f-f* type. The correspondence of the altered components in the *f-f* type to the components in *E. coli* type *C* ribosomes was determined as described in Materials and Methods (Table 2); a part of the results was reported previously (2, 8).

The ribosomal protein compositions of *E. anindolica* ATCC 6879 were found to be indistinguishable from the *f-f* type.

E. freundii type f-m. The ribosomal protein compositions of *E.* (*Citrobacter*) *freundii* ATCC 6750, the original strain of *E. intermedia*, are the same as those of the *f-f* type except that the 50-4 protein component of the *f-m* [designated as 50-4(f-m)] is eluted much earlier than the corresponding component of the *f-f* [50-4(f-f)] on the chromatogram. We have named the ribosomes found in strain 6750 the *f-m* type.

Among the three *E. freundii* types, the *f-m* type seems to be most widely distributed in nature. Four strains previously identified as *E. freundii*, four strains designated as *P. intermedium*, and two strains labeled as *E. coli* (IAM 1132; IAM 1182 = *R* type in reference 8) contained this type of ribosomes (Table 1). The chromatographic difference of the *f-m*-type ribosomes (IAM 1182) from *E. coli C*-type ribosomes was shown in a previous paper (8).

E. freundii type f-i. The *f-i*-type ribosomes are the same as the *f-f* or *f-m* types except that the 50-4 protein component designated here as 50-4(f-i) is modified so as to be situated between 50-4(f-m) and 50-4(f-f). This type was found only in one strain of *P. intermedium*, 49761.

E. paraintermedia group ribosomes. Two strains both previously identified as *E. intermedia* possess ribosomes considerably different from the *E. coli* or *E. freundii* group in their protein compositions. Ribosomes from these two *E. intermedia* strains are similar and thus are classified as the *E. paraintermedia group*. Since there exist differences in some components between them, the *E. paraintermedia* group may be divided into two types.

E. paraintermedia type p. The type *p* ribosomes were found only in *E. intermedia* AHU 1412. In Fig. 2 are shown the chromatograms of ribosomal proteins of the type *p* (^{3}H) in comparison with those from *E. coli* type *C* (^{14}C). A similar comparison was also made between AHU 1412 and *E. freundii f-m*. The *p* type differs from the *E. coli C* type in having at least eight 50*S* and five 30*S* components and from the *f-m* in having seven 50*S* and five 30*S* components. The components common to neither the *E. coli* nor the *E. freundii* group were designated by the symbol *p*. The correspondence of some of the *p*-specific components (question mark in Fig. 2 and Table 2) to the *E. coli* or *E. freundii* components is not definite because of their complicated chromatographic profile.

E. paraintermedia type j. The *j*-type ribosomes were found only in *E. intermedia* ATCC 21073. When the ribosomes of the *j* type were compared with those of the *p* type, no difference was found in their 30*S* ribosomal proteins. On the other hand, the 50*S* proteins of the *j* type possess at least three specific components, i.e., 50-4(j), 50-5(j), and 50-7(j), which are clearly distinguishable from the corresponding components of the *p* or other types on the chromatogram.

E. adecarboxylata group ribosomes. The ribosomes from the type strain of *E. adecarboxylata*, ATCC 23216, designated here as the *E. adecarboxylata* group, are considerably different in their protein compositions from those so far de-

scribed. At present this group is represented by a single strain, ATCC 23216, the ribosomes of which are thus classified as *E. adecarboxylata* group type *a*. To characterize the *a* type, ribosomal proteins of *E. adecarboxylata* were compared with those of *E. coli* type *C*, *E. freundii* type *f-f*, and *E. paraintermedia* type *p*. Some of the components of the *a*-type ribosomes are indistinguishable from components in *E. coli*, *E. freundii* (shown by the symbol f-f in Table 2), or *E. paraintermedia* (shown by p). Some others are specific to this *a*-type ribosomes (shown by a).

Ribosomal protein compositions in genera closely related to Escherichia. To see the relation of ribosomes in the genus *Escherichia* to those in other genera which have been supposed to be near *Escherichia*, we collected a series of strains so far classified as *Shigella*, *Salmonella*, and *Arizona* (including *Paracolobactrum arizonae*) and analyzed their ribosomal protein compositions. The grouping of the ribosomes here adopted is only tentative, since our collection of the strains is very incomplete as compared with the tremendous number of "species" or serotypes so far described.

Shigella group ribosomes. Ribosomal protein compositions were analyzed only for one strain of *S. dysenteriae*, ATCC 13313 (neotype strain of this species). The chromatographic behavior of the *Shigella* 30*S* proteins was indistinguishable from that of *E. coli* type *C* 30*S* proteins, whereas there were two distinct differences between the 50*S* proteins of these two strains. The *Shigella* 50-10 component, i.e., 50-10(Sg), is eluted a little later than the *E. coli* 50-10, and the *Shigella* 50-12 component, i.e., 50-12(Sg), is eluted a little earlier than the 50-12 of *E. coli*.

Salmonella group ribosomes. Simultaneous analyses of the ribosomal proteins from *S. heidelberg* (^{3}H) and those of *E. coli* type *C* or *E. freundii* type *f-f* (^{14}C) were performed. The chromatograms indicated the presence of at least five 50*S* and three 30*S* ribosomal protein components which were distinct between *Salmonella* and *E. coli*, and five 50*S* and one 30*S* components differed between *Salmonella* and *E. freundii* (Fig. 3). Several components of *Salmonella* are identical either to those of *E. coli C* type or to those of *E. freundii f-f* (shown by f-f in Fig. 3 and Table 2). The other components shown by S are specific to *Salmonella* in the sense that these were not found in any ribosome type so far described.

The *Salmonella*-type ribosomes were found in two strains of *S. typhimurium* (cf. 1), in *S. abony* SW 803 (cf. 9), in *S. heidelberg* SW 1092, and in three strains of *P. arizonae* (see Table 1).

Arizona group ribosomes. The 50*S* and 30*S* ribosomal proteins from *A. arizonae* ATCC 13314, the neotype strain for this species, were compared with those from *E. coli* C and *S. heidelberg*. A close resemblance of the protein compositions of these bacteria was found: two components, 50-7(Az) and 30-3(Az) are specific to *Arizona*; 50-a is common to *E. coli* C but not to *Salmonella*; 50-13 is probably the same as the 50-13(f-f) from *E. freundii* but is not the same as

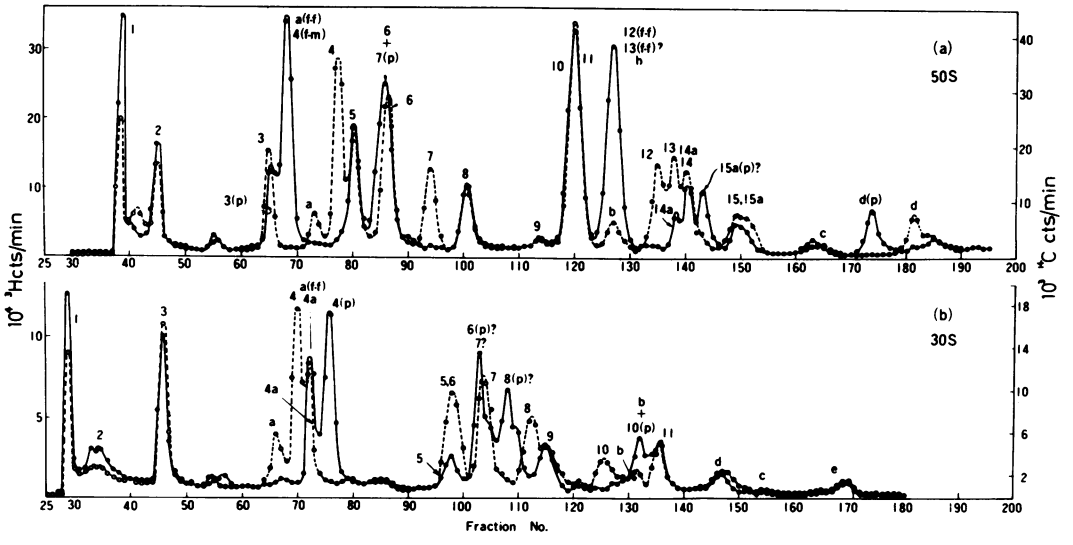

FIG. 2. *Chromatography on CMC columns of ribosomal proteins from ATCC 11775 (E. coli C) and AHU 1412 (E. paraintermedia p). (○) ^{14}C-lysine-labeled E. coli C protein; (●) ^{3}H-lysine-labeled E. paraintermedia p protein; (a) 50S; (b) 30S.*

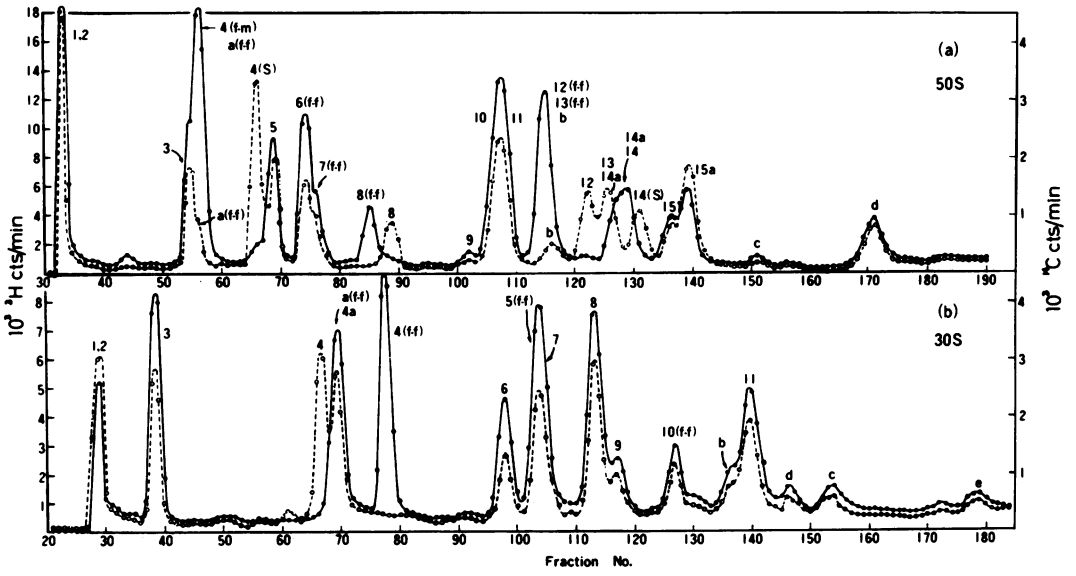

FIG. 3. *Chromatography on CMC columns of ribosomal proteins from ATCC 6750 (E. freundii f-m) and Salmonella heidelberg SW 1092.* (○) *¹⁴C-lysine-labeled E. freundii f-m protein;* (●) *³H-lysine-labeled Salmonella protein; (a) 50S; (b) 30S.*

the corresponding 50-13 from *E. coli* or *Salmonella*. The other components were indistinguishable from those of *Salmonella* (Table 2).

Besides their occurrence in ATCC 13314, the *Arizona*-type ribosomes were found in *P. arizonae* LYO 10313.

Ribosomal protein compositions in other genera of Enterobacteriaceae. Two strains belonging to other genera of *Enterobacteriaceae* were examined preliminarily: *Paracolobactrum aerogenoides* LYO 3565 and *Proteus morganii* IFO 3848. Their chromatographic profiles were very different from that of *E. coli* C; the similarity between these bacteria and *E. coli* C is in all cases estimated to be at most 10%. Profiles of 50S proteins from *P. aerogenoides* (³H; Fig. 4a) and from *P. morganii* (Fig. 4b) are shown in comparison with those from *E. coli* C (¹⁴C).

A list of bacterial strains used in this study together with their ribosome groups and types are collectively shown in Table 1. The detailed protein compositions of these groups and types are summarized in Table 2 in which chromatographically ill-defined components (50-1, 50-9, 50-b, 50-14a, 50-c, 30-1, 30-2, 30-d, and 30-c) are omitted.

DISCUSSION

The degree of relatedness among different bacteria would be best expressed by the extent of homology in their gene compositions. This may be approximated by the direct comparison of the gene products in terms of the amino acid sequences of the proteins. We have assumed that the overall relatedness of gene compositions among various bacteria is closely represented by the relatedness of their ribosomal protein compositions. This assumption is based on our observation that bacteria which reveal considerable similarity in their ribosomal protein compositions are also similar in their soluble protein compositions when analyzed with diethylaminoethyl cellulose chromatography, whereas those having very different ribosomal protein compositions are different in their soluble proteins (*unpublished data*).

We have constructed a similarity matrix for the ribosome groups and types (subgroups) based on the data shown in Table 2. This matrix would represent the difference in the overall ribosomal protein species and in the overall gene compositions as well. Several possible sources of error in the above comparisons should be pointed out. First, we have at present no precise way to compare the overall gene compositions, and the parallelism of the similarity between ribosomal and soluble proteins mentioned above is only very approximate. Second, we are dealing with the product of structural genes, proteins, and are ignoring regulatory genes or genes for ribosomal and transfer ribonucleic acids. However, this would not influence our discussion too seriously, since the proportion of regulatory genes would be small in comparison with the structural genes. Third, not all of the ribosomal protein components considered here have been shown to consist of a single protein species, although most of them revealed a single band in gel electrophoresis

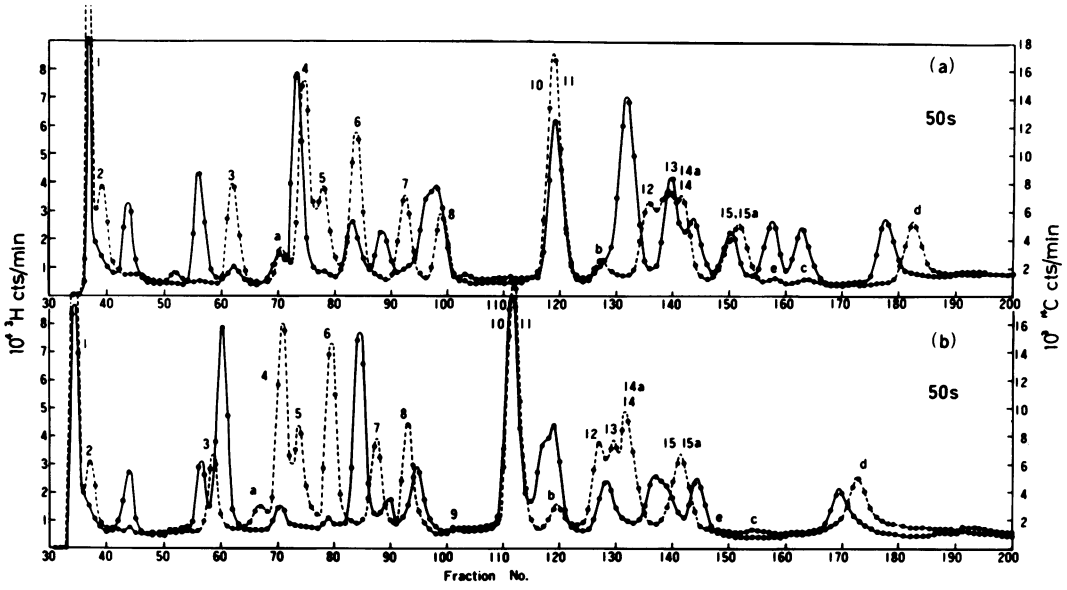

FIG. 4. *Chromatography on CMC columns of 50S ribosomal proteins from (a) ATCC 11775 (E. coli C) and Paracolobactrum aerogenoides LYO 3565, and (b) ATCC 11775 and Proteus morganii IFO 3846.* (○) *^{14}C-lysine-labeled E. coli C protein;* (●) *^{3}H-lysine-labeled Paracolobactrum aerogenoides or Proteus morganii protein to which no component number was given in the figure.*

(9). Fourth, we assume that the identity of the protein components determined by CMC chromatography reflects the identity in their amino acid sequence. To see the validity of this assumption, the 30-4 and 50-8 protein components were isolated from three bacterial strains, i.e., *E. coli* (type *K*), *Salmonella*, and *E. freundii* (f-f). The 30-4 (or 50-8) component from *E. coli* type *K* and *Salmonella* reveal no difference on the chromatogram, whereas the 30-4 (or 50-8) component from *E. freundii* is eluted later (or earlier in the case of 50-8) than that from *E. coli* type *K* or *Salmonella*. Tryptic peptide analyses of these proteins done by use of a column of Beckman type PA 35 resin did not show any significant difference between the 30-4 (or 50-8) of *E. coli* type *K* and that of *Salmonella*. On the other hand, the peptide map of the 30-4 (or 50-8) revealed several definite differences from those of *E. coli* type *K* or *Salmonella* (*unpublished data*). Thus, the identity or difference of the protein components determined by CMC chromatography would reflect the identity or difference in their amino acid sequence so far as the 30-4 and 50-8 from the above bacteria are concerned. Whether or not this is applicable to the other protein components is not known. Fifth, suppose there exist allelic proteins A, A′, and A″ from three strains which differ from one another chromatographically. In the present study, we treat them all as "equivalently" different without considering the nature of the difference. In some cases, it might be that the polypeptide chain A′ differs from that of A in one amino acid, whereas A″ differs in more than one, etc. At the moment, we have to leave this problem to the future study.

With the above technical limitations in mind, we see from the matrix (Table 3) that the similarity of *E. coli C*, *B*, *K*, *KW*, *D*, and *Shigella* ribosomes, of *E. freundii f-f*, *f-m*, and *f-i* ribosomes, or of *E. paraintermedia p* and *j* ribosomes is in all cases 90% or more, indicating only a small genetic differentiation of ribosomal (and probably other) protein cistrons among them. The results are in agreement with the previous reports that *E. coli* and *Shigella*, or *Salmonella* and *Arizona*, are genetically or taxonomically very close (3, 10). On the other hand, *E. coli-Shigella*, *Salmonella-Arizona*, *E. freundii*, *E. paraintermedia*, and *E. adecarboxylata* ribosome groups revealed only 40 to 70% similarity, suggesting a considerable genetic differentiation among these groups of bacteria. As far as the matrix indicates, the *E. freundii* group is more closely related to the *Salmonella-Arizona* group than to the *E. coli* group. Although we have not discussed the genus designations of the bacterial strains considered here, it would be necessary to rearrange them in light of the relationships of gene compositions.

It has been pointed out that *Escherichia aures-*

TABLE 3. *Similarity matrix of ribosome groups and types*[a]

Ribosome group or type	*E. coli C*	*E. coli B*	*E. coli K*	*E. coli KW*	*E. coli D*	*Shigella*	*Salmonella*	*Arizona*	*E. freundii f-f*	*E. freundii f-m*	*E. freundii f-i*	*E. paraintermedia p*	*E. paraintermedia j*	*E. adecarboxylata a*
E. coli C	100													
E. coli B	97	100												
E. coli K	97	97	100											
E. coli KW	93	90	97	100										
E coli D	93	90	90	87	100									
Shigella	93	90	90	87	87	100								
Salmonella	73	69	69	66	66	66	100							
Arizona	69	66	66	62	62	62	87	100						
E. freundii f-f ..	59	55	55	52	52	55	76	69	100					
E. freundii f-m .	59	55	53	52	52	55	76	69	97	100				
E. freundii f-i ..	59	55	55	52	52	55	76	69	97	97	100			
E. paraintermedia p	55	52	52	48	48	55	62	52	55	59	55	100		
E. paraintermedia j	48	45	45	41	41	52	52	45	55	55	55	90	100	
E. adecarboxylata a ...	45	41	41	38	38	38	52	45	59	59	59	62	62	100

[a] Similarity (%) = ribosomal components in common/ribosomal components examined × 100; the number of components examined was 29. Calculations were based on the data shown in Table 1. Components designated by a question mark with no group or type symbol in Table 1 were treated tentatively as those common to *E. coli* C.

cens, *Shigella alkalescens*, *Paracolobactrum coliforme*, anaerogenic *E. coli* strains (*E. anaerogenes* examined here would be one of them), and various *E. coli* "subspecies" are all synonyms of, or very close to, *E. coli* (3; American Type Culture Collection Catalogues of Strains; *Bergey's Manual*). The results reported here support this conclusion.

It seems to be generally recognized that *E. intermedia* and *P. intermedium* are synonyms of *Citrobacter freundii* (American Type Culture Collection Catalogues of Strains). This conclusion is based upon the facts that the neotype strain of *C. freundii* (ATCC 8090) agrees in all respects examined by the American Type Culture Collection with the original strain of *E. intermedia* (=*Citrobacter freundii* ATCC 6750) and with the neotype strain of *P. intermedium* (=*Citrobacter freundii* ATCC 11606). However, we could classify the ribosomes from bacteria identified as *E.* (*C.*) *freundii*, *E. intermedia*, and *P. intermedium* into two groups, *E. freundii* group and *E paraintermedia* group, which are further differentiated into three types, *f-f*, *f-m*, and *f-i*, and *p* and *j*, respectively. Since the *E. paraintermedia* group ribosomes are considerably different from the *E. freundii* group, the strains having ribosomes of the former would have to be treated as a species distinct from *E. freundii* in the taxonomic sense. Our analyses further suggest that two strains of "*E. coli*," IAM 1132 and IAM 1182, might very well be *E. freundii* (with *f-m*-type ribosomes), and that *E. anindolica* ATCC 6879 could be a synonym of *E. freundii*.

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