Immunological Comparison and Characterization of Ribosomes of Xanthomonas vesicatoria

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ABSTRACT

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Ribosomes from 25 isolates of *Xanthomonas vesicatoria* have been compared and characterized immunologically. Using Ouchterlony double-diffusion tests, two lines of precipitin were detected. One precipitin, designated R-1, was always present in homologous systems, was specific at the subspecies level, and was identified as a trypsin, ribonuclease-insensitive immunogen of the 50S subunit core particle. The other precipitin, designated R-2, was not always present in homologous systems, and therefore was of no comparative value. The R-2 precipitin was identified as a ribonucleic acid species of the 50S subunit. Based on the R-1 immunogen, the 25 isolates of *X. vesicatoria* were grouped into three serotypes. Ribosomes of *X. vesicatoria* failed to react with ribosomes from 15 other closely and distantly related bacteria. Therefore, it is suggested that the molecular structure of ribosomes of different species is distinct. It is further suggested that differences in ribosomal structure can be used to distinguish bacterial strains. The possible application of ribosomal immunology as a tool in identifying specific strains of pathogenic bacteria is discussed.

Ribosomes are the most prominent structural component of bacterial cytoplasm. Since all ribosomes regardless of their species origin function in protein synthesis, it has been suggested that all prokaryotic ribosomes are structurally the same (11). The complete reconstitution between 16S RNA of *Escherichia coli* and 30S ribosomal protein (r protein) of *Bacillus stearothermophilus* into a functional 30S subunit (17), and the immunogenic cross-reactions between antisera to individual (7, 9) or total (10) subunit proteins of *E. coli* and total subunit protein of *B. stearothermophilus* does suggest a structural and functional similarity between ribosomes of different species. Antiserum to ribosomes, subunits, and subunit proteins of *E. coli* reacted as strongly with ribosomes from *Proteus, Salmonella,* or *Serratia* as in the homologous system and nearly as strongly with ribosomes from *Azotobacter* (26). Also, ribosomal proteins from seven species of *Enterobacteriaceae* have been reported to be immunologically similar (8). In contrast, I found that the immunogenicity of intact ribosomes of seven species of the *Enterobacteriaceae,* including several plant pathogenic ones, was dissimilar (20). The first report on the immunogenicity of ribosomes suggested that they were highly immunogenic and species specific (19). Later Friedman et al. (5) reported that 50S subunits were species specific. With the exception of a positive reaction between mouse and chicken ribosomes, Noll and Bielka (16) reported that ribosomes from different species of animals reacted only with homologous antisera. They also reported that intact ribosomes were more specific than the extracted proteins.

Until now, a detailed study of the structure of ribosomes of a plant pathogenic bacterium has not been made. Ribosomes of 25 isolates of *Xanthomonas vesicatoria,* the causal agent of bacterial spot of tomato and pepper, have been examined and compared by Ouchterlony double-diffusion to ribosomes of 15 other closely and distantly related bacteria. The results showed that the structure of intact ribosomes was similar at the subspecies level, but very dissimilar at the specific and generic level. The 25 isolates of *X. vesicatoria* were grouped into three serotypes.

MATERIALS AND METHODS

Bacterial strains and preparation of cells.—Strains of the *Xanthomonas* spp. used in this study are listed in Table 1. All other strains of bacteria were described earlier (20), except for those identified in the text. Bacteria were stored by lyophilization and maintained on yeast extract-dextrose-calcium carbonate agar (24). Cells were grown, harvested, and washed (0.85% saline) as previously described (20).

Preparation of ribosomes.—Ribosomes were obtained from 6-7 g (packed wet weight) of cells. The frozen cells were suspended at 5 C in 20 volumes of TKM/3 buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris); Sigma Chemical Co., 0.05 M KCl, 0.005 M $MgCl_2$] at pH 7.4 (20). Deoxyribonuclease was added to a final concentration of 2 mg/ml and the cells were disrupted at 363 kg/mm^2 (20,000 psi) in a cold French pressure cell (American Instruments Co.). Unbroken cells and cell debris were removed by centrifugation in a Sorvall SS34 rotor at 17,000 *g* (12,000 rpm) for 20 minutes at 2-3 C.

Intact ribosomes were obtained from the low-speed supernatant by centrifuging at 177,000 *g* (40,000 rpm) in a Spinco 42.1 rotor for 2 hours at 2-3 C using a Spinco Model L3-40 ultracentrifuge. The pellets were washed overnight in TNM/3 buffer (0.01 M Tris, 0.5 M NH_4Cl, 0.005 $MgCl_2$) at pH 7.4 (20) to remove loosely bound proteins (21, 26), pooled, and centrifuged at 17,000 *g*. The

ammonium chloride-washed ribosomes were then pelleted by centrifuging at 177,000 *g* in a 42.1 rotor for 2 hours. The sedimented ribosomes were suspended in TKM/3 buffer, pooled, and centrifuged at 17,000 *g*. The absorbance was recorded at 235, 260, and 280 nm, and the ribosomes were stored frozen or used for further purification.

Washed ribosomes were obtained by sucrose density-gradient centrifugation of intact ribosomes as described (20), except that a SW 27.1 rotor at 131,000 *g* was used in place of a SW 25.1 rotor. Also, the ribosomes were washed in TKM/3 buffer and concentrated to approximately 5 ml by the Diaflo ultrafiltration method (Amicon Corp., Lexington, Mass.) using a PM 30 membrane.

Subunits were obtained by dialyzing intact ribosomes against TKM/4 (3×10^{-4} M $MgCl_2$) buffer and centrifuging on sucrose density gradients as described (20), except a SW 27.1 rotor was used in place of a SW 25.1 rotor. A PM 30 and a PM 10 membrane were used to wash and concentrate the 50S and 30S subunits, respectively.

Crude ribosomes were obtained from extracts disrupted with ultrasonic sound waves as described (20).

Immunological procedures.—Washed ribosomes and 30S and 50S subunits were used as immunizing antigens to prepare antisera in New Zealand White rabbits (20). Ouchterlony double-diffusion experiments were conducted as described (20). In all experiments, unless stated otherwise, outer well number 1 contained

TABLE 1. Bacterial strains employed in the immunological comparison and characterization of ribosomes of *Xanthomonas vesicatoria*[a]

Species	Strain	Host origin	Date	Source (Scientist; State)
X. vesicatoria	B-200	Tomato	1971	Author; Florida
	B-201	Tomato	1971	Author; Florida
	B-202	Tomato	1971	Moore; Oregon
	B-203 (069-663)	Tomato	1971	Miller; Florida
	B-204	Pepper	1971	Sowell; Georgia
	B-206	Pepper	1971	Miller; Florida
	B-207	Pepper	1971	Sowell; Georgia
	B-208 (XV-69-13)	Tomato	1972	Stall; Florida
	B-209 (XV-69-23)	Tomato	1972	Stall; Florida
	B-210 (XV-68-4)	Tomato	1972	Stall; Florida
	B-213 (555-81)	Pepper	1973	Sasser; Delaware
	B-214	Tomato	1973	Sasser; Delaware
	B-215 (P-22)		1973	Sutton; Canada
	B-218		1973	Sasser; Delaware
	B-219 (X-1)		1973	Dye; New Zealand
	B-220 (X-5)		1973	Dye; New Zealand
	B-222 (X-7)		1973	Dye; Catania, Italy
	B-223 (X-13)		1973	Dye; New Zealand
	B-226 (5091)	Pepper	1973	Lai; California
	B-227 (5093)	Pepper	1973	Lai; California
	B-228 (ENA-76)	Tomato	1974	Robbs; Brazil
	B-229 (ENA-734)	Pepper	1974	Kimura; Brazil
	B-230 (ENA-804)	Pepper	1974	Kimura; Brazil
	B-233 (ATCC 11551)[a]	Tomato	1975	American Type Culture Collection
	B-234 (ATCC 11633)[a]	Pepper	1975	American Type Culture Collection
X. campestris	B-1 (J.C.)	Brussels Sprouts	1971	Grogan; California
	B-4	Broccoli	1971	Author; Georgia
	B-18	Cabbage Soil	1972	Author; Florida
	B-24	Broccoli	1972	Moore; Oregon
	B-12	Cabbage	1971	Author; Georgia
X. translucens	B-410 (XY-104)	Wheat	1972	Schnathorst; California
	B-426 (537)	Wheat	1974	Otta; South Dakota
	B-427 (550)	Wheat	1974	Otta; South Dakota
	B-429 (551)	Wheat	1974	Otta; South Dakota
X. malvacearum	B-412 (XM-5)	Cotton	1972	Schnathorst; California
	B-414 (R-4)	Cotton	1972	Goodman; Missouri
	B-416 (R-10)	Cotton	1972	Goodman; Missouri
X. dieffenbachia	B-400	Philodrendron	1971	Knauss; Florida
X. pelargonii	B-411 (XP-2)		1972	Schnathorst; California
X. fragariae	B-420 (070-1277)	Strawberry	1972	Miller; Florida
X. juglandis	B-423 (X.J.)	Walnut	1972	Hildebrandt; California

[a]ATCC = American Type Culture Collection, Rockville, Maryland.

ribosomes or subunits of homologous subunits or ribosomes. Detailed discussions of the interpretation of precipitin band patterns were given by Gasser and Gasser (6) and Williams and Chase (23).

Characterizing and identifying immunogens.—Thirty mg of 50S subunits of isolate 215 were divided into approximately equal and largely mutually exclusive fractions, called core particles and split proteins, by extracting with 2 M LiCl according to the method of Atsmond et al. (1). The core particles were then divided into protein and RNA by extracting with acetic acid (20). The resulting core particle protein was lyophilized and suspended in 3 M urea in TKM/3 buffer pH 6.7 at a concentration of 3 mg/ml. The acid-extracted RNA was suspended in TKM/3 and solubilized with sodium hydroxide prior to gel diffusion. Ribonucleic acid also was extracted from 50S subunits with phenol-SDS according to Muto et al. (15). Core particles and 70 S ribosomes were incubated with trypsin and RNase (20).

RESULTS

Immunogenicity of homologous ribosomes and ribosomal subunits.—Ribosomes and their subunits were highly immunogenic. Antisera prepared against washed 70S ribosomes (AR) of *Xanthomonas vesicatoria* produced one or two lines of precipitin when reacted against homologous intact or crude ribosome preparations (Fig. 1-A, B, C, D: well 1). The stronger immunoprecipitin or R-1 antigen was nearer the antigen

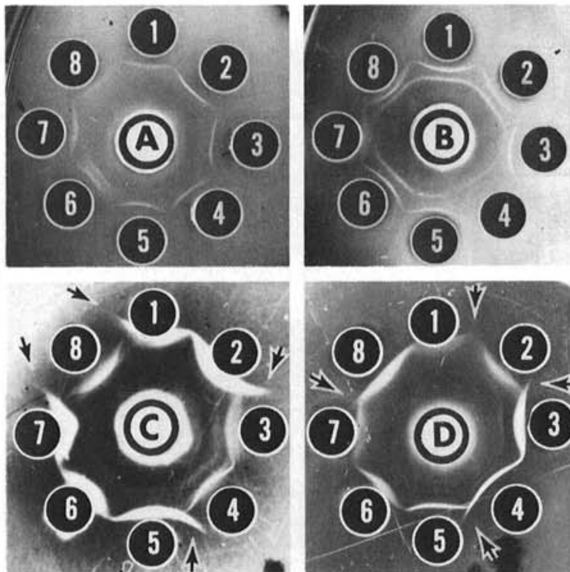

Fig. 1-(A to D). Gel diffusion patterns of ribosomes of different isolates of *Xanthomonas vesicatoria*. Center well contained 10 μliters of antiserum to ribosomes of isolates: **A**) 210, **B**) 206, **C**) 218, and **D**) 201. **Outer wells of plate A**) contained ribosomes of isolates: 1) 210, 2) 201, 3) 202, 4) 204, 5) 206, 6) 208, 7) 209, and 8) 211. **Outer wells of B**) contained ribosomes of isolate 1) 206, 2) 200, 3) 202, 4) 203, 5) 204, 6) 205, 7) 208, and 8) 209. **Outer wells of C**) contained ribosomes of isolate 1) 218, 2) 219, 3) 226, 4) 227, 5) 220, 6) 222, 7) 228, and 8) 230. **Outer wells of D**) contained ribosomes of isolate 1) 201, 2) 219, 3) 226, 4) 227, 5) 220, 6) 222, 7) 218, and 8) 230.

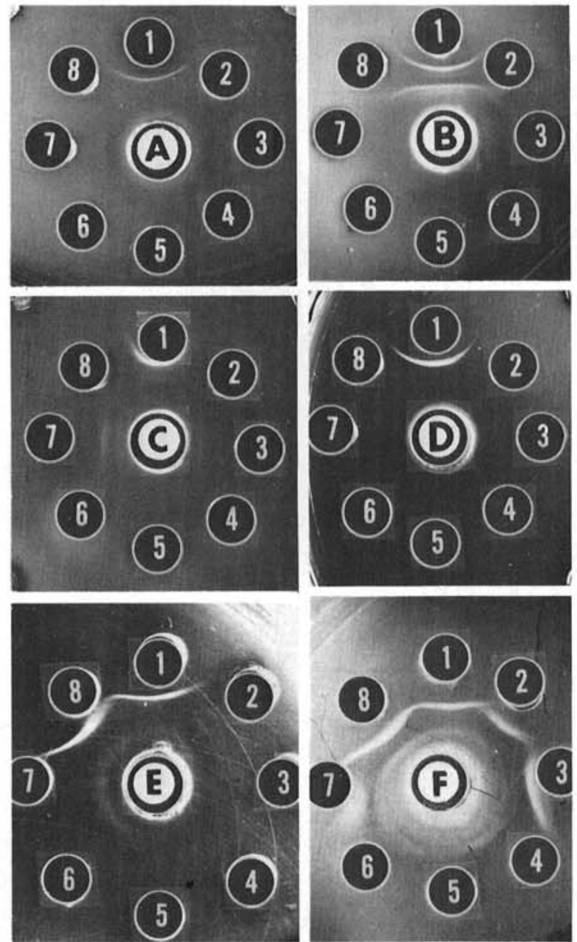

Fig. 2-(A to F). Gel diffusion patterns of ribosomes of *Xanthomonas vesicatoria* and several distantly related bacteria. Center wells contained 10 μliters of antiserum to ribosomes of: **A**) *Xanthomonas vesicatoria*, isolate 214; **B**) *Escherichia coli* isolate Ola; **C**) *X. vesicatoria*, isolate 201; **D**) *X. vesicatoria* isolate 213; **E**) *X. vesicatoria*, isolate 201; and **F**) *X. vesicatoria* isolate 202. **Outer wells of A**) contained ribosomes of: 1) *X. vesicatoria* 214; 2) *Staphylococcus epidermidis* 14990; 3) *Klebsiella pneumoniae* 15; 4) *Escherichia coli* Ola; 5) *Salmonella typhimurium* 4066; 6) *Enterobacter cloaceae;* 7) *Pectobacterium carotovorum* 105; and 8) *Erwinia rubrifaciens* 533. **Outer wells of B**) contained ribosomes of: *E. coli*, isolate Ola 1) and *X. vesicatoria* isolates 2) 201; 3) 200; 4) 202; 5) 203; 6) 204; 7) 206; and 8) 208. **Outer wells of C**) contained ribosomes of: 1) *X. vesicatoria* 201; 2) *Pseudomonas syringae* B-3; 3) yellow-pigmented bacterium G-29; 4) *Xanthomonas*-like 8004; 5) *Enterobacter agglomerans* 25D31; 6) yellow-pigmented bacterium G-57; 7) yellow-pigmented bacterium G-11; and 8) *Xanthomonas* sp. YE-2. **Outer wells of D**) were the same as C except that well 1) contained ribosomes of *X. vesicatoria* 210. **Outer wells of E**) contained ribosomes of: 1) *X. vesicatoria* 201; 2) *Xanthomonas translucens* XT-104; 3) *X. translucens* 537; 4) *X. translucens* 551; 5) *X. translucens* 550; 6) *Xanthomonas malvacearum* XM-5; 7) *X. malvacearum* R-4; and 8 *X. vesicatoria* 201. **Outer wells of F**) contained ribosomes of: 1) *X. vesicatoria* 202; 2) *Xanthomonas campestris* 4; 3) *X. campestris* 22; 4) *Xanthomonas juglandis* XJ-1; 5) *Xanthomonas pelargonii* XP-2; 6) *Xanthomonas dieffenbachia* 400; 7) *X. campestris* 18; and 8) *X. vesicatoria* 202.

well (Fig. 1-A) and was present in all homologous systems. The weaker immunoprecipitin or R-2 antigen was nearer the antiserum well (Fig. 1-B). Since the R-2 line of precipitin was not always present in homologous systems, results will be limited to the R-1 line of precipitin unless stated otherwise. Antisera prepared against 50S subunits produced a reaction of identity when reacted against homologous 70S ribosomes or 50S subunits, but failed to react against 30S subunits. Finally, antisera prepared against 30S subunits produced a line of precipitin against 30S subunits and 70S ribosomes but not against 50S subunits.

Homologies between ribosomes of different isolates of Xanthomonas vesicatoria.—The precipitin lines produced by ribosomes of isolates 210, 201, 202, 204, 206, 208, 209, and 211 (wells 1 to 8, respectively) all fused with AR of isolate 210 (Fig. 1-A). Identity reactions occurred with ribosomes of isolates 206, 200, 202, 204, 201, 208, and 209 (wells 1, 2, 3, 5, 7, and 8, respectively) and AR of isolate 206 (Fig. 1-B). Ribosomes of isolate 203 failed to react with AR of isolate 210 or 206 (Fig. 1-B, well 4). Results of testing AR of isolates 201, 202, 203, 206, 210, 213, 214, 215, and 218 against ribosomes of the 25 isolates tested are summarized in Table 2. Based on these results, ribosomes of *X. vesicatoria* can be grouped into three serotypes (ser.) as follows: (i) ser. Doidge (isolates 200, 201, 202, 204, 206, 207, 208, 209, 210, 222, 226, 227, 229, 230, and 234; (ii) ser. Dowson (isolates 213, 214, 215, 218, 219, 220, 222, 223, 228, and 233); and (iii) ser. Burkholder (isolate 203). An indication of the degree of antigenic homology is given by the pattern of spur formation between the precipitin lines of ribosomes from the various isolates.

Precipitin lines formed by ribosomes of the same serotype in adjacent wells always fused (Fig. 1-C, wells 1-2, 3-4, 5-6-7 and Fig. 1-D, wells 3-4, 5, 6, 7, 8-1) whereas those lines formed by ribosomes of ser. Doidge and Dowson in adjacent wells always formed spurs. Several examples of such spur formation (arrows) are shown in Fig. 1-C (wells 2-3, 4-5, 7-8, 8-1) and Fig. 1-D (wells 1-2, 2-3, 4-5, and 7-8). Spurs were formed between ribosomes of all ser. Doidge and Dowson isolates, using AR of either ser. Doidge or Dowson. On the other hand, no precipitin bands were observed between ribosomes of ser. Burkholder and AR of ser. Doidge (Fig. 1-B, well 4) or Dowson. Also, ribosomes of ser. Doidge and Dowson failed to form precipitin bands when reacted against AR of ser. Burkholder.

It is evident from these results that ribosomes from isolates of a single bacterial species can have quite different molecular structures.

Relationships between serotype, starch hydrolysis, and pathogenicity.—Because different isolates of *X. vesicatoria* differed in their ability to hydrolyze starch and to infect pepper and tomato, the 25 isolates were tested for ability to hydrolyze starch and to infect pepper and tomato. All ser. Dowson and Burkholder isolates hydrolyzed starch, whereas ser. Doidge isolates did not. No correlation existed between serotype and pathogenicity.

Homologies between ribosomes of Xanthomonas vesicatoria and ribosomes of several distantly related bacteria.—Several experiments were conducted to determine if there were structural homologies between ribosomes of *X. vesicatoria* and ribosomes from other bacteria. The 70S ribosomes (AR) of the nine isolates of

TABLE 2. Homologies between ribosomes of different isolates of three serotypes of *Xanthomonas vesicatoria* as detected by Ouchterlony immunodiffusion[a]

Ribosomes from	Antiserum to ribosomes of serotype (Ser.)								
	Ser. Doidge				Ser. Dowson				Ser. Burkholder
	201	202	206	210	213	214	215	218	203
200	++	++	++	++	+	+	+	+	−
201	++	++	++	++	+	+	+	+	−
202	++	++	++	++	+	+	+	+	−
204	++	++	++	++	+	+	+	+	−
206	++	++	++	++	+	+	+	+	−
207	++	++	++	++	+	+	+	+	−
208	++	++	++	++	+	+	+	+	−
209	++	++	++	++	+	+	+	+	−
210	++	++	++	++	+	+	+	+	−
226	++	++	++	++	+	+	+	+	−
227	++	++	++	++	+	+	+	+	−
229	++	++	++	++	+	+	+	+	−
230	++	++	++	++	+	+	+	+	−
234	++	++	++	++	+	+	+	+	−
213	+	+	+	+	++	++	++	++	−
214	+	+	+	+	++	++	++	++	−
215	+	+	+	+	++	++	++	++	−
218	+	+	+	+	++	++	++	++	−
219	+	+	+	+	++	++	++	++	−
220	+	+	+	+	++	++	++	++	−
222	+	+	+	+	++	++	++	++	−
223	+	+	+	+	++	++	++	++	−
228	+	+	+	+	++	++	++	++	−
233	+	+	+	+	++	++	++	++	−
203	−	−	−	−	−	−	−	−	++

[a]Symbols defined: ++ = reaction of identity; + = partial or nonidentity; − = no reaction.

X. vesicatoria (listed above) were tested by immunodiffusion for reaction with ribosomes of *Escherichia coli* 01a, *Klebsiella pneumoniae* 15, *Salmonella typhimurium* 4066, *Enterobacter cloacae, Pectobacterium (Erwinia) carotovorum* 105, *Erwinia rubrifaciens* 533, *Pseudomonas syringae* B-3, *Staphylococcus epidermidis* 14990 a gram-positive bacterium, and several yellow-pigmented bacteria nonpathogenic to tomato or pepper. The following yellow-pigmented bacteria were tested: isolate G-29 (author) isolated from jointed charlock (*Raphanus raphanistrum*), Xanthomonas-like isolate B 8004 (P. S. Riley, Center for Disease Control, Atlanta, Ga.) isolated from a clinical source, *Xanthomonas* sp. YE-2 (A. von Graevenitz, Yale University) isolated from human blood, isolate G-57 (author) isolated from tomato, isolate G-11 (author) from pond water, and *Enterobacter agglomerans* 25D31 (C. I. Kado, U. C. Davis).

In reciprocal tests, AR of *E. coli, K. pneumoniae, S. typhimurium, E. cloacae, E. agglomerans, P. carotovorum, E. rubrifaciens,* and *P. syringae* were tested for immunoprecipitin against ribosomes from all 25 isolates of *X. vesicatoria*. All the tests were negative. Whereas AR of *X. vesicatoria* reacted with homologous ribosomes (Fig. 2-A, C, D; well 1), ribosomes from the enterobacteria (Fig. 2-A; wells 3 to 8), *S. epidermidis* (Fig. 2-A; well 2), yellow-pigmented bacteria (Fig. 2-C, D, wells 3 to 8), and *P. syringae* (Fig. 2-C, D; well 2) failed to react. The absence of a reaction between ribosomes of seven isolates of *X. vesicatoria* (wells 2 to 8) and AR of *E. coli* is shown in Figure 2-B.

Homologies between ribosomes of closely related species.—Crude or intact ribosomes of *Xanthomonas dieffenbachia, Xanthomonas juglandis, Xanthomonas fragariae, Xanthomonas pelargonii,* three isolates of *Xanthomonas malvacearum,* and five isolates each of *Xanthomonas translucens,* and *Xanthomonas campestris* were tested. AR of ser. Burkholder failed to react with any of the other xanthomonad ribosomes. The 70S ribosomes (AR) of ser. Dowson (Fig. 2-E, F) and ser. Doidge failed to react with ribosomes of *X. translucens* (2-E; wells, 2, 3, 4, 5), *X. malvacearum* (2-E; wells 6, 7), *X. juglandis* (2-F; well 4), *X. fragariae, X. pelargonii* (2-F; well 5), and *X. dieffenbachia* (2-F; well 6) but did cross-react with ribosomes of *X. campestris* (Fig. 2-F; wells 2, 3, and 7). Reciprocal tests using AR of *X. campestris* isolates 18 and 24 also showed strong cross-reactions with ribosomes of *X. vesicatoria*.

To determine if the observed cross-reaction between AR of *X. vesicatoria* and ribosomes of *X. campestris* was a result of genuine structural similarities of ribosomes or to contamination of injected ribosome preparations, ribosomes and ribosomal subunits were subjected to a more rigorous scheme of purification. Prior to the first high-speed centrifugation, cell extracts were centrifuged for 1 hour at 177,000 *g* to pellet cellular membranes. The supernatant was then used to obtain washed ribosomes and subunits, as described. To eliminate soluble proteins and polysaccharides having a molecular weight of less than 2×10^7 and 5×10^6, respectively, washed ribosome preparations which were slightly yellow and subunits were chromatographed on 1.5×15 cm, TKM/3-equilibrated Sepharose 4B (Pharmacia Fine Chemicals, Uppsula, Sweden) columns. Eluants were monitored at 254 nm using an ISCO UA-2 absorbance monitor with a flow-through adapter. Both subunits and 70S ribosomes (Fig. 3) eluted in the void volume as a single peak, but with a trailing shoulder. The preparations were visibly clear and ultraviolet absorption spectra and sedimentation profiles indicated that the main peak contained ribosomes and ribosomal subunits only. After concentrating ribosomes on a PM 30 and subunits on a PM 10 membrane they were stored frozen to be used as antigens for injections.

Results of testing antiserum to ribosomes or subunits obtained from Sepharose columns showed the same specificity and cross-reactivity as the AR from washed ribosomes, indicating a lack of low molecular weight contamination.

Characterization and identification of the R-1 and R-2 immunogens.—When antisera to 30S subunits were reacted against homologous 30S and 50S subunits, a line of precipitin occurred only with 30S subunits. Results of reciprocal tests were the same, that is, no line of precipitin occurred between antiserum of one subunit and antigen of the other subunit. To determine if the R-1 and R-2 immunogens were associated with one or both subunits, purified subunits were reacted against antiserum to homologous 70S ribosomes. The results were that both subunits produced lines of precipitin. Whereas the lines of precipitin produced by 50S subunits fused with those

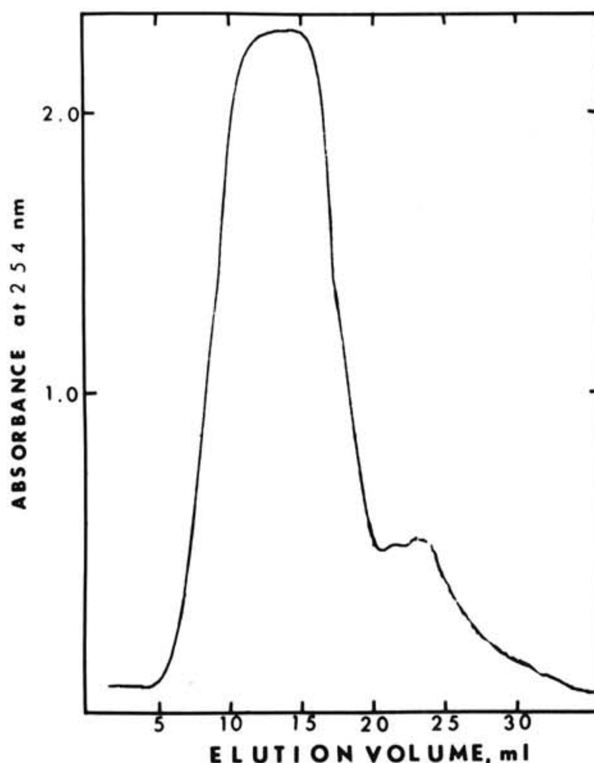

Fig. 3. Chromatographic profile of washed 70S ribosomes of *Xanthomonas vesicatoria* on a column of Sepharose 4B. A 0.8-ml sample containing 4.3 mg of ribosomes per ml was applied to a 1.5×15-cm column equilibrated with TKM/3 pH 7.4 buffer. The sample was eluted with the same buffer. The absorbance of the column effluent was monitored with an ISCO UA-2 absorbance monitor at 254 nm.

produced by 70S ribosomes (Fig. 4-A; wells 1, 2, and 3), the single line produced by 30S subunits had two spurs (Fig. 4-A, well 8), indicating nonidentity. Reciprocal tests using antiserum to 30S subunits also showed a reaction of nonidentity between 30S subunits and 70S ribosomes (Fig. 4-B). Therefore, it was concluded that the R-1 and R-2 immunogens were associated only with the 50S subunit.

To further characterize and identify the R-1 and R-2 immunogens, 50S subunits were separated into their component core particles, split proteins, core proteins, and RNA and tested by gel diffusion tests against antiserum to homologous ribosomes. The R-1 precipitin was produced only with the core particles, whereas the R-2 precipitin was produced by both core particles and RNA. Further evidence that the R-2 immunogen was RNA was determined by incubating core particles and RNA with trypsin and RNase. The R-2 precipitin was completely eliminated by RNase but unaffected by trypsin in gel diffusion tests. The failure of proteins or RNA of core particles to produce the R-1 precipitin was confirmed by treating core particles with trypsin and RNase. The R-1 immunogen was unaffected by either enzyme.

DISCUSSION

The present results support earlier conclusions (19, 20) that the structure of intact ribosomes was immunologically specific at the subspecies level. Ribosomes of the 25 isolates of *X. vesicatoria* were grouped into three distinct serotypes, based on the R-1 precipitin. No similarities existed between ribosomes of *X. vesicatoria* and ribosomes of five species of the *Enterobacteriaceae,* several yellow-pigmented bacteria, *Pseudomonas syringae,* a gram-positive coccus, or six species of *Xanthomonas*. The only ribosomes from other bacteria for which evidence of relatedness was found was with a closely related species, *Xanthomonas campestris*. Thus, the structure of intact ribosomes of *X. vesicatoria* and *X. campestris* has been partially conserved. The negative results with other ribosomes, however, do have a measure of uncertainty. There may be homologies less than sufficient to constitute determinants (27). Moreover, homologies might exist and not be detected by the immunodiffusion techniques used. For example, the inconsistent results with the R-2 determinant needs to be investigated further. Could the R-2 determinant (RNA) be common to all xanthomonads?

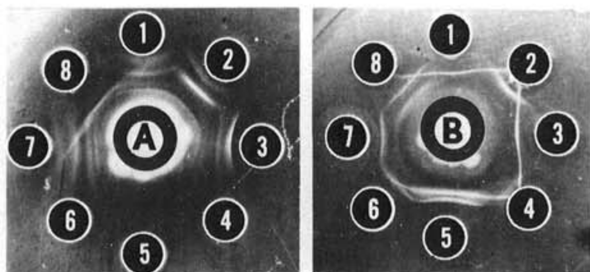

Fig. 4. Immunodiffusion reactions between ribosomes and ribosomal subunits of *Xanthomonas vesicatoria* isolate 215. The center wells contained: **A**) antiserum to 70S ribosomes, and **B**) 30S subunits. **Outer wells of A**) contained: 1), 3), and 7) 70S ribosomes; 2) 50S subunits; and 8) 30S subunits. Wells 4, 5, and 6 were empty. **Outer wells of B**) contained: 1) and 3) 30S subunits of isolate 215; 2) and 4) 70S ribosomes of isolate 215; and 70S ribosomes of isolates 5) 230, 6) 227, 7) 226, and 8) 234.

The failure of purified ribosomal proteins to react with antiserum to homologous ribosomes suggests that the R-1 immunogen is not a complete protein species, but is instead a specific amino acid sequence involved in the secondary or tertiary structure of a protein(s). This would help explain why the immunogenicity of ribosomes is destroyed during protein extraction, and also why only one or two lines of precipitin are evident in double-diffusion tests. Since bacterial ribosomes contain over 50 proteins (8, 25), the presence of only two immunoprecipitins in diffusion tests indicates that ribosomal proteins are either poor immunogens in their natural state or that their immunogenic portion is unexposed. However, the results could also indicate immunogenic competition, that is, the R-1 immunogen might be inhibiting antibodies from being formed against other antigens. Immunogenic competition is supported by the fact that 30S subunits were highly immunogenic (a single immunogen) when injected into rabbits but failed to cross-react with either the R-1 or R-2 immunogens of 70S ribosomes.

Another possible explanation for the failure of extracted protein to react with antiserum to homologous ribosomes is that the R-1 immunogen is not a protein. It is possible that the R-1 immunogen is a high-molecular-weight nonribosomal molecule which is firmly attached to the 50S subunit.

The absence of a cross-reaction between 30S and 50S subunits of *X. vesicatoria* agrees with the heterogeneity of subunits of ribosomes of *Escherichia coli* (25).

It is tempting to speculate on the taxonomic merit of ribosomal immunology. Because members of the genus *Xanthomonas* form a uniform group with respect to their cultural, physiological, and biochemical characters their identification at the species level depends solely upon host specificity. Although many attempts (2, 4, 12, 13) have been made to distinguish members of the genus *Xanthomonas* by agglutination tests of their water soluble, somatic antigens ("0" antigens), none has been successful.

Link and Sharp (13) reported that *X. campestris* and *X. phaseoli* could be differentiated serologically. Brooks et al. (2) reported a close immunological relationship between *X. campestris, X. malvacearum, X. phaseoli, X. pelargonii,* and *X. vitians*. They further stated that several yellow-pigmented non-xanthomonads showed no relationship to the xanthomonads. Link and Link (12) reported that *X. campestris* produced an antiserum highly specific for its homologous organisms. Elrod and Braun (4) divided 36 species of *Xanthomonas* into five serological groups based on cross-agglutinations. On the other hand, Wernham (22) concluded that somatic antigens were of no value for determining species of *Xanthomonas,* and that pathogenicity was needed in order to differentiate the species.

In contrast, results of this study indicate that different species of *Xanthomonas* can be differentiated on the basis of structural antigens, such as ribosomes. Although others (4, 14, 18) have concluded that pepper and tomato strains of *X. vesicatoria* were distinguishable on the basis

of somatic antigens, my results with ribosomal immunogens agree instead with the negative correlation reported by Charudattan et al. (3).

It is concluded that the molecular structure of ribosomes of the phytopathogenic bacterium *X. vesicatoria* has not been conserved during evolution and that ribosomal immunology should be a useful tool to distinguish species and subspecies of bacteria.

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