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Exopolysaccharide Colanic Acid and Its Occurrence in the *Enterobacteriaceae*

W. D. GRANT[1], I. W. SUTHERLAND, AND J. F. WILKINSON
Department of General Microbiology, University of Edinburgh, Edinburgh, Scotland

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A study of strains from the genera *Salmonella*, *Escherichia*, and *Aerobacter* has shown that under appropriate conditions many strains produce an exopolysaccharide slime of identical composition, which has been identified as colanic acid on the basis of its chemical composition and its sensitivity to certain bacteriophage-induced depolymerase enzymes. Chemical analysis shows that the polysaccharide contains *O*-acetyl groups in addition to the sugars glucose, galactose, fucose, and glucuronic acid. Mild acid hydrolysis has led to the isolation of a β-glucosylfucose in addition to glucuronic acid containing oligosaccharides. Many strains were found to synthesize colanic acid under normal conditions of growth or under conditions favoring polysaccharide synthesis, whereas others only synthesized colanic acid when the control mechanism was derepressed by *p*-fluorophenylalanine.

During Kauffmann's studies on the antigens of the *Enterobacteriaceae*, he reported an antigen common to several mucoid strains of *Salmonella paratyphi* B (17). This antigen was subsequently shown to be a polysaccharide (4), and Kauffmann later reported that it was common to all mucoid *Salmonella* species (18). Henriksen (14) was able to show that mucoid *Escherichia coli* strains also produced this "M" antigen; Anderson (1, 2) later showed that a large number of not normally mucoid strains in the *Salmonella-Escherichia* group could be induced to form slime exopolysaccharides when grown at 15 to 20 C in high phosphate concentration. The slime exopolysaccharides produced in high phosphate concentration could be shown in all cases to contain the sugars glucose, galactose, fucose, and glucuronic acid (2). Other reports have described material of the same chemotype from *E. coli* strains (3, 34), including strains secreting an additional exopolysaccharide K antigen (12, 23), and from *Aerobacter cloacae* strains (28). Goebel (12) called the exopolysaccharide of this chemotype isolated from an *E. coli* strain, "colanic acid," and isolated antigenically similar material from the *E. coli* K-12 line (25), suggesting that colanic acid may be the M antigen of Kauffmann and Henriksen, and identical with the exopolysaccharides described by other workers. Recently Markovitz (20, 21) investigated the synthesis of colanic acid in *E. coli* K-12 strains and showed the involvement of a regulator gene which appeared to control certain enzymes believed to be involved in the synthesis of colanic acid. Under normal conditions in most strains, the synthesis of colanic acid was repressed, and the bacteria were nonmucoid. The product of the regulator gene could be inactivated by growth in the presence of *p*-fluorophenylalanine, under which conditions the strains became mucoid (16).

These results have led several investigators to suggest that there is an exopolysaccharide common to many strains within the *Enterobacteriaceae* and that it may be colanic acid. However, the suggestion is based largely on incomplete chemical characterizations of the reported exopolysaccharides, and the distribution of colanic acid within the genera of the *Enterobacteriaceae* is ill-defined. Accordingly, we have reexamined the occurrence of colanic acid within several groups of the *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacterial strains. The following strains of *E. coli* K-12 were used: S22, S23, S45, S56, S53, S53C (capsulate variant of S53), S61 (derived from S53), S33, S5, S7, and S8. Strains CA3 and CA10 were uridine diphosphate glucose pyrophosphorylaseless and uridine diphosphate galactose-4-epimeraseless and were obtained from S. Brenner, Laboratory of Molecular Biology, Cambridge, England.

The *Salmonella* strains listed in Table 1, other than the three *S. typhimurium* LT2 derivatives, were isolated from pathological material at the Department of Bacteriology, Edinburgh Medical School. *S. typhi-*

[1] Present address: McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wis. 53706.

murium LT2 trp/ was a departmental strain. *S. typhimurium* strains SL 1543 and SL 1098, both sublines of LT2, were obtained from B. A. D. Stocker, Department of Microbiology, Stanford University, Calif.

Other strains used were *Shigella flexneri* NCTC 9725, *S. flexneri* NCTC 8522, *A. cloacae* NCTC 5920, *Klebsiella aerogenes* type 1, *K. aerogenes* type 54, and *K. aerogenes* A4 [used by Wilkinson, Duguid, and Edmunds (33)].

Media. All basic media were sterilized by autoclaving at 121 C for 15 min. Supplements were sterilized separately by membrane filtration and added aseptically. Nutrient agar was prepared by dissolving 25 g of Oxoid No. 2 Nutrient Broth powder, and 15 g of Oxoid No. 2 Ionagar in 1 liter of distilled water. The minimal medium described by Davis and Mingioli (6) was used, supplemented where necessary with threonine, leucine, methionine, proline, thiamine, tryptophan, and arginine, which were added to give a final concentration of 0.002% (w/v), and solidified by the addition of 1.5% (w/v) Ionagar. The minimal medium was also used supplemented with DL-*p*-fluorophenylalanine as described by Kang and Markovitz (16) at a concentration of 5×10^{-6} M in solid media. Nitrogen-deficient yeast extract medium, described by Sutherland and Wilkinson (28), was used as a medium enhancing polysaccharide production.

Purification of exopolysaccharides. Bacteria were grown on the solid medium which gave maximal exopolysaccharide production. The isolation and purification of the exopolysaccharide slime has been described previously (28).

Preparation of phage-induced depolymerase enzymes. The source of all the phages isolated was untreated Edinburgh sewage. The host organism was *E. coli* S53 which produced large amounts of exopolysaccharide on all types of media. Depolymerase-producing bacteriophages were isolated by a modification of the method described by Sutherland and Wilkinson (28). Sewage was membrane-filtered (Millipore U.K. Ltd., Wembley, Middlesex, England; pore size, 0.45 or 0.22 nm), and 0.5-ml samples were inoculated into 20 ml of nutrient broth shake cultures of exponentially growing S53. Incubation was continued for 4 hr at 37 C with gentle shaking; the phage lysate obtained was membrane-filtered. Plates of yeast extract medium were uniformly seeded with overnight broth cultures of S53 and allowed to dry. Dilutions of the phage lysates in broth were spotted on the plates, and the plates were incubated at 30 C for 48 hr. Those plaques showing dissolution of the thick layer of exopolysaccharide surrounding and overlying them were picked into 20 ml of nutrient broth cultures of exponentially growing S53. Replating and repicking was carried out several times to obtain pure cultures. Nutrient broth lysates of the phages so obtained were prepared by using exponentially growing S53 as host; they were then membrane-filtered and kept at −20 C. Six such isolates from different sewage samples were made, and the phages were designated B_1 to B_6.

Shake cultures (200 ml) of exponentially growing S53 in liquid yeast extract medium were inoculated with 2 to 3 ml of the phage lysates, and the flasks were incubated overnight at 30 C with gentle shaking. Any remaining cells were centrifuged off at 20,000 × *g*, and the supernatant fluid was dialyzed against frequent changes of distilled water for 5 days at 4 C. A 100-fold concentration of the supernatant fluid was achieved by removing the dialysis casings from the distilled water and surrounding them with solid polyethylene glycol (molecular weight 6,000) at 4 C. Such preparations were kept at −20 C without further purification. In the absence of any serological data at the present, it is not known whether the six enzyme preparations differ from one another or from the other preparations previously reported (28). The six preparations exhibited no visible difference in enzymatic activity.

Enzymatic activity was tested by preparing confluent cultures of various strains on plates of solid media and placing drops of all six depolymerase preparations on the surface of such cultures. After incubation at room temperature for 18 hr, the plates were examined for depolymerase activity, evidenced by large depressions in the layer of exopolysaccharide in the area of the drops, indicating depolymerization.

Analytical methods. Exopolysaccharide preparations were hydrolyzed in sealed ampoules in concentrated formic acid for 18 hr at 100 C. The formic acid was removed by lyophilizing, and the residue was rehydrolyzed for 2 hr with 1.0 N H_2SO_4 at 100 C to remove formyl esters. The solution was then diluted with distilled water and neutralized with Amberlite IRA-410 (HCO_3^- form). The resin was removed by filtration and the solution was concentrated to small volume. Ascending chromatography of the hydrolysates was carried out on thin-layer plates of 0.3-mm thickness prepared from MN 300 cellulose (Macherey, Nagel and Co., Düren, W. Germany) in the following solvents: (A) pyridine-ethyl acetate-acetic acid-water, 5:5:1:3 (v/v/v/v; reference 11); (B) pyridine-butanol-water, 6:4:3 (v/v/v; reference 31); (C) ethyl acetate-acetic acid-formic acid-water, 18:3:1:4 (v/v/v/v; reference 10). High-voltage electrophoresis was carried out on Whatman no. 1 or 3MM paper in pyridine-acetic acid-water, 5:2:43 (v/v/v, *p*H 5.3; reference 27). Sugars were detected with the alkaline silver nitrate reagent of Trevelyan et al. (30) and identified by comparison with standards. For microchemical work, constriction micro pipettes (H. E. Pedersen, Copenhagen) were used, and all glassware was cleaned with concentrated HNO_3 and glass-distilled water. All spectrophotometric measurements were carried out in a Zeiss PMQ2 spectrophotometer. Glucose was determined in hydrolysates with glucose oxidase reagent (The Boehringer Corp.); galactose was determined in hydrolysates with galactostat reagent (Worthington Biochemical Corporation); fucose and glucuronic acid were determined on unhydrolyzed material by the method of Dische and Shettles (7) and Bowness (5), respectively.

Enzymes. Yeast α-glucosidase, almond β-glucosidase, and mollusk β-glucuronidase were commerically available.

RESULTS

Exopolysaccharide production. Plates of media were inoculated from overnight streak cultures

of the same medium incubated at 30 C. They were incubated at 30 C for 24 hr or at 20 C for 7 days and then examined for exopolysaccharide production. Strains which produced a large amount of exopolysaccharide were easily recognized on solid media, forming large, raised viscous colonies in contrast to the more commonly encountered small flat colonies characteristic of most of the coliform group. The exopolysaccharide production of the strains tested is shown in Table 1. Of 13 strains of *E. coli* K-12 examined, 9 produced exopolysaccharide on one or more types of media, ranging from the copiously mucoid S53, S53C, S61, S8, and S33 types to others such as S7 and S56, which produced only small amounts of exopolysaccharide after long incubation at low temperature. Of 21 *Salmonella* species, 13 produced exopolysaccharide in varying amounts. Although yeast extract medium was the most useful medium for enhancing exopolysaccharide production, none of the media used enhanced exopolysaccharide production in all strains. Examination of all the strains which produced exopolysac-

TABLE 1. *Exopolysaccharide production*

Organism	Conditions of growth[a]					
	Nutrient agar		Yeast extract		Minimal medium	
	30 C, 24 hr	20 C, 7 days	30 C, 24 hr	20 C, 7 days	30 C, 24 hr	20 C, 7 days
CA3	−	−	−	−	−	−
CA10	−	−	−	−	−	−
S5	−	−	−	−	−	−
S7	−	−	−	±	−	±
S8	++[b]	++[b]	++[b]	++[b]	++[b]	++[b]
S22	++[b]	++[b]	++[b]	++[b]	++[b]	++[b]
S23	−	−	−	−	−	−
S33	±	+[b]	++[b]	++[b]	±	+
S45	−	−	−	±	−	±
S53	++[b]	++[b]	++[b]	++[b]	++[b]	++[b]
S53c	++[b]	++[b]	++[b]	++[b]	++[b]	++[b]
S56	−	−	−	±	−	−
S61	++[b]	++[b]	++[b]	++[b]	++[b]	++[b]
Aerobacter cloacae 5920	++[b]	++[b]	++[b]	++[b]	++[b]	++[b]
Salmonella anatum	0	0	−	+	+	+
S. bareilly	0	0	−	+[b]	+[b]	+[b]
S. blackley	0	0	−	−	−	−
S. braenderup	0	0	−	−	−	−
S. bredeney	0	0	−	−	−	−
S. choleraesuis var. *kunzendorf*	0	0	−	+	+	++[b]
S. enteriditis	0	0	−	±	+	+
S. heidelberg	0	0	−	−	±	+[b]
S. montevideo	0	0	−	−	+	+
S. paratyphi B	0	0	−	+[b]	+[b]	++[b]
S. poona	0	0	−	−	−	−
S. potsdam	0	0	±	+	+	+
S. saint-paul	0	0	−	−	−	−
S. stanley	0	0	−	−	−	−
S. tuebingen	0	0	−	−	−	−
S. typhi	0	0	−	±	−	±
S. typhimurium 1543	−	±	−	±	±[b]	±[b]
S. typhimurium 1098	++[b]	++[b]	++[b]	++[b]	++	++
S. typhimurium LT2	±	+	++[b]	++[b]	++[b]	++[b]
S. worthington	0	0	−	−	−	−
Salmonella sp. M28246	0	0	−	−	−	−
Shigella flexneri 9725	−	−	−	−	−	−
S. flexneri 8522	−	−	−	−	−	−

[a] Symbols: +, visible exopolysaccharide production; −, no visible exopolysaccharide production; 0, not tested.

[b] Indicates tested with phage-induced depolymerase enzymes.

charide revealed, by the india ink method of Duguid (8), that all of the strains were noncapsulate, with the exception of S53c which had been originally isolated as a colony resistant to several nondepolymerase-producing coliphages (28). Two strains of *S. flexneri* did not not produce visible exopolysaccharide, whereas *A. cloacae* was mucoid as previously reported (28).

Chemical composition of exopolysaccharides. Exopolysaccharide preparations from a number of strains grown on different types of media at 20 and 30 C were examined for sugar composition. Chromatographically, hydrolysates of all the exopolysaccharide preparations examined showed the same components in solvents A, B, and C, namely, glucose, galactose, fucose, and glucuronic acid. To determine whether this similarity was reflected in the quantitative composition, analyses were made on the exopolysaccharides of 15 strains, including the identification and assay of *O*-acetyl groups in some of the preparations by the methods of Thompson (29) and Hestrin (15), respectively (Table 2). There appears to be little significant difference in the quantitative analyses of the sugar components, and of the *O*-acetyl content of the preparations examined.

The average per cent composition of the sugars found, namely, 27.6% fucose, 29.4% galactose, 15.6% glucose, and 18.5% glucuronic acid, infers a molar ratio of 2:2:1:1, respectively, agreeing well with figures previously reported for colanic acid (12, 25).

To provide further indications of the similarities of the exopolysaccharides produced by strains in different genera, 1% (w/v) solutions of exopolysaccharides from *E. coli* S53, *A. cloacae* NCTC 5920, and *S. typhimurium* SL 1543 were passed through amberlite IRA 120 H^+ resin to convert them to the acid form. Portions (100 ml) of each were heated in sealed tubes at 100 C for 16 hr and then dialyzed against distilled water. The dialyzable material was lyophilized and subjected to electrophoresis at 80 to 100 ma, 2,000 v, for 1 hr to separate neutral and changed material.

On chromatography of the neutral material in solvent B, each preparation was found to contain fucose, glucose, galactose, and a spot moving slower than galactose. This proved to be an oligosaccharide containing glucose and fucose in equimolar proportions. All the fucose was reduced to fucitol on treatment with sodium borohydride. Under conditions where cellobiose was 100% hydrolyzed by β-glucosidase, this fraction was 48% dissociated to its monosaccharide components; α-glucosidase had no effect.

The fastest-moving charged oligosaccharide in all three cases proved to contain equal amounts of galactose and glucuronic acid, all of the former sugar being converted to galactitol by borohydride treatment. The enzyme β-glucuronidase caused 85% hydrolysis to the component sugars. The same charged oligosaccharide was obtained from all three exopolysaccharide preparations by hydrolysis [1% (w/v) solutions] in

TABLE 2. *Quantitative analyses of exopolysaccharides*[a]

Organism	Conditions of growth	Fucose	Galactose	Glucose	Glucuronic acid	*O*-acetyl
		%	%	%	%	%
S53	Yeast extract, 30 C, 24 hr	29.3	30.9	13.3	17.2	7.3
S53C	Minimal medium, 20 C, 7 days	32.0	29.1	14.7	17.1	5.9
S61	Yeast extract, 30 C, 24 hr	26.8	28.7	13.9	18.3	6.0
S8	Yeast extract, 20 C, 7 days	27.1	31.2	16.3	17.4	
S22	Yeast extract, 30 C, 24 hr	31.0	31.7	17.0	19.1	
S33	Yeast extract, 20 C, 7 days	27.9	28.4	16.0	19.2	
S45	Yeast extract, 20 C, 7 days	26.1	27.4	15.1	17.9	
Aerobacter cloacae 5920	Nutrient agar, 20 C, 7 days	26.2	28.9	15.4	16.1	6.3
Salmonella bareilly	Minimal medium, 20 C, 7 days	29.0	32.0	17.3	21.2	
S. choleraesuis var. *kunzendorf*	Minimal medium, 30 C, 24 hr	24.0	25.2	17.1	17.2	
S. enteriditis	Minimal medium, 20 C, 7 days	28.1	29.1	19.2	23.5	
S. paratyphi B	Minimal medium, 30 C, 24 hr	23.5	27.2	15.2	15.9	
S. typhimurium 1543	Yeast extract, 20 C, 7 days	27.8	31.0	14.9	21.0	5.6
S. typhimurium LT2	Minimal medium, 30 C, 24 hr	29.0	31.2	13.3	19.3	
S. typhimurium 1098	Yeast extract, 30 C, 24 hr	26.1	29.2	16.1	17.4	5.5

[a] Results are expressed as the average of at least four estimations.

1.0 N H_2SO_4 at 100 C for 30 to 45 min, followed by neutralization with $Ba(OH)_2$ and electrophoresis.

Sensitivity of exopolysaccharides to depolymerase enzymes. The apparent high specificity of phage-induced depolymerase preparations was reported previously (26, 28). The specificity of the preparations available was indicated by testing three types of *K. aerogenes* exopolysaccharides. Two such exopolysaccharides, produced by strains A4 and type 54, were known to have different chemotypes from the exopolysaccharides under investigation (32, 33), whereas type 1 had the same chemotype (9). Confluent cultures of the three strains on plates of yeast extract medium were prepared and tested with drops of all six depolymerase preparations. It was clearly indicated that all three *K. aerogenes* exopolysaccharides were insensitive to all of the depolymerase preparations, whereas, in the strains which elaborated sufficient exopolysaccharide to be tested (Table 1), the exopolysaccharides were sensitive to all the depolymerases.

Production of exopolysaccharides in the presence of p-fluorophenylalanine. Strains which appeared nonmucoid or only slightly mucoid on the media already tested were grown on minimal *p*-fluorophenylalanine medium, supplemented if necessary with growth factors, as described by Kang and Markovitz (16), at 37 C for 24 hr, followed by 7 days at 20 C. The plates were examined for production of exopolysaccharide (Table 3). Of eight strains of *E. coli* K-12 considered to be nonmucoid on ordinary media or producing only small amounts of exopolysaccharide, three produced a large amount of exopolysaccharide in the presence of *p*-fluorophenylalanine and two of the others appreciably more than on ordinary media. Of 13 strains of *Salmonella* considered to be nonmucoid or only slightly mucoid on ordinary media, 7 became mucoid in the presence of *p*-fluorophenylalanine, and 2 others elaborated more exopolysaccharide than on ordinary media. Of the strains producing exopolysaccharide, those that elaborated sufficient exopolysaccharide were tested with depolymerase preparations, and in all cases the exopolysaccharides were sensitive (Table 3). The two *S. flexneri* strains did not produce exopolysaccharide under these conditions.

TABLE 3. *Exopolysaccharide production in the presence of p-fluorophenylalanine*

Organism	Conditions of growth[a]	
	Minimal medium (20 C, 7 days)	Minimal PFA[b] medium (37 C, 24 hr followed by 20 C, 7 days)
CA3	−	−
CA10	−	−
S5	−	++[c]
S7	±	+[c]
S23	−	−
S34	±	++[c]
S45	±	+
S56	±	++[c]
Salmonella blackley	−	±
S. braenderup	−	+
S. bredeney	−	++[c]
S. montevideo	+	+
S. poona	−	++[c]
S. potsdam	+	+
S. saint-paul	±	±
S. stanley	−	+
S. tuebingen	−	+
S. typhi	±	+
S. typhimurium 1543	±	±[c]
S. worthington	±	±
Salmonella sp. M28247	−	+[c]
Shigella flexneri 9725	−	−
S. flexneri 8522	−	−

[a] Symbols: +, visible exopolysaccharide production; −, no visible exopolysaccharide production.

[b] *p*-Fluorophenylalanine.

[c] Indicates tested with phage-induced depolymerase enzymes.

DISCUSSION

The results suggest that a large number of strains in the *Escherichia-Salmonella-Aerobacter* group have the genetic potential for synthesis of an identical exopolysaccharide. Analysis of a representative marker of the exopolysaccharides produced showed that in each case the qualitative and quantitative composition of the product corresponded well with the figures quoted for the exopolysaccharide given the name "colanic acid" (12, 25). Furthermore, partial cleavage of purified exopolysaccharide preparations from strains of three genera, *E. coli* S53, *S. typhimurium* SL 1543, and *A. cloacae* NCTC 5920, by autohydrolysis and partial acid hydrolysis has shown an identical pattern of oligosaccharides, including a β-glucosylfucose and an aldobiuronic acid containing galactose and glucuronic acid, which may be identical with the 3-*O*-β-D-glucuronosylgalactose isolated from colanic acid by Rodén and Markovitz (24).

Sutherland and Wilkinson (26, 28) obtained results which suggested that depolymerase enzymes induced by bacteriophages were active only on exopolysaccharides identical to that of

the host cell. By using an *E. coli* K-12 strain as host, several depolymerase preparations were obtained which were shown to be active on all the exopolysaccharides from the *Salmonella-Escherichia-Aerobacter* group tested. However, the preparations were inactive on three *K. aerogenes* exopolysaccharides, two of different chemotype from that of the host cell and one of the same chemotype, suggesting high specificity. In view of the evidence that the exopolysaccharide of *E. coli* K-12 is colanic acid (10), it seems likely that the depolymerase preparations are specific for colanic acid and that their use as a "typing" mechanism for exopolysaccharides affords an accurate and convenient method for determining similarities in the absence of a reliable precipitating antiserum. We have been unable, however, to produce such an antiserum in a number of different types of experimental animals.

It is probable that those exopolysaccharides produced by several *Salmonella* species (Table 1) which we have not fully characterized are also colanic acid. The results suggest that there is a pattern of repression of colanic acid synthesis within the *Salmonella* genus which is similar to that in the *E. coli* K-12 line described by Kang and Markovitz (25).

A number of *E. coli* strains did not appear to synthesize colanic acid even in the presence of *p*-fluorophenylalanine, and it may be that in these strains a deficiency exists in one or more of the enzymes involved in colanic acid synthesis. In particular, *E. coli* K-12 strains CA3 and CA10 are uridine diphosphogalactose (UDPGal)-4-epimeraseless and uridine diphosphoglucose (UDPG) pyrophosphorylaseless mutants, respectively. Since colanic acid contains galactose and glucose, the inability of these strains to synthesize one or other of these sugars as uridine derivatives, and the concomitant inability of either strain to synthesize colanic acid, suggests the involvement of UDPG and UDPGal in the synthesis of the polymer. In a later paper, the isolation of nucleotide derivatives of the sugars found in colanic acid and the levels of enzymes involved in their synthesis will be described more fully.

We have not examined any of the other groups within the *Enterobacteriaceae*, such as the genera *Erwinia* or *Serratia*, which are known to produce exopolysaccharides, but we are not aware of any material resembling colanic acid being isolated. Similarly, to our knowledge there are no reports of mucoid *Shigella* being isolated, and two strains of *S. flexneri* tested failed to become mucoid even in the presence of *p*-fluorophenylalanine. However, the transfer from *S. dysenteriae* to *E. coli* of a factor which repressed colanic acid synthesis has been reported (22). In addition, it was reported (13) that several factors, including mucoidness, could be rapidly transferred among various *E. coli* strains, but, whereas the other factors were expressed on transfer to *S. flexneri*, the mucoid characteristic was not. The possession of a repressor of colanic acid synthesis suggests that the *Shigellae* may have the genetic potential for the synthesis of colanic acid but may normally be repressed in the same way as some *Salmonella* and *Escherichia* strains. In this case, it seems that the repression cannot be overcome in the presence of *p*-fluorophenylalanine. The genus *Klebsiella* encompasses a large number of antigenically distinct capsulate strains, and despite the plethora of information available about the composition of the capsular material, there are few reports of material of the same chemotype as colanic acid. We have shown that one such exopolysaccharide is quite different from colanic acid. It has been said (19) that colanic acid is the M antigen of the *Enterobacteriaceae*, but it seems that the spread of colanic acid may be restricted within the *Enterobacteriaceae* to those bacteria which are normally found as inhabitants of the intestine, such as the *Salmonella-Escherichia-Aerobacter* group. This may be a reflection of the easy genetic exchange between some of the members (13, 22). Colanic acid may thus be the M antigen of the enterobacteria sensu strictu, rather than of the *Enterobacteriaceae*.

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