The Synthesis of Polysaccharides by Bacteria Isolated from Soil

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SUMMARY: Bacteria capable of synthesizing polysaccharides are present in various agricultural, moorland and forest soils, and may form from 5–16% of the viable bacterial population, as estimated by the plate-dilution counting technique. Chemical examination of the polysaccharides synthesized distinguished four types: levans, glucose-uronic acids, glucose-mannose-uronic acids and glucose-mannose-rhamnose-uronic acids. Bacteria synthesizing these polysaccharides are well distributed in the soils studied, those forming the first two types being by far the most common. There is no clear relationship between morphology of the organisms and type of polysaccharide synthesized.

It has been suggested (Norman, 1942) that the uronide carbon of soil organic matter may be in part derived from microbial polysaccharides. Indirect evidence in support of this hypothesis has been brought forward by Fuller (1947), and Forsyth (1947) isolated from soils a definite polysaccharide fraction which contained uronic acid units. Although an important role in determining the physical structure of soils has been suggested for bacterial polysaccharides (Martin, 1946), little has been done in isolating the bacteria in soil which are capable of synthesizing polysaccharides, or in determining the nature of the polysaccharides produced. Martin (1945) has made a start in this direction. He found, using a medium containing egg-albumin, or sodium nitrate and glucose or sucrose, and pouring plates from a high dilution, that nearly every plate contained from one to five slimy colonies. The majority of the organisms forming these colonies were capsulated. We have surveyed different soils (agricultural, moorland, and forest) to determine (*a*) the presence, and if possible the proportion, of gum-producing bacteria, and (*b*) the nature of the polysaccharides produced by these organisms.

METHODS

Bacteriological methods. The medium used throughout this work was that of Cooper & Preston (1937), called by them Medium II. For counting purposes the CaCO_3 which this medium ordinarily contains can be omitted. Soil samples were taken with sterile precautions and plated by the dilution technique of Cutler, Crump & Sandon (1922) on the day the samples were collected, and usually incubated from 3–4 days at 25°. With longer incubation the plates were frequently overgrown by spreading organisms. The sucrose, used in 2% concentration in the medium, was sterilized separately at double strength and 4 ml. added to an equal quantity of double-strength mineral base + agar.

The proportion of slimy colonies was noted and each colony examined for capsulated organisms before inclusion in the count. From among these colonies (developing on the five replicate plates) random isolations were then made on

to Cooper & Preston's solid medium. Pure cultures obtained on this medium were grown for 2–4 days at 25° on four plates of the same medium and the growth harvested for investigation.

Chemical methods. The polysaccharides were purified and hydrolysed as described by Forsyth & Webley (1949) and the constituent sugars identified by the paper chromatography technique (Partridge, 1946, 1948; Forsyth, 1948) as follows.

Fructose. After hydrolysis with 0.1 N-H₂SO₄ for ½ hr. at 100°, fructose was identified from its R_F values (*n*-butanol, 0.10; phenol, 0.51; *s*-collidine, 0.42), and confirmed by the ketose reaction on spraying the chromatograms with naphthoresorcinol or resorcinol reagent. When fructose was present it was destroyed by dilute acid hydrolysis of another sample of the gum and the residual polysaccharide, if any, reprecipitated with ethanol and subjected to stronger hydrolysis (N-H₂SO₄ for 4 hr.) to test for other sugars. When fructose was not present, another sample of gum was directly hydrolysed with the N-H₂SO₄ for the other sugars. Only 10–20 mg. samples were required.

Glucose. R_F values: *n*-butanol, 0.07; phenol, 0.39; *s*-collidine, 0.39. That the sugar was an aldohexose was confirmed by resorcinol spray.

Mannose. R_F values: *n*-butanol, 0.10; phenol, 0.45; *s*-collidine, 0.46. That the sugar was an aldohexose was confirmed by resorcinol spray.

Rhamnose. R_F values: *n*-butanol, 0.22; phenol, 0.59; *s*-collidine, 0.59. That the sugar was a methylpentose was confirmed by naphthoresorcinol and resorcinol spray.

Uronic acids. These were detected both by the naphthoresorcinol test on the intact polysaccharide and by spraying chromatograms of the hydrolysate with this reagent. They also give spots close to the starting line with the silver nitrate reagent.

RESULTS

From Table 1 it will be seen that the polysaccharide-producing bacteria were present in all three types of soil examined. The proportion varied from about 5 % of the total viable bacterial flora in the B_1 layer of the moorland soil to about 16 % in the top 6 in. of the agricultural soil. From the soils listed in Table 1 and from one other agricultural soil 40 strains were isolated at random from the slimy colonies on the plates. Table 2 gives a complete list of the

Table 1. *Gum-producing bacteria in soils*

Soil sample		Dilution used for plating	Mean count (3–5 plates)		Proportion of gum-pro- ducing bacteria (%)
Location and type	Depth		Total	Gummy colonies	
Moorland (Dinnet)	A_2^* layer	1/2,500	120	13	10.8
	B_1^* layer	1/2,500	73	5	4.5
Broad-leaved wood- land (Dunottar)	2–4 in.	1/25,000	98	9	9.5
	12–15 in.	1/25,000	76	11	15
Agricultural 3rd year year grass (Stonehaven)	0–6 in.	1/250,000	131	21	16

* A_2 = Upper leached layer of the soil (usually grey in colour).

* B_1 = Upper layer of less strongly leached sub-soil (usually reddish or yellowish in colour).

Table 2. *The morphological characters of soil bacteria and the constituent sugars of the polysaccharides synthesized by them*

Soil sample		Morphology	Components of the polysaccharides
Location and type	Depth		
Dinnet Moor	Layer A_2	Spore former, Group 1	Fructose
		Short non-sporing rod	Glucose, mannose, rhamnose, uronic acid
		Short non-sporing rod	Glucose, uronic acid
		Spore former, Group 1	Fructose
	Layer B_1	Spore former, Group 1	Fructose
		Spore former, Group 2	Fructose, glucose, mannose, uronic acid
		Short non-sporing rod	Glucose, mannose, rhamnose, uronic acid
		Spore former, Group 1	Glucose, uronic acid
Tilliecorthy; agricultural soil	12-15 in.	Spore former, Group 1	Glucose, uronic acid
		Spore former, Group 2	Fructose, glucose, uronic acid
		Spore former, Group 2	Glucose, mannose, uronic acid
		Short non-sporing rod	Glucose, uronic acid
Dunottar Woods; broad-leaved woodland soil	2-4 in.	Spore former, Group 2	Fructose, glucose, mannose, uronic acid
		Short non-sporing rod	Glucose, mannose, rhamnose, uronic acid
		Medium non-sporing rod	Glucose, mannose, uronic acid
		Spore former, Group 2	Glucose, mannose, uronic acid
	12-15 in.	Short non-sporing rod	Glucose, uronic acid
		Spore former, Group 1	Fructose
		Short non-sporing rod	Glucose, uronic acid
		Short non-sporing rod	Glucose, uronic acid
		Short non-sporing rod	Glucose, mannose, rhamnose, uronic acid
		Short non-sporing rod	Fructose, glucose, uronic acid
		Short non-sporing rod	Glucose, uronic acid
		Short non-sporing rod	Glucose, uronic acid
Stonehaven; agricultural soil	0-6 in.	Short non-sporing rod	Glucose, uronic acid
		Spore former, Group 1	Fructose
		Short non-sporing rod	Glucose, uronic acid
		Spore former, Group 1	Fructose
		Spore former, Group 1	Fructose
		Pleomorphic type	Fructose
		Short non-sporing rod	Glucose, uronic acid
		Short non-sporing rod	Glucose, uronic acid
		Short non-sporing rod	Glucose, mannose, rhamnose, uronic acid
		Pleomorphic rods	Glucose, uronic acid
		Pleomorphic rods	Glucose, uronic acid
		Medium non-sporing rod	Fructose, glucose, uronic acid
		Pleomorphic rods	Glucose, mannose, uronic acid
		Spore former, Group 1	Glucose, uronic acid
		Spore former, Group 1	Fructose
		Spore former, Group 1	Fructose

bacteria, their morphological character, and the constituent sugars of the polysaccharides synthesized by each. The spore-forming bacteria have been divided into groups after Smith, Gordon & Clark (1946). The pleomorphic organisms were similar morphologically to those described by Conn & Dimmick (1947) under the genus *Arthrobacter*.

DISCUSSION

It is apparent from Table 1 that gum-producing bacteria are present in reasonable numbers in all the soils studied, and from Table 3 that the levan and glucose-uronic types of polysaccharides predominate in the soils studied. A glucose-mannose-uronic type is also present. A group of organisms synthesizing a polysaccharide containing rhamnose is moderately prevalent and well distributed. Such a polysaccharide has not hitherto been isolated from a non-pathogenic bacterium.

Table 3. *The different types of polysaccharide gum produced by soil bacteria; distribution among the soils*

The different types of bacterial gums give on hydrolysis the following sugars: *A*, fructose; *B*, fructose, glucose, uronic acid; *C*, fructose, glucose, mannose, uronic acid; *D*, glucose, uronic acid; *E*, glucose, mannose, uronic acid; *F*, glucose, mannose, rhamnose, uronic acid.

Soil	Type of polysaccharide synthesized						Total
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	
	No. of isolates						
Dinnet moorland	3	0	1	3	0	2	9
Dunottar woodland	1	1	1	6	2	2	13
Stonehaven agricultural	6	1	0	6	1	1	15
Tilliecorthy agricultural	0	1	0	1	1	0	3
Totals	10	3	2	16	4	5	40

It is known that the Group 1 bacilli of Smith *et al.* (1946) produce levans (Harrison, Tarr & Hibbert, 1930; Challinor, Haworth & Hirst, 1934; Lyne, Peat & Stacey, 1940; Forsyth & Webley, 1949). Group 2 bacilli produce glucose-uronic and glucose-mannose-uronic types (Forsyth & Webley, 1949). Soil organisms such as *Rhizobium* spp. and *Azotobacter* spp. (Cooper, Daker & Stacey, 1938) and possibly the *Cytophaga* group (Walker & Warren, 1938) also produce polysaccharides of the glucose-uronic type. Martin (1945) has isolated from soil non-spore-forming rods which synthesize polysaccharides of this type.

Table 4. *Morphology of soil bacteria and type of polysaccharide synthesized*

Morphological group	Type of polysaccharide* synthesized						Total
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	
Spore-former Group 1	9	0	0	3	0	0	12
Spore-former, Group 2	0	1	2	0	2	0	5
Short non-sporing rods	0	1	0	11	0	5	17
Medium non-sporing rods	0	1	0	0	1	0	2
Pleomorphic rods	1	0	0	2	1	0	4

* As in Table 3.

When a comparison is made of the morphological characteristics and the type of polysaccharide synthesized by the soil bacteria, it is seen (Table 4) that there is no clear correlation between morphology and type of polysaccharide synthesized; different types of polysaccharide are produced by members of a given morphological group. The results for the spore-formers are in close agreement, as regards the type of polysaccharide produced within each group, with the findings of Forsyth & Webley (1949) for type cultures of species of the genus *Bacillus*. The predominant group of short non-spore-forming rods produce gums chiefly of the glucose-uronic type.

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