DISCUSSION

We believe that our procedure is an improvement on those of other workers for the following reasons. We are in agreement with Najjar & Ketron (1944) that nicotinamide methochloride is partly destroyed by sodium sulphite and that the procedure suggested by Mason & Williams (1942) gives too high results. Najjar & Ketron (1944) and Coulson (1944) allow for the interference of nicotinamide methochloride by assuming that the galvanometer deflexion due to F_2 in the NaOH blank bears a fixed relation to that due to F_3 in the unknown. No such assumption is necessary in our method as we are able to measure F_3 directly.

In the method of Mickelsen *et al.* (1945) the isobutanol extraction is carried out at a pH 8.0–9.5, at which F_2 in the NaOH blank does not fluoresce, but the fluorescence of thiochrome in the unknown is unimpaired. Table 4 confirms this but shows that this treatment does not affect F_3 , so that in the presence of nicotinamide methochloride both thio-

chrome and F_3 contribute to the fluorescence. Table 5 shows that for this reason the method of Mickelsen *et al.* (1945) gives too high results when applied to rat and human urine; e.g. the urine of vitamin B₁-deficient rats was found by this method to contain 0.45 μ g. vitamin B₁/100 ml., whereas none was found by our method. For the sample of human urine the discrepancy was less since less nicotinamide was present.

SUMMARY

A modification of the thiochrome method for the fluorimetric determination of vitamin B₁ in urine in the presence of nicotinamide methochloride is described. Since treatment of the isobutanol extract with HCl removes the thiochrome fluorescence, the difference between readings taken before and after this treatment is a measure of the vitamin B₁ content of the urine.

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Aggregate Formation in Soil

1. INFLUENCE OF SOME BACTERIAL POLYSACCHARIDES ON THE BINDING OF SOIL PARTICLES

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For maximum crop production it is essential that the soil should have a good crumb structure and that the crumbs should be stable and resistant to the dispersing effects of rain, frost, etc. Aggregate formation facilitates cultivation, drainage and aeration, increases the moisture-holding capacity of soil and reduces erosion. It also maintains sufficient cohesion in the soil to give anchorage to plants, and yet sufficient incoherence to facilitate root penetration and emergence of seedlings. Large additions of organic matter and the growth of grassland vegetation are

credited with having an ameliorative effect on the physical state of the soil, but there is considerable diversity of opinion as to the exact processes involved in the formation of water-stable aggregates.

Stable structure formation under natural conditions is possibly a gradual process influenced by many factors. It is reasonable to expect that physical, chemical and biological agencies are involved. The biological agencies include plants, animals and micro-organisms; and of these the micro-organisms are of great importance in pro-

ducing cementing or stabilizing substances, and filamentous forms like fungi, actinomyces and some algae may have a mechanical binding effect.

Geoghegan & Brian (1946) reported that bacterial polysaccharides of the levan and dextran types have a marked aggregating effect on soil particles. Levans in which fructofuranose units are joined by 2:6 linkages are formed by a wide variety of aerobic organisms including plant pathogens. Dextrans, polyglucoses in which the units are joined by 1:6 glucosidic linkages, are formed by various species of *Leuconostoc* bacteria and possibly other organisms.

As a preliminary to finding how these bacterial products aggregate soil particles, exceptionally pure samples of levans and dextrans have been prepared and examined in some detail.

EXPERIMENTAL

Bacterial strains used. An organism, isolated from garden soil and identified as a strain of *Bacillus subtilis*, was used for levan synthesis. Cultures of *Leuconostoc dextranicum* (N.C.T.C. Cat. no. 3354) and *Leuconostoc mesenteroides* (N.C.T.C. Cat. no. 3351) were obtained from the National Collection of Type Cultures and used for dextran production.

Media used. Media having the following composition were employed:

A		B	
KH ₂ PO ₄	1.5 g.	K ₂ HPO ₄	1.0 g.
NaNO ₃	2.0 g.	Na(NH ₄)HPO ₄ ·4H ₂ O	4.9 g.
MgSO ₄ ·7H ₂ O	0.5 g.	MgSO ₄ ·7H ₂ O	0.5 g.
KCl	0.3 g.	KCl	0.5 g.
Distilled water	1000 ml.	FeSO ₄ ·7H ₂ O	0.01 g.
		Bactopeptone	2.0 g.
		Distilled water	1000 ml.

Sucrose was added to (A) in amounts sufficient to give 2, 4, 6, 8 and 10% (w/v) concentrations of sucrose in the final medium and to (B) to give 2 and 10% (w/v). The media were adjusted to pH 7.0, dispensed in litre quantities in 1500 ml. Erlenmeyer flasks and sterilized by autoclaving under 15 lb./sq.in. pressure for 15 min. To avoid precipitation it was necessary to sterilize the MgSO₄·7H₂O separately, and after cooling to add it to the rest of the cooled medium employing aseptic technique.

Production of levans. The media were inoculated with the washings of 48 hr.-old agar-slant cultures of the organism, and after 8–10 days' incubation at 27° large amounts of polysaccharides were formed. The gross impurities were removed by filtering the solutions through no. 1 Whatman filter paper and the liquids were concentrated *in vacuo*. Following centrifugation and dialysis, sufficient ethanol was added to give 70% (v/v) in the final solutions, and the precipitated polysaccharides were obtained as white products. These crude materials, resuspended in water, formed viscous opalescent solutions, which contained large amounts of bacterial cells and 'cell debris'. In case nucleoproteins were present the solutions were adjusted to pH 4.0 and centrifuged. The precipitate was discarded and the supernatant fluid dialyzed until it had a pH of 7.0. Finally, proteins and bacterial cells were removed by the method

of Sevag (1934) and the purified levans were precipitated with ethanol, dried, and ground to fine powders.

Production of dextrans. The media were inoculated with 5 ml. of 4-day-old sucrose-broth cultures of the organisms and, except in the case of the product from *Leuconostoc dextranicum*, the method of isolation and purification of the dextrans was the same as for levans. The dextran produced by *L. dextranicum* was insoluble in water after ethanolic precipitation; hence in the isolation of this polysaccharide the first precipitation with ethanol was omitted.

Hydrolysis of levans. The purified levans were dissolved in excess 0.1 N-H₂SO₄ and heated under reflux for 30 min. The hydrolysates were cooled to 60° and neutralized with solid BaCO₃, rapidly cooled and the BaSO₄ removed by centrifugation. The clear solutions were then made up to the required volumes.

Hydrolysis of dextrans. The method of hydrolysis in this case was identical with that used for levan, except that 2 N-H₂SO₄ was used and heating was continued for 3 hr.

Estimations

(1) **Total reducing sugars.** According to the Shaffer-Somogyi method (Heinze & Murneek, 1940).

(2) **Fructose.** By the 'ferricyanide method' of Becker & Englis (1941). Methyl red was used as indicator instead of sodium diphenylaminesulphonate.

(3) **Total N.** By the micro-Kjeldahl method (Pregl, 1930).

(4) **Aggregating effect.** A soil, the structure of which in terms of water-stable crumbs is extremely poor, was air-dried and ground to pass through a 52-mesh B.S.S. sieve. The polysaccharides, in colloidal suspension, were applied so that different samples of soil contained from 0.05 to 0.75% polysaccharide. Sufficient moisture was used to bring the soil to the 'sticky point'. In this state it was well stirred with a glass rod, placed on a 3 mm. sieve and pressed through it.

The aggregates were dried at 50° and their stability was tested by the following technique: 25 g. of aggregates were placed in 600 ml. of water in a 1 l. cylinder. The cylinder was inverted once and then allowed to stand for 30 min. After this the cylinder was inverted 30 times, the operation taking about 1 min. The contents were then poured on to a 52-mesh B.S.S. sieve (8 in. diameter) standing in a shallow bowl. Extra water was added to bring the level above that of the soil on the sieve. The sieve was then moved up and down 30 times, the water allowed to drain out each time; the sieve was gently tilted each time. The sieve was then removed, the contents washed into a basin and the whole evaporated to dryness (105°). The weight of water-stable aggregates is expressed as a percentage of the air-dried soil. The control samples (moistened with water, granulated and dried) were completely unstable when they were wet-sieved. Apparently, wetting and drying or pressing of moist soil through a sieve, *per se*, is unable to produce water-stable aggregates.

The advantages of the above method are that there is very good agreement between replicates, results are reproducible and small differences in the aggregating abilities of the polysaccharides are easily measured.

Viscosity measurements. To avoid terminological confusion, so common in this field, we adopted throughout the symbols and nomenclature proposed by Cragg (1946).

The measurement of viscosities of levan and dextran solutions involved the determination of the rate of flow of the solutions in an Ostwald viscometer. All solutions were thoroughly centrifuged before being run into the viscometer. The flow time and density (pyknometer method) of levan and dextran solutions, varying in concentration from 1.5 to 0.125% (w/v), and weaker dilutions where necessary, were determined at $20 \pm 0.1^\circ$, and from the data the relative viscosity (η_r) was calculated.

If d_1 = density of solvent,
 d_2 = density of polysaccharide solution,
 t_1 = flow time of solvent,
 t_2 = flow time of polysaccharide solution,

then $\eta_r = \frac{t_2 d_2}{t_1 d_1} = \frac{t_2 d_2}{t_1}$ when the solvent is water, and the specific viscosity (η_{sp}) = $\eta_r - 1$.

Hence the reduced viscosity (η_{sp}/c) may be calculated, and if this is plotted against the corresponding concentration c (in primary moles) the intrinsic viscosity

$$\lim_{c \rightarrow 0} \left(\frac{\eta_{sp}}{c} \right)$$

is obtained.

RESULTS

Bacillus subtilis was cultured in a number of different media in which the sucrose concentration and source of nitrogen were varied, and in every case there was marked opalescence and an increase in viscosity associated with the formation of a polysaccharide which was a levan (97–98% fructose after hydrolysis). The levan from each medium was thoroughly purified, and several samples of the products from 2 and 10% (w/v) sucrose media were prepared.

Leuconostoc dextranicum when cultured in media A and B containing 10% (w/v) sucrose produced large quantities of dextran (96–98% glucose) while *L. mesenteroides*, being apparently more fastidious in its requirements, produced dextran in medium B only. After ethanolic precipitation the dextran from *L. dextranicum* was insoluble in water, while the product from *L. mesenteroides* gave a colloidal suspension similar to that of levans. However, when the material was worked into a paste and then diluted by adding more water a suspension was obtained which was fairly stable on standing, but precipitation occurred when it was centrifuged at a low speed.

Aggregation of soil particles

The ability of each product to form water-stable soil crumbs was estimated. Results are presented in Figs. 1, 2 and 3. The amounts of the different levans and dextrans applied to soil are expressed as a percentage of the air-dried soil and the plotted values of percentage aggregation are the means of duplicate results, except in the case of levans 107 and F7 where only single estimations were made. For substances giving an average aggregation of 10–80%, S.E. (single determination) = ± 2.46 , and S.E. (mean

of 2 determinations) = ± 1.74 . For aggregations less than 10% or greater than 80%, S.E. falls towards zero.

Fig. 1. Effect of increasing quantities of various levans on aggregation of soil particles. (Levans produced by *B. subtilis* when cultured in medium A containing from 2 to 10% sucrose.)

Levan preparation	Culture medium
147, 152, 129	A + 2% (w/v) sucrose
141	A + 4% (w/v) sucrose
136	A + 6% (w/v) sucrose
135	A + 8% (w/v) sucrose
131, 107	A + 10% (w/v) sucrose

Up to 0.25%, the aggregating effect bears an approximately linear relationship to the amount of levan or dextran added. Consequently, 0.25% appears to be the most suitable concentration at which to appraise the aggregating effect of the different products.

All the levans and dextrans had a marked ameliorative effect on the physical condition of soil, but some levan products had a more pronounced effect than others. Apparently the aggregating effect of the levan decreases with increase in sucrose content of the medium. Also it appears that, when two or

more samples of levan are prepared from the same medium but from different lots of culture solution, their aggregating effects are not exactly alike.

absorb a certain amount of levan, and, having absorbed this amount, the degree of aggregation produced is dependent on the activity of the substance.

Fig. 2. Effect of increasing quantities of various levans on aggregation of soil particles. (Levans produced by *B. subtilis* when cultured in medium B containing 2 and 10% sucrose.)

Levan preparation	Culture medium
130 A, 145 A	B + 2% (w/v) sucrose
MS 1, F7, 151	B + 10% (w/v) sucrose

Effect of applying large doses of levan to soil. In the case of those levans which possess a relatively low aggregating effect, it was thought that the degree of aggregation might be increased by applying larger quantities of levan to the soil. This was investigated (Table 1) by treating samples of soil with product 131, until the soil contained 2 and 5% levan, and comparing the degree of aggregation produced with that obtained when the soil contained from 0.05 to 0.75% levan. All determinations were done in duplicate and mean results are shown.

Apparently, further application of levan beyond a certain point has very little effect on aggregation of the soil particles, which suggests that soil can only

Fig. 3. Effect of increasing quantities of various dextrans on aggregation of soil particles.

Dextran preparation	Synthesizing organism	Culture medium
148	<i>Leuconostoc dextranicum</i>	B + 10% (w/v) sucrose
149	<i>Leuconostoc dextranicum</i>	A + 10% (w/v) sucrose
154	<i>Leuconostoc mesenteroides</i>	B + 10% (w/v) sucrose

Table 1. *Effect of applying levan to soil in large and small amounts*

Levan (no.)	Amount added to soil (%)	Aggregation (%)
131	5.0	76.1
	2.0	65.6
	0.75	69.4
	0.50	61.4
	0.25	37.0
	0.125	13.6
	0.05	3.3
	Control	0.8

Table 2. *Effect of medium and of percentage sucrose in the medium on the intrinsic viscosity and molecular weight of levans produced by B. subtilis*

(K _m = 5 × 10 ⁻⁴)						
Polysaccharide (no.)	Medium in which polysaccharide was produced	Concentration c (in primary moles)	Flow time (sec.)	Density d	$\frac{\eta_{sp}}{c}$	Mol. wt.
147	A + 2% (w/v) sucrose	0.093	286.6	1.007	36.2	26,800
		0.062	158.8	1.005	23.0	
		0.031	97.5	1.003	15.5	
		0.0155	79.6	1.001	13.4	
		0.0093	74.2	1.000	13.4	
		(Water = 66.0)				
152	A + 2% (w/v) sucrose	0.093	169.6	1.007	17.0	18,800
		0.062	117.3	1.005	12.7	
		0.031	86.5	1.003	10.13	
		0.0155	75.8	1.001	9.68	
		0.0078	70.9	1.000	9.48	
		(Water = 66.0)				
129	A + 2% (w/v) sucrose	0.093	112.4	1.007	14.30	16,000
		0.062	82.4	1.005	11.43	
		0.031	61.6	1.003	9.00	
		0.0155	54.3	1.001	7.79	
		0.0093	51.5	1.000	8.38	
		(Water = 48.5)				
141	A + 4% (w/v) sucrose	0.093	131.0	1.007	10.73	14,000
		0.062	99.8	1.005	8.40	
		0.031	80.6	1.003	7.26	
		0.0155	73.4	1.001	7.28	
		0.0093	70.2	1.000	6.90	
		(Water = 66.0)				
136	A + 6% (w/v) sucrose	0.093	71.1	1.007	5.12	8,000
		0.062	62.2	1.005	4.70	
		0.031	54.7	1.003	4.23	
		0.0204	52.4	1.002	4.00	
		(Water = 48.5)				
135	A + 8% (w/v) sucrose	0.093	67.5	1.007	4.31	7,200
		0.062	60.2	1.005	4.00	
		0.031	53.9	1.003	3.71	
		0.0155	51.3	1.001	3.80	
		(Water = 48.5)				
107	A + 10% (w/v) sucrose	0.083	56.4	1.006	4.42	6,400
		0.062	50.1	1.005	3.44	
		0.031	45.6	1.003	3.29	
		(Water = 41.5)				
131	A + 10% (w/v) sucrose	0.093	64.6	1.007	3.67	6,200
		0.062	58.6	1.005	3.45	
		0.031	53.0	1.003	3.10	
		0.0155	50.8	1.001	3.10	
		(Water = 48.5)				
145 A	B + 2% (w/v) sucrose	0.093	134.5	1.007	19.3	18,000
		0.062	90.1	1.005	14.0	
		0.031	64.3	1.003	10.65	
		0.0155	55.1	1.001	8.71	
		0.0093	52.8	1.000	9.46	
		(Water = 48.5)				
130 A	B + 2% (w/v) sucrose	0.093	101.5	1.007	11.80	15,600
		0.062	79.8	1.005	10.53	
		0.031	61.1	1.003	8.48	
		0.0155	54.2	1.001	7.68	
		0.0093	52.1	1.000	7.96	
		(Water = 48.5)				
MS1	B + 10% (w/v) sucrose	0.093	76.8	1.007	6.39	9,400
		0.062	65.6	1.005	5.81	
		0.031	55.9	1.003	5.00	
		0.0155	52.0	1.001	4.77	
		(Water = 48.5)				
F7	B + 10% (w/v) sucrose	0.083	65.5	1.006	7.15	9,600
		0.062	55.8	1.005	5.66	
		0.031	47.8	1.003	5.00	
		(Water = 41.5)				
151	B + 10% (w/v) sucrose	0.093	121.8	1.007	9.23	12,200
		0.062	97.2	1.005	7.74	
		0.031	78.9	1.003	6.42	
		0.0155	72.2	1.001	6.13	
		(Water = 66.0)				

Table 3. *Intrinsic viscosity and molecular weight of the dextran produced by Leuconostoc mesenteroides*

$(K_m = 5 \times 10^{-4})$						
Dextran (no.)	Medium in which dextran was produced	Concentration c (in primary moles)	Flow time (sec.)	Density d	$\frac{\eta_{sp}}{c}$	Mol. wt.
154	B + 10% (w/v) sucrose	0.111	185.6	1.009	16.5	14,000
		0.055	100.7	1.004	9.65	
		0.028	81.1	1.003	8.29	
		0.014	72.5	1.001	7.14	
		0.007	69.2	1.000	7.00	

(Water = 66.0)

Viscosity measurements

Since the aggregating effects of levans from different media were not alike, it was thought that the ability to stabilize soil crumbs might be related to some chemical or physical property of the polysaccharides. This idea was supported by the observation that after ethanolic precipitation the products from '2% (w/v) sucrose' media were tough and when stretched showed pronounced recoil, while those from '10% (w/v) sucrose' medium were viscous and capable of being drawn into threads. The chain length of the polymers, which influences their physical properties, was therefore investigated by viscosity measurements.

The intrinsic viscosities of solutions of the various levans and the dextran produced by *L. mesenteroides* were determined, and the values correlated with the corresponding aggregating effects. Whilst the value $\lim_{c \rightarrow 0} \left(\frac{\eta_{sp}}{c} \right)$ yielded all the information required, the molecular weights were found from the Staudinger relationship $\frac{\eta_{sp}}{c} = K_m M$, where K_m is a constant estimated to have the value 10^{-3} for cellulose in Schweitzer's reagent ($c = 1.4\%$ (w/v) (Staudinger, 1933)). More recently the constant $K_m = 5 \times 10^{-4}$ has been used by Staudinger (1938).

The constants for our levans and dextran were not determined and $K_m = 5 \times 10^{-4}$ was selected. The use of a constant which applies to cellulose in Schweitzer's reagent in calculating the molecular weights of levans and dextrans is admittedly an approximation but gives information on the order of the molecular weights. The results are shown in Tables 2 and 3.

The intrinsic viscosities and hence the molecular weights of the levans decreased with increasing sucrose content of the medium. In the case of '2% sucrose' media the form of nitrogen supplied to the organism did not materially influence the intrinsic viscosities of the products. The intrinsic viscosity of the dextran produced by *L. mesenteroides* is similar to that of some levans.

There appears to be a relationship between the value of $\lim_{c \rightarrow 0} \left(\frac{\eta_{sp}}{c} \right)$ and the percentage aggregation.

From the values for intrinsic viscosity and aggregating effect of 13 levans and 1 dextran a correlation coefficient was calculated and found to be 0.809. This value indicates a significant correlation (odds 1 in 1000).

A dot diagram, in which the value of $\lim_{c \rightarrow 0} \left(\frac{\eta_{sp}}{c} \right)$ is plotted against the degree of aggregation produced when soil contains 0.25% of the various levans and dextrans, is given in Fig. 4. No curve is fitted to the

Fig. 4. Relationship between the intrinsic viscosities and the aggregating effects of 13 levans and 1 dextran.

points on the diagram; this cannot be done satisfactorily until the shape of the curve as it approaches zero and 100% aggregation can be forecast for substances of lower and higher intrinsic viscosities than those so far considered.

Nitrogen content of levans and dextrans

Solutions of bacterial polysaccharides are very viscous and separation of the metabolic products from bacterial cells is very difficult. Levans and dextrans as usually prepared contain a small amount of nitrogen (0.2–0.5%) which may be present as an impurity or as a constituent of the polysaccharide

molecule. Stacey (1943) has suggested that dextrans consist of polyglucose chains linked together by units of the synthesizing enzyme which remains as an integral part of the complex mucopolysaccharide. In our earlier investigations we found that the levan obtained when *B. subtilis* was cultured in '10% sucrose' media contained 0.09% N, whereas that from '2% sucrose' media contained 0.2 to 0.3% N, and since the product from the '10% sucrose' media was not as effective an aggregator of soil as the product from '2% sucrose' medium it appeared that the aggregating effect was related to the nitrogen

increases with increase in sucrose content of medium A. This increase in yield of levan is accompanied by a lowering of the nitrogen content of the 'crude levan', which suggests that the ratio of levan to bacterial cells in the culture solution is increased with increase in sucrose content of the medium. Microscopical examination of levan solutions has shown that, even after most careful purification, a small amount of bacterial cells remains. It is reasonable to expect that the amount of bacterial cells that remains is influenced by the ratio of levan to bacterial cells in the crude material.

Table 4. *Nitrogen content, molecular weight and aggregating effect of some levans and dextrans*

Polysaccharide (no.)	Medium in which polysaccharide was formed	Nitrogen (%)	Mol. wt.	Aggregating effect when soil received 0.25% polysaccharide
Levan				
147	A + 2% (w/v) sucrose	0.12	26,800	90.9
152	A + 2% (w/v) sucrose	0.13	18,800	80.1
129	A + 2% (w/v) sucrose	0.05	16,000	75.1
130 A	B + 2% (w/v) sucrose	0.18	15,600	56.0
145 A	B + 2% (w/v) sucrose	0.09	18,000	88.9
141	A + 4% (w/v) sucrose	0.09	14,000	80.9
136	A + 6% (w/v) sucrose	0.06	8,000	54.1
135	A + 8% (w/v) sucrose	0.03	7,200	52.9
131	A + 10% (w/v) sucrose	0.02	6,200	37.0
107	A + 10% (w/v) sucrose	0.10	6,400	20.5
MS 1	B + 10% (w/v) sucrose	0.10	9,400	35.8
F7	B + 10% (w/v) sucrose	0.09	9,600	41.4
151	B + 10% (w/v) sucrose	0.06	12,200	82.7
Dextran				
148	B + 10% (w/v) sucrose	0.22	—	67.9
149	A + 10% (w/v) sucrose	0.00	—	70.1
154	B + 10% (w/v) sucrose	0.11	14,000	69.7

content. However, by improving the technique used for separating the polysaccharides from the bacterial cells, the nitrogen content of the products was lowered appreciably without degrading the products and without lowering the aggregating effects. In Table 4 are shown the nitrogen content, molecular weight and aggregating effect of some of our most purified levans and dextrans.

It appears that the aggregating effects and the molecular weights of these products are not related to their nitrogen contents. This is most evident in the case of levans from the same medium.

In the case of levans 141, 136, 135 and 131 there appears to be a decline in molecular weight and aggregating effect as the nitrogen content of the products decreases, but these materials are from media containing different concentrations of sucrose, and it has been shown that the molecular weight and aggregating effect decrease with increasing sucrose concentration of the medium. The decline in nitrogen content, accompanying the decrease in molecular weight and aggregating effect, is possibly due to the fact that the yield of levan/l. of culture solution in-

In another experiment levans were isolated in crude form and samples of the crude material were purified to different degrees. The nitrogen content and molecular weight of each product were determined. Results are recorded in Table 5.

Table 5. *Nitrogen content and molecular weight of 'crude' and 'purified' levans*

Levan (no.)	Nitrogen content (%)	Molecular weight
151 Crude	0.13	12,200
Purified	0.06	12,200
139 Crude	0.43	6,200
Purified	0.11	7,600

Relatively large amounts of nitrogen were removed without degrading the substances, and it appears that there is no correlation between molecular weight and nitrogen content of levans produced by *B. subtilis*.

Table 6 shows the results of an experiment designed to test how the aggregating effect of a levan was affected when its nitrogen content was lowered.

Table 6. *Relation between aggregating effect and nitrogen content of a levan*

Levan (no.)	Amount added to soil (%)	Aggregation (%)	
		Sample 1, N=0.25 %	Sample 2, N=0.09 %
124	0.75	73.8	83.3
	0.50	69.8	78.6
	0.25	48.8	60.8

As the nitrogen content decreased the aggregating effect increased, which shows that purification removed only extraneous matter and did not affect the ability of the substance to form water-stable soil crumbs.

It will be recalled that it has been suggested that polysaccharides owe certain of their immunological properties to the presence in them of a nitrogenous constituent, e.g. Fitzgerald (1933) found from serological studies that the antigenic activity of a polysaccharide produced by *Leuconostoc mesenteroides*

separate actions, and it is not necessary to degrade the levan to remove nitrogen.

Since the intrinsic viscosities, and hence the molecular weights of levans and dextrans, were not affected by removal of nitrogen, it is concluded that the nitrogen content of the polysaccharides examined is due to the presence of bacterial cells, as impurities, in the substances.

Stability of soil aggregates made with bacterial levan

It is well known that unstable soil aggregates disintegrate when they are placed in water. However, when aggregates containing 0.5 % levan were submerged in water for 20 days it was observed that they were more or less unchanged. They had absorbed a large amount of moisture but showed no sign of crumbling.

The data in Table 7 show that the method of wetting aggregates containing levan does not materially influence their stability.

Table 7. *Effect of maintaining aggregates in a moist condition for long and short periods before wet sieving*

Levan (no.)	Amount added to soil (%)	Aggregation (%)	
		Aggregates absorbing moisture by capillary action for 17 hr. before wet sieving	Aggregates submerged for 30 min. before wet sieving
151	0.5	84.7	88.5
	0.125	54.4	68.8

disappeared when its nitrogen content was reduced to less than 0.2%. However, Evans, Hawkins & Hibbert (1941) found that anti-*L. mesenteroides* sera (produced in rabbits) gave precipitin reactions with relatively high dilutions of the homologous polysaccharide, dextran, having a maximum nitrogen content of 0.08%. This suggests that the small amount of nitrogen present in the dextran may not be responsible for the immunological reactions of the polysaccharide.

When our levans were treated with hot dilute NaOH, a flocculent precipitate appeared in the solution, and when it was removed a degraded levan was recovered from the solution which had a reduced nitrogen content and a very low aggregating effect (cf. Stacey, 1943). A similar precipitate was formed when solutions of the levan from *B. subtilis* were dialyzed. The precipitate settled to the bottom of the dialysis tube and was easily removed. Microscopic examination showed that it consisted, almost entirely, of bacterial cells. The levan recovered from the dialyzed solution had a much lower nitrogen content and a slightly higher aggregating effect. Apparently, the effect of NaOH on levan solutions is twofold: it facilitates removal of nitrogen by precipitating the bacterial cells and 'cell debris', and it degrades the levan. It is thought that these are

In another experiment aggregates containing 0.5% levan (product 151) were dropped into boiling water and held at a temperature of 95° for 10 min. with and without stirring. They were then cooled and the degree of aggregation measured by the wet-sieving technique. It is rather remarkable that, without stirring, a large proportion (over 65 %) of the aggregates remained stable after heating, and that, with stirring, as much as 35 % still remained stable.

Soil aggregates, containing 0.5 % levan, were broken down when shaken in water for 1 hr. However, when the fine soil was removed from the shaking bottle, dried to the 'sticky point' and pushed through a 3 mm. sieve, water-stable aggregates were reformed. Approximately 40 % of the polysaccharide applied to the soil was present in the water with which the soil was shaken. These results suggest that a certain amount of levan is fixed on soil particles.

Table 8 gives the results of an experiment designed to discover if levan, present on soil particles, was resistant to microbial decomposition. Aggregates containing 0.5 and 0.125 % levan were moistened with a soil suspension and incubated at 27°. At intervals samples were removed and the percentage of water-stable aggregates estimated.

It is concluded that levan present on soil particles is slowly decomposed by micro-organisms.

Table 8. *Effect of microbial activity on stability of 'levan-soil aggregates'*

Levan (no.)	Amount added to soil (%)	Aggregation (%)			
		Before incubation	After 5 days' incubation	After 11 days' incubation	After 31 days' incubation
151	0.5	88.5	82.8	61.0	44.1
	0.125	68.8	23.7	8.2	7.2

DISCUSSION

Polysaccharides are normal metabolic products of micro-organisms. Numerous species of bacteria, fungi and actinomyces are capable of producing a wide variety of complex polysaccharides as cell constituents or as extracellular products. Hence it is reasonable to expect that, under natural conditions, the diverse flora of the soil may synthesize many polysaccharides from the constituents of vegetable material, and that these substances may be of considerable importance in the formation of crumb structure in soil.

Polysaccharides are probably only one of the groups of microbial metabolic products having an ameliorative effect on soil structure, and only when further knowledge is available will it be possible to estimate the full extent to which biological activity influences the physical condition of soil.

SUMMARY

1. It has been shown that bacterial polysaccharides of the levan and dextran types exert a pronounced binding effect on soil particles.

2. *Bacillus subtilis* was cultured in a number of different media, in which the sucrose concentration

and source of nitrogen were varied, and it was observed that the yield of levan increased with increase in sucrose content of the medium from 2 to 10% (w/v). The aggregating effect of the levan decreased, however, with increasing sucrose content of the medium, and was paralleled by a decrease in intrinsic viscosity and molecular weight of the levans.

3. It appears that the nitrogen content of levans and dextrans is due to the presence of bacterial cells, as impurities, in the substances. There was no correlation between aggregating effect and nitrogen content, or between molecular weight and nitrogen content of either levans or dextrans.

4. Soil crumbs, containing small amounts of levan, are extraordinarily stable to the dispersing actions of water, heat and shaking, but are eventually broken down by the activities of micro-organisms.

5. Preliminary investigations indicate that levans are fixed on soil particles.

6. It is not suggested that polysaccharides are the sole agents concerned in aggregating soil, but it does appear that they may be of considerable importance.

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