CCLXVIII. ENZYME FORMATION AND POLYSACCHARIDE SYNTHESIS BY BACTERIA.

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It is only comparatively recently that precise knowledge has been obtained in regard to the important problem of polysaccharide synthesis by bacteria. This advance has been due to the work of Hibbert and co-workers [1930-34], who succeeded in isolating in the pure state and also on a large scale the polysaccharides formed by the activity of the micro-organisms *B. mesentericus*, *B. subtilis*, and *Acetobacter xylinum*, and ascertaining their chemical structure.

The polysaccharides synthesised by the closely related organisms, *B. mesentericus* and *B. subtilis* from sucrose and raffinose [1930; 1931, 1, 3; 1932] were found to be identical in chemical nature and to be "fructosans" of the laevan type. These laevans, consisting entirely of fructofuranose units are formed synthetically by the activity of a bacterial enzyme through the condensation of molecules of fructofuranose liberated in the nascent state in the initial hydrolysis of the sucrose and raffinose. The foregoing organisms were thus only able to produce a polysaccharide from carbohydrates, *e.g.* sucrose and raffinose, containing fructofuranose units; and such sugars as maltose, lactose, glucose, fructose (fructopyranose) were found to be unsuitable starting materials for synthesis. *Ac. xylinum* (the sorbose bacillus) synthesised cellulose [1931, 2, 4; 1934] from various carbohydrates and polyhydric alcohols, and it was discovered that the forms of cellulose produced by bacterial synthesis from glucose, fructose, galactose, sucrose, mannitol and glycerol were chemically identical, and also identical with cotton cellulose.

The experimental work described in the present paper deals mainly with the problem of enzyme formation and polysaccharide synthesis by (1) bacteria related to *B. mesentericus* and (2) bacteria pathogenic to plants. Apart from the work of Ruhland [1906], no systematic researches appear to have been carried out, so far, in connection with polysaccharide formation in the latter group of micro-organisms.

Ruhland studied the synthetic activities of *B. spongiosus*, an organism causing disease in cherry trees in Germany, and stated that a pentosan of an araban type was formed by the growth of this organism in sucrose and raffinose agar media. On account, therefore, of the possible interest of the subject in plant pathology, and also because of the chemical interest of the phenomenon of pentosan formation by a micro-organism, an investigation on polysaccharide synthesis by plant pathogens has been carried out. Furthermore, very little information is available concerning the rôle of synthesising enzymes, the nutritional conditions essential for their formation in bacteria and also their activity. An investigation of the biochemical conditions influencing the develop-

ment and activity of the synthesising enzymes in bacteria has therefore been undertaken, and the results obtained have been correlated with corresponding observations made with the hydrolytic enzymes, invertase and diastase.

METHODS OF INVESTIGATION.

The general procedure consisted in growing the organisms at 37° or 30° according to the optimum temperature of the species in a sugar-peptone-salt medium of the following composition.

Medium I [Hibbert *et al.*, 1930].

	%
Sucrose or other carbohydrate	10
Peptone	0.1
Sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) ...	0.2
Potassium chloride	0.5

The preliminary experiments were carried out on a small scale in sterilised test-tubes or conical flasks. To avoid decomposition of the carbohydrates in consequence of boiling with the peptone, the sugar solutions were initially made up to 20 % strength with distilled water only and sterilised by intermittent steaming for 30 min. on three successive days. The peptone-phosphate-salt mixture was made up separately to double strength and similarly sterilised. The two media were then transferred by means of sterile pipettes with aseptic precautions and mixed in equal volume, thus giving a resulting medium of the above composition. The following carbohydrates and polyhydric alcohols were employed in order to test the capacity of the organisms to synthesise polysaccharides: sucrose, raffinose, maltose, lactose, galactose, glucose, fructose, mannose, arabinose, xylose, mannitol, glycerol. Raffinose being less soluble was used in 7.5 % concentration.

The organism under investigation was first cultivated in the sucrose-peptone medium (10 ml.) by transferring a small amount of the growth from the agar stock culture and incubating at the optimum temperature for 48 hours. One drop of this culture was then introduced by means of a platinum loop into each of the carbohydrate media employed, and these were then incubated for a period of 10 days. Vigorous growth usually took place, but notwithstanding the turbidity due to growth, where polysaccharide synthesis had occurred, it was generally possible to detect the bluish opalescence characteristic of a colloidal solution. The cultivations were then filtered through glass-wool or filter-paper, to remove the gross bacterial masses, and allowed to flow into three times the volume of methyl or ethyl alcohol. If a polysaccharide had not been produced, only a slight turbidity resulted, but where synthesis had taken place a white precipitate was thrown down. The supernatant liquid was poured off, the polysaccharide product dissolved in a small volume of water and reprecipitated twice with alcohol to remove adherent sugar and mineral matter. It was then weighed and examined by chemical methods discussed later in this paper.

An investigation of the influence of various carbon compounds on polysaccharide formation by *B. mesentericus* and other organisms from sucrose was first undertaken (a) to throw light on the conditions essential for the formation of the synthesising enzyme, (b) to ascertain whether new polysaccharide derivatives could be obtained by the linking of other sugars with the nascent fructofuranose. Culture media were therefore prepared, consisting of the usual 10 % sucrose-peptone-salt constituents, but containing in addition 7.5 % concentrations of one of the following compounds: glycerol, mannitol, arabinose, rhamnose, galactose, mannose, lactose, maltose. *B. mesentericus*, *B. megaterium*, or

the plant-pathogen, *Ps. pruni*, was subcultured into these media, and incubation was continued for 10 days. The results obtained were unexpected; maltose and mannitol increased growth and polysaccharide formation, whilst glycerol, rhamnose, arabinose and mannose entirely inhibited both these functions, and galactose slightly inhibited them. The mechanism of inhibition has been especially studied in the cases of mannose and arabinose. At first it was supposed that these inhibiting substances either suppressed the formation or retarded the activity of the invertase normally secreted by the organisms, thus preventing the hydrolysis of sucrose and formation of monosaccharides for growth and polysaccharide synthesis. This view had a certain measure of support by analogy with the results of some preliminary observations concerning the influence of certain carbon compounds on the diastatic action of *B. megaterium*. This organism was cultivated in a basal medium, containing 0.2% peptone, 0.2% sodium chloride, 0.2% starch, to which varying concentrations of polyhydric alcohols from 0.5% to 4% could be added. The diastatic action was measured by the incubation period at 37° necessary for the disappearance of the starch and intermediate dextrans in the culture media, as indicated by the absence of a blue or red colour when a drop was removed and mixed with iodine solution on a tile. It was found that glycerol, mannitol and sorbitol inhibited diastatic action when present in the above concentrations, whilst glycol and erythritol, and also sucrose, glucose, galactose, mannose and arabinose were without effect.

In the absence of the alcohols hydrolysis of the starch was complete within 48 hours, but in the presence of glycerol, mannitol or sorbitol 96 hours were usually necessary. The inhibitory action of glycerol and mannitol was still observed in the presence of phosphate buffer (p_H 7.5), and was thus not due to the deleterious influence of p_H changes on growth or enzymic activity.

In some further experiments the organism was first of all grown in the peptone-medium for 48 hours in the absence of starch and polyhydric alcohols. These were then introduced into the cultures together with chloroform or a crystal of thymol to inhibit growth, and diastatic action was measured. It was now found that the presence of the alcohols was without effect.

The organism was next grown in the peptone medium containing the polyhydric alcohol and phosphate buffer, but without starch. The latter was then added after 48 hours' incubation together with thymol, and diastatic action was determined. It was now found that the cultures containing the mannitol or glycerol hydrolysed the starch less rapidly than the control peptone culture.

These experimental results lead to the conclusion that the inhibitory effect of the polyhydric alcohols on diastatic power is not due to any interference with the actual activity of the diastatic enzyme but is associated with a depressing action on the process of formation of the enzyme in the bacterial cells. The influence of mannitol and glycerol on the rate of growth of *B. megaterium* was therefore next investigated both by observing the turbidity of the cultures and by actual enumeration. It was found that in the initial stages of the growth of the cultures multiplication of the organism was somewhat inhibited, but in the later stages growth was increased in the presence of the alcohols, and the results are difficult to interpret.

The observations made on diastatic action, however, suggested the possibility that the inhibitory effect of mannose and arabinose on polysaccharide formation already referred to might be due to their interference with the invertase secreted by the organism, and this hypothesis was tested by employing a culture medium in which the sucrose was entirely replaced by glucose (10%), and also by using

the peptone-salt medium free from either sucrose or glucose, and containing only the inhibitory carbohydrate under examination. It was found that arabinose and mannose were still strongly antagonistic to growth. These results pointed to the conclusion that their inhibitory power was not due to interference with the invertase function, since inhibition still took place in the absence of sucrose, and it would seem to be of the nature of antiseptic action. In this connection it is interesting to note that Huang [1934] has found that mannose is more toxic to fibroblasts cultivated *in vitro* than is glucose, fructose or galactose. This interpretation is supported by the further observations that arabinose, glucose and galactose and also mannitol and glycerol in 2% concentration had no influence on the rate of hydrolysis of 2% sucrose by *B. megaterium*. The method employed consisted in estimating the increase in the amount of reducing sugar after incubation at 37° for 3 days.

We have also investigated the effect of the presence of various carbohydrates on the formation of cellulose from sorbitol by *Ac. xylinum*. The sorbose bacillus was grown in yeast-water, to which had been added 5% of sorbitol, at 20° for 10 days. The yeast-water was prepared by boiling 1 part of yeast by weight with 10 parts of water for 10 min., and filtering through a folded filter. 50 g. of sorbitol were then dissolved in 720 ml. of the yeast extract, 182 ml. of distilled water were added, followed by 40 ml. of red wine and 8 ml. of glacial acetic acid, and the mixture was sterilised by boiling for 10 min. The culture medium was distributed in large Erlenmeyer flasks to a height of 2 cm. to ensure good aeration and again sterilised. The sorbose bacillus was then subcultured from the stock culture into the flasks by means of a platinum loop, and the flasks were allowed to stand at 20° for 10 days. The red wine was added to supply alcohol to the organism, in order to encourage growth, and the acetic acid was added to inhibit the growth of any contaminating moulds, which might possibly gain access [Schlubach and Vorwerk, 1933; Reichstein and Grüssner, 1934].

After 10 days, a thick pellicle or membrane of cellulose was produced in the liquids. It was found that the addition of glucose, arabinose or mannose in 5% concentration prevented the formation of a cellulose membrane, whilst galactose and fructose increased cellulose formation. In some further experiments arabinose in 3%, and mannose even in 0.1%, concentration perceptibly inhibited the growth of the organism and polysaccharide formation in 5% sorbitol-yeast-water medium.

Hibbert *et al.* [1931, 2, 4] showed that *Ac. xylinum* formed cellulose readily from many carbon compounds, *e.g.* glycerol, fructose *etc.*, but in only relatively small amount from sucrose. This is explained by the observation of Hermann and Neuschul [1931] that the organism has only a limited capacity to invert sucrose, thus producing only small amounts of monosaccharide for synthesis to the polysaccharide. *Ac. xylinoides*, however, exerted a much more powerful invertase action, and it was thus of interest to compare the synthetical capacity of this organism in building cellulose from sucrose.

Ac. xylinoides was therefore cultivated in yeast-water media, containing respectively 5% sorbitol, glycerol or sucrose, at 20° for 10 days. In all cases a membrane was produced, and it was produced as strongly in the presence of sucrose as of either glycerol or sorbitol. A membrane was also formed from raffinose. The pellicles were collected and purified as before and retained for chemical examination and comparison with the corresponding material synthesised by the sorbose bacillus.

The possibility of synthesis of cellulose from fatty acids by *Ac. xylinum* has also been investigated. As this organism produces a small amount of cellulose

from yeast-water, a basal medium of the following composition was employed [Hibbert *et al.*, 1931, 2]:

1 g. asparagine,
5 g. potassium dihydrogen phosphate,
1 g. sodium chloride,

dissolved in 1 litre distilled water.

5% solutions of succinic, citric and malonic acids were prepared in this medium and the p_H was adjusted to 5.0 with *N* sodium hydroxide. Ethyl alcohol was added to 0.5% concentration and *Ac. xylinum* subcultured into the media, which were then allowed to stand at 20° for 3 weeks. No membranes were produced, although a control experiment with 5% glucose and sucrose yielded a delicate cellulose pellicle. The experiments showed that under these conditions *Ac. xylinum* was unable to synthesise cellulose from the fatty acids. Further experiments using 2% solutions of other acids, *e.g.* tartaric, maleic, sebacic, adipic and glutaric acids, also gave negative results.

B. megaterium was also unable to synthesise polysaccharides from fatty acids, *e.g.* 2.5% sodium acetate, malonate or succinate, nor did these substances influence the formation of polysaccharide from sucrose. The fatty acid salts also had no effect on diastatic activity in the case of *B. megaterium* grown in a peptone medium.

With the exception of the section of work dealing with *Acetobacter*, the conventional peptone medium (Medium I) has been employed throughout this investigation. It was now considered of interest to vary the form in which nitrogen was supplied in the media, as this change might influence the yield of polysaccharide produced and throw light on the question as to the nutritional conditions essential for the formation of the synthesising enzymes.

For this purpose the peptone was replaced by amino-acids, amides and amine-derivatives, 0.1% solutions being employed. The following results have been obtained:

In the cases of *B. megaterium*, *Ps. pruni* and *B. syringae* asparagine was quite as efficacious as peptone as a source of nitrogen for polysaccharide formation. The addition of asparagine (0.1%) to peptone, however, did not increase the yield of polysaccharide.

The following results were obtained with *B. megaterium*:

(1) Ammonium chloride, potassium nitrate, urea and a tetraethylammonium salt were not suitable sources of nitrogen, no polysaccharide at all being produced and no growth when these substances were used in the place of peptone.

(2) Only very slight growth and formation of polysaccharide occurred with glycine as source of nitrogen.

(3) *D*-Alanine and *L*-leucine were generally as efficacious as peptone for the synthesis of the polysaccharide, but not consistently so, as occasionally the yield of polysaccharide was distinctly less than that obtained when peptone was employed.

It is seen that growth and polysaccharide formation both depend fundamentally on the source of nitrogen supplied, but whenever growth occurred there was evidence of the simultaneous production of the synthesising enzyme. Analogous results were obtained in the case of the hydrolytic enzymes, diastase and invertase, secreted by *B. megaterium*, diastatic action being measured by the colour changes with iodine and formation of reducing sugar in a starch medium, and invertase activity by the production of reducing sugar from

sucrose. Whenever growth occurred, formation of the enzymes took place, as in the case of the synthesising enzyme.

The organism *Achromobacter viscosum*, however, is much more limited in its growth capacity than *B. megaterium* as we have found that, whilst it develops satisfactorily in the sucrose-peptone medium, when the peptone is replaced by *l*-leucine growth is only slight, and growth does not take place at all when asparagine, glycine, *d*-alanine or synthetic alanine is employed as source of nitrogen.

The organisms so far employed have been mainly *B. mesentericus*, *B. megaterium*, and *Ps. pruni*. *B. mesentericus* was shown by Hibbert to form a polysaccharide of the laevan type, and we have now shown that a similar product is formed by *B. megaterium* and *Ps. pruni*. In both cases the polysaccharides after hydrolysis with acid yielded a sugar, which gave Selivanoff's reaction for fructose, formed glucosazone with phenylhydrazine and was laevorotatory (see Tables I and III).

It was thought that the investigation would be of greater interest if it were possible to study enzyme formation in organisms synthesising polysaccharides of wider physiological interest than fructosans, such as pentosans, dextrans and galactans. Organisms of three classes have therefore been studied in regard to their capacity to synthesise polysaccharides.

A. Aerobic sporing organisms from soil, air and water. Hibbert and co-workers showed that the polysaccharides obtained by the action of *B. mesentericus* and *B. subtilis* were of the laevan type, containing fructofuranose as the only sugar unit. We have carried out experiments with several related organisms and in some cases were able to isolate a polysaccharide.

B. megaterium, *B. mycoides*, *B. panis*, *B. ruminatus*, and an unidentified organism resembling *B. subtilis* and isolated from the gum of a cherry tree, all produced when cultivated in the sucrose-peptone medium a polysaccharide yielding only fructose on hydrolysis. As in the cases of *B. mesentericus* and *B. subtilis* other sugars used in the place of sucrose gave entirely negative results. The results with *B. mycoides* were somewhat variable, as this organism occasionally failed to yield a polysaccharide.

B. tumescens, on the other hand, consistently failed to synthesise a polysaccharide, probably because it is deficient in invertase. It is thus unable to hydrolyse sucrose and liberate the nascent fructofuranose, necessary for the synthesis of the polysaccharide.

Table I.

Organism	Specific rotations	
	Polysaccharide	Product of hydrolysis
<i>B. megaterium</i>	-47.0°	-91.5°
<i>B. mycoides</i>	-38.0°	-80.0°
<i>B. panis</i>	-37.0°	-87.0°

B. Plant pathogens. Investigations with these important organisms have been undertaken as already pointed out primarily on account of (1) the possible immunological significance of their specific polysaccharides, and (2) the chemical interest centred on the work of Ruhland [1906], who concluded that *B. spongiosus*, causative of cherry tree bacteriosis in Germany, produced a polysaccharide of an araban type from sucrose. We have not been able to obtain a culture of this particular organism, but have studied certain micro-organisms closely allied to *B. spongiosus* and also several other bacteria known to be

Table II. *Plant-pathogens.*

(a) Organisms readily forming a polysaccharide from sucrose.

Ps. pruni
Ps. prunicola (from plum and cherry trees)
Ps. mors-prunorum (from plum and cherry trees) [Wormald 1930; 1932]
Ps. aptatum
Ps. campestre
B. syringae

(b) Organisms occasionally yielding a polysaccharide

<i>Ps. phaseoli</i>	<i>B. tabacum</i>
<i>Ps. solanacearum</i>	<i>B. marginale</i>
<i>B. caratovorius</i>	

(c) Organisms consistently giving negative results

<i>B. amylovorus</i>	<i>Ps. stewarti</i>
<i>B. barkeri</i>	<i>Ps. juglandis</i>
<i>B. cannas</i>	<i>Ps. tumefaciens</i> , and also
<i>B. malvacearum</i>	the non-pathogenic
<i>B. phytophthorus</i>	<i>Radiobacter</i>

pathogenic to plants (Table II). From the results set out in Table II it will be seen that in quite a number of cases a polysaccharide was synthesised in a sucrose medium. With two organisms, *Ps. pruni* and *B. syringae*, raffinose was employed and also yielded a polysaccharide, but in every case all the other carbohydrates previously mentioned gave entirely negative results. The capacity to form a polysaccharide from sucrose (or raffinose) only and not from any other carbohydrate employed is thus a specific property of all the plant-pathogens so far found to exhibit synthetical activity. A few of these organisms, e.g. *B. caratovorius*, only occasionally yielded a polysaccharide product. The reasons for this variability, however, have not yet been investigated. A certain number of these pathogenic organisms, on the other hand, consistently failed to produce a polysaccharide from sucrose or any other carbohydrate under the experimental conditions. A typical case is that of *Ps. tumefaciens*, and it is interesting to note that the non-pathogenic form *Radiobacter* is apparently closely related with *Ps. tumefaciens* [Sager *et al.*, 1934], and is also deficient in synthetical power.

Ps. tumefaciens being of pathological importance in inducing a form of cancer in plants, it was considered that it would be of interest to investigate its action on the metabolism of other bacteria. Accordingly, the influence of *Ps. tumefaciens* on polysaccharide formation by other organisms has been studied. Cultures of *Ps. pruni*, *B. syringae*, *B. megaterium* in 10% sucrose-peptone media were therefore set up, and into a certain number of these *Ps. tumefaciens* was introduced, incubation then being continued for 10 days. It was found that polysaccharide formation by the foregoing organisms was entirely suppressed in the presence of *Ps. tumefaciens*. However, it was subsequently observed that *B. coli* also interfered with the formation of polysaccharide by *B. megaterium* so that this is not specific to *Ps. tumefaciens*. It is of interest to note that when *B. megaterium* was cultivated in sucrose-peptone, the p_H changed from the initial value of 8 to 6.5, owing to the metabolic production of organic acids. In the presence of *Ps. tumefaciens* or *B. coli*, however, the p_H remained unchanged. The amount of reducing sugar produced by *B. megaterium* through the inversion of the sucrose was also diminished in the presence of these interfering organisms. It would appear therefore that the organisms inhibited the growth of each other when cultivated in mixed culture.

The polysaccharide products obtained in the above way were next examined chemically, with the following results:

(i) The polysaccharides themselves were nitrogen-free and without reducing action on Fehling's solution, but after hydrolysis with boiling dilute acids and neutralisation with NaOH marked reduction took place. Hydrolysis seemed to proceed rapidly, as the opalescence due to the colloidal state soon disappeared.

(ii) A portion of the acid solution (obtained by hydrolysing the polysaccharide with acid) was treated with solid sodium acetate and a few drops of phenylhydrazine, and the mixture heated in the water-bath for 20 min. Yellow crystals were deposited and were identified by microscopical examination and M.P. as glucosazone.

(iii) The polysaccharides gave Selivanoff's test when heated with resorcinol and HCl, suggesting the presence of fructose units in their molecules. Pentoses were shown to be absent by failure to obtain either the colour reactions or the absorption spectra tests with orcinol and phloroglucinol.

(iv) *Optical activity.* All the polysaccharides after hydrolysis with oxalic acid yielded a sugar, which had a specific rotation approximating to that of fructose.

Table III.

Organism	Specific rotations	
	Polysaccharide	Product of hydrolysis
<i>Ps. pruni</i>	-42.4°	-95.5°
<i>Ps. prunicola</i> (plum)	—	-96.5°
" (cherry)	—	-98.0°
<i>Ps. mors-prunorum</i>	—	-92.5°
<i>Ps. aptatum</i>	—	-82.0°
<i>Ps. phaseoli</i>	—	-85.0°
<i>B. syringae</i>	-40.0°	-89.0°
<i>B. tabacum</i>	—	-80.0°

Ruhland [1906] carried out his experiments on solid media, and in view of his results with *B. spongiosus* we therefore repeated our work with *Ps. pruni* and *Ps. prunicola* [Wormald, 1930] using 10 % sucrose-nutrient agar. After 10 days' incubation *Ps. pruni* had formed a hard horny growth on the surface of the agar, whilst that of *Ps. prunicola* was more fluid and gummy. The growths were carefully washed off with a little cold distilled water and the filtered washings poured into methyl alcohol. In the case of *Ps. pruni* only a small amount of an impure gummy material was isolated. Better results were obtained with *Ps. prunicola*, but even in this case the polysaccharide was less pure than when prepared in liquid media. The product, however, after hydrolysis yielded glucosazone with phenylhydrazine and gave Selivanoff's reaction. It was not pure enough, however, for measurement of optical activity; even after heating with acid it still gave a turbid solution. These preliminary observations pointed to the conclusion that the polysaccharides derived from the various plant-pathogens tested in this investigation were fructosans, *i.e.* contained fructose as the only constituent unit. There was no evidence that a pentosan was formed, as stated by Ruhland [1906] in the case of *B. spongiosus*.

Two of the polysaccharides (from *Ps. pruni* and *Ps. prunicola*) have been prepared on a large scale, in order to obtain more precise knowledge of their chemical nature. In the case of *Ps. pruni* experiments were therefore carried out to ascertain the optimum concentration of sucrose for polysaccharide synthesis. Concentrations of 5, 10, 15, 25 and 30 % were used in the peptone-salt medium already mentioned. 30 ml. of each concentration were inoculated with a

48-hours' sucrose-peptone culture of *Ps. pruni* and incubated for 16 days at 37°. 10 ml. of each solution were then run into 35 ml. of methyl alcohol, the polysaccharide was allowed to settle down, the supernatant alcohol poured off and the residual polysaccharide dried at 37° for 1 hour and then in a vacuum over calcium chloride, and finally weighed.

Table IV.

Sucrose concentration %	Weight of polysaccharide per 10 ml.	
	8 days g.	16 days g.
5	0.038	0.065
10	0.069	0.118
15	0.114	0.177
25	0.145	0.134
30	0.189	0.392

The yield of polysaccharide thus increased as the concentration rose and the period of incubation was extended. In the large scale preparation 20 % sucrose was selected and 14 days' incubation.

Similar experiments were carried out to discover whether peptone was the most desirable source of nitrogen for polysaccharide synthesis. It was found that there was no advantage in substituting asparagine for peptone, or in supplementing the peptone supply with 0.1 % concentration of asparagine.

Preparation of the polysaccharides.

Ps. pruni. Seven litres of the 20 % sucrose-peptone-salt medium were distributed into large Erlenmeyer flasks and sterilised, inoculated with a 48 hours' culture of *Ps. pruni* (in sucrose-peptone), and incubated at 37° for a fortnight. The opalescent solution was then filtered and concentrated to 1/4 bulk under reduced pressure at 45°. The residual syrup was poured slowly with mechanical stirring into 2.5 volumes of methyl alcohol. The precipitated polysaccharide was dissolved in warm water and reprecipitated twice with methyl alcohol. It was then ground with alcohol to a fine powder, filtered, washed with alcohol and ether and dried in a vacuum desiccator. 146 g. of the polysaccharide were obtained, as a fine pinkish white powder free from reducing sugar.

A further 6 litres of 20 % sucrose-peptone medium was inoculated with *Ps. pruni*, and an additional amount of polysaccharide prepared to complete the chemical investigation.

Ps. prunicola [Wormald, 1930]. Six litres of 20 % sucrose-peptone medium were inoculated with this organism and incubated at 25° for 3 weeks. The polysaccharide was isolated by the same method as for *Ps. pruni*. The solutions after incubation were extraordinarily opalescent and the isolated polysaccharide was less soluble in water than that of *Ps. pruni*; yield 106 g.

A chemical investigation of these two apparently different polysaccharides is at present being undertaken by Prof. W. N. Haworth and Dr E. L. Hirst, and their chemical structure is being compared with that of the "laevan" synthesised by *B. mesentericus* and *B. subtilis*.

C. *B. coli* and related organisms. *B. coli* and *B. lactis aerogenes* failed to produce a polysaccharide from any of the sugar-peptone media previously mentioned. In the presence of calcium carbonate to prevent the accumulation of organic acids in the cultures, *B. lactis aerogenes* synthesised a small amount of

a polysaccharide product from sucrose and galactose [cf. Emmerling, 1900]. It was not possible to isolate a sufficient amount for chemical investigation.

An apparently similar organism [Beckwith, 1931; Sanborn, 1933] isolated from paper-slime also yielded an insignificant amount of polysaccharide in sucrose and galactose media.

The following organisms failed to form a polysaccharide under the foregoing experimental conditions: *Lactobacillus pentoaceticus*, *Staphylococcus cremoris viscosi*, *Achromobacter* sp. I., *Proteus vulgaris*, *B. macerans*.

SUMMARY.

1. Mannose and arabinose inhibit polysaccharide formation from sucrose by *B. mesentericus*, *B. megaterium* and *Ps. pruni*; and also by *Acetobacter xylinum* from sorbitol. This phenomenon seems to be due to a direct toxic effect on the cell and not to an interference with enzyme mechanism.

2. Glycerol, mannitol and sorbitol diminish the diastatic power of cultures of *B. megaterium*, and this inhibitory effect appears to be due to a depression in the rate of formation of the diastatic enzyme in the bacterial cell. The action of invertase on the other hand is unaffected.

3. *B. megaterium* and *Ac. xylinum* do not synthesise polysaccharides from fatty acids, and these in the case of *B. megaterium* are without effect on diastatic action.

4. The presence of peptone is not essential for polysaccharide formation from sucrose by *B. megaterium*, *B. syringae* and *Ps. pruni*, as this occurs as freely with asparagine, and usually with *D*-alanine and *L*-leucine, as sole sources of nitrogen. The synthesising enzyme can thus be formed in the presence of a single amino-acid, and this is also true for the formation of invertase and diastase by *B. megaterium*. With this organism on the other hand ammonium and tetraethylammonium salts, potassium nitrate, urea and glycine are not suitable sources of nitrogen for growth, enzyme formation or polysaccharide synthesis.

5. Bacteria pathogenic to plants [ref. Table II] in many cases synthesise polysaccharides when grown in culture media containing sucrose. The polysaccharides are of the fructosan type, containing fructose as the constituent unit, and appear to be analogous to the laevans formed by *B. mesentericus* and *B. subtilis*. So far no evidence has been obtained of the formation of dextrans or pentosans by plant-pathogens.

6. Several aerobic spore-forming organisms, related to *B. mesentericus* and *B. subtilis*, also form similar polysaccharide products.

7. Methods for the large scale preparation of the polysaccharides synthesised by the plant-pathogens, *Ps. pruni* and *Ps. prunicola* are described.

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