STUDIES ON THE PECTINOLYTIC ANAEROBES *CLOSTRIDIUM FLAVUM* AND *CLOSTRIDIUM LANIGANII*

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Pectin-fermenting bacteria of the genus *Clostridium* constitute a group of considerable economic importance. Their ability to dissolve middle lamella material, thereby bringing about disintegration of plant tissues, is the basis of the retting process used in the recovery of such major textile fibers as flax and hemp. Several retting clostridia have been described in the literature but, owing to the incompleteness of many descriptions, the taxonomic relationships of these organisms have remained obscure. Only one of them, namely, *Clostridium felsineum*, was accorded specific rank in the 6th edition of *Bergey's Manual of Determinative Bacteriology*, (Breed *et al.*, 1948). Prévot (1948), on the other hand, recognizes several retting species in his system of classification and Raynaud (1949) has published a valuable review of the knowledge then available regarding these bacteria. In keeping with their more liberal policy in regard to recognition of bacteria of practical importance, the editors of the 7th edition of *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1957) now list eight pectinolytic species in their greatly enlarged genus *Clostridium*. Among them are *C. flavum* and *C. laniganii*.

These two species were first described by Lanigan (1951) in an account of flax retting studies in Australia. There, they were referred to as retting types IV and II, respectively, and were considered to be new species of *Clostridium*. Strains of *C. laniganii* have now been isolated from flax grown in all major producing districts in Australia, as well as from samples grown in Belgium and New Zealand and from Australian and New Guinea jute. In all, this organism has been recovered from more than three quarters of the rets studied and it is obviously a retting agent of major importance. *C. flavum*, on the other hand has been found much less commonly, although its retting ability is at least equal to that of *C. laniganii*. Only five strains of *C. flavum* have been isolated by the author to date. They have, however, been found on material grown in widely separated localities, flax from Belgium, Victoria, and Western Australia and jute from Queensland.

Representative strains of retting types II and IV as they were then known, were sent to Professor L. S. McClung, Indiana University, who had expressed interest in them, and who agreed with the author's contention that they were previously undescribed species of *Clostridium*. Subsequently they were accorded specific rank and were given the names already referred to in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1957).

The purpose of the present communication is to record certain additional information regarding these two species, which has been obtained from more recent work. In particular, quantitative data for products of carbohydrate metabolism are presented and some factors governing pectin utilization are described. A reasonably clear picture of the relationships of *C. flavum* and *C. laniganii* to other species of the genus *Clostridium* now emerges.

MATERIALS AND METHODS

Bacterial cultures. Strains 11-2, 11-4, 11-6, 11-9, and 11-11 of *C. laniganii* and 13-1 and 13-2 of *C. flavum* were used in the work described.

Bacteriological media and methods. Procedures used for the isolation and routine cultivation of these bacteria have been described previously (Lanigan, 1951). The basal medium for pectin utilization studies consisted of peptone, 0.5 per cent; yeast extract, 0.5 per cent; and bromcresol purple indicator; initial pH level 6.8. It was used either in liquid form or solidified with 1.5 per cent of agar powder, as the occasion demanded. It was further supplemented with various combinations of glucose and pectin or sodium

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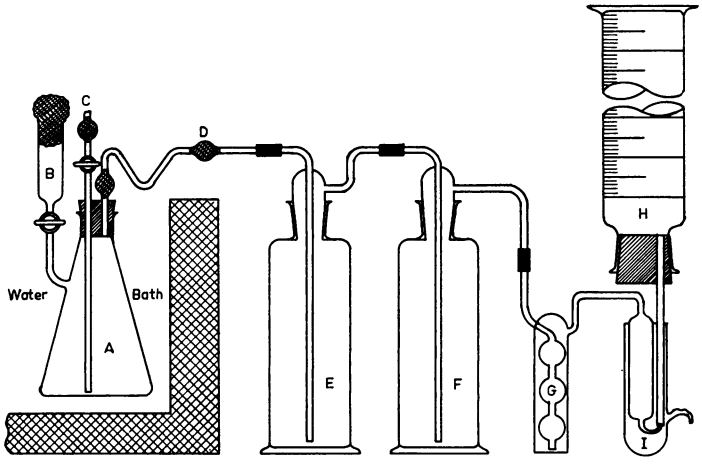

Figure 1. Apparatus for carbon balance fermentations with anaerobic bacteria

pectate as required. Unless otherwise stated, all cultures were incubated in anaerobic jars in an atmosphere of hydrogen and nitrogen with 5 per cent added carbon dioxide at 37 ± 1 C. The pectin used was prepared by purification of commercial, 100 grade, citrus pectin. Purification was achieved by repeated (3) precipitations from 2 per cent aqueous solutions with acidified ethanol at a final concentration of 60 per cent, v/v. The final precipitate was dried by washing with acetone and heating in a vacuum oven at 55 C. Sodium pectate was prepared by de-esterification of purified pectin at pH 8.5, at room temperature, according to Kertesz (1951), followed by precipitation with neutral ethanol and drying as above.

Washed spore suspensions were prepared from 7-day cultures on a glucose, yeast extract, peptone agar and heated at 80 C for 20 min prior to storage at 2 to 4 C. Such suspensions were stable for at least 6 months.

Carbon balance fermentations were carried out in 250-ml volumes of a medium of the following composition (in per cent): glucose, 2.0; peptone, 0.5; yeast extract, 0.5; cysteine·HCl, 0.02; pH before autoclaving, 7.0. The apparatus used for these fermentations is shown in figure 1. The glucose required (5.0 g of reagent grade glucose, 100 per cent pure by analysis) was weighed directly into the fermentation flask, *A*, dissolved in 150 ml of distilled water and sterilized at 121 C for 15 min. The remaining constituents of the medium were separately dissolved in distilled water at 2.5 times their concentrations in the final medium and after pH adjustment sterilized similarly to the glucose solution. One hundred ml of this concentrated medium were then added, aseptically, through the side arm, *B*, to the glucose solution. The fermentation flask was then placed in the water bath, at a temperature of 37 ± 0.1 C and connected to the gas-trapping train. This comprised two Dreschel bottles, *E* and *F*, each containing 100 ml of N NaOH of known carbonate content, a caustic soda tube, *G*, to protect the CO_2 traps from atmospheric CO_2, and an inverted 1-L, graduated cylinder, *H*, filled with water, to collect evolved hydrogen. The whole system, including the medium, was then flushed with a stream of sterile nitrogen, through tube *C*, for 15 min, the hydrogen collecting cylinder being displaced from over the delivery spout during this process.

Vegetative inocula were used for the carbon balance fermentations. They were prepared as follows. Spore stocks kept on soil were plated out and single, typical colonies were transferred to tubes of glucose yeast extract peptone water. Three, successive, daily transfers were made in the latter medium. The final cultures were centrifuged, the supernatant discarded, and the cells resuspended in a small volume of the fermentation medium less the glucose. An amount of this suspension equivalent to 5 ml of vegetative culture was used to inoculate each fermentation flask, the inoculum being introduced through the side arm *B*, taking precautions to avoid admittance of air.

The fermentation flasks were incubated for

approximately 24 hr after gas evolution had ceased. Duplicate fermentations were carried out with each culture. In each case, however, virtually identical results were obtained with regard to final pH level, glucose consumed, and CO_2 and hydrogen evolved. Therefore, detailed analyses were made on only one of each pair of duplicates.

Analytical methods. When fermentation was complete, the water bath was cooled to below 5 C, in order to minimize loss of volatile compounds during subsequent treatment of the culture. The volume of hydrogen evolved was then read directly from the graduated cylinder and corrected to standard temperature and pressure, making due allowance for the hydrostatic head in the collecting vessel. Residual CO_2 was transferred to the caustic soda traps by passing a slow stream of CO_2-free air through the system for ½ hr. The pH of the culture fluid was determined by means of a glass electrode pH meter. One ml of 20 N sulfuric acid was then added as a preservative and the fluid then diluted to 500 ml. This stock solution was stored in a glass-stoppered bottle, at 2 to 4 C, while awaiting analysis.

The CO_2 evolved during fermentation was determined by potentiometric titration of the trapping liquids between pH 8 and 4, using 2 N hydrochloric acid, with appropriate correction for the initial CO_2 content of the NaOH. Residual glucose was determined by the micro-copper method of Stiles *et al.* (1926) after clarifying the sample of culture with lead acetate and disodium phosphate. Lactic acid was estimated by the method of Friedemann and Graeser (1933) following pretreatment of the sample with copper-lime reagents, as recommended by these authors, in order to remove residual glucose and protein. Acetone was separated from the culture by distillation and was determined in the distillate by a modification of Ravin's (1936) colorimetric method. The classical hypo-iodite method (*see* Goodwin, 1920) proved unsatisfactory for the small amounts of acetone produced by some of these bacteria, owing to interference by some unknown constituents of the cultures.

Volatile acids were steam distilled in the presence of magnesium sulfate (Friedemann, 1938), the distillate neutralized, excess caustic soda added, and evaporated to dryness. Individual fatty acids were determined by the gas-liquid chromatographic method of James and Martin (1952).

Alcohols were distilled from the culture solution by Friedemann's (1938) method and oxidized at room temperature overnight as described by the same author. Isopropanol was determined by distilling a portion of the oxidized solution and estimating acetone formed by the hypo-iodate method of Goodwin (1920). Butanol and ethanol were determined by analysis of the mixed fatty acids produced by their oxidation. This analysis was made in the same manner as used for free fatty acids in the culture.

All analytical methods employed were checked in advance using known amounts of pure compounds. This proved particularly important in the case of butanol and ethanol. Chromatography of the products of butanol oxidation revealed that they included propionic acid and not just acetic and butyric acids as stated by the authors of the method. A further complication arose in that the ratio of acetic:propionic:butyric varied with the concentration of butanol initially present in the oxidation mixture. Further investigation showed, however, that the molar ratio of butyric: acetic plus propionic formed was sufficiently constant over quite a wide range of butanol concentrations to permit analyses of mixtures of butanol and ethanol to be made with adequate precision. It is proposed to publish details of this study at a later date.

EXPERIMENTAL METHODS AND RESULTS

Pectin fermentation. Quantitatively, the major change brought about during retting of fiber-bearing plants is a reduction in their content of pectic substances. It is axiomatic, therefore, that bacteria capable of retting such plants have the ability to hydrolyze pectin under certain conditions. The converse is not necessarily true and, moreover, pectinolytic microorganisms may be expected to vary in their effectiveness as retting agents with different plants. This is probably due to differences in the physicochemical properties of the naturally occurring pectic substances and in the nature and quantities of fiber-encrusting polysaccharides which must also undergo some degradation for the retting process to be really effective. To the author's knowledge, this aspect of retting has received little if any attention from bacteriologists.

Flax retting bacteria isolated from Australian

TABLE 1

Growth of Clostridium flavum and Clostridium laniganii in pectate media from washed spore inocula, incubated at 37 C for 6 days

Medium	*C. flavum* (2 strains)	*C. laniganii* (4 strains)
1	Vigorous growth, acid and gas, pectate hydrolysis complete.	Vigorous growth, acid and gas, pectate hydrolysis complete.
2	As in 1.	Slight growth only, trace of acid and gas, pectate still present.
3	Well developed, typical colonies, acid zone.	Skeleton colonies only, no acid zone.*
4	No growth.	No growth.
5	No growth.	No growth.

* There was a faint acid reaction around regions of heavy confluent growth, but not around discrete colonies.

flax have been shown previously to ferment purified citrus pectin to a greater or lesser degree (Lanigan, 1951). At that time, irregular results were frequently encountered in replicated tests and it was noted that regularity could only be ensured by use of heavy vegetative inocula: actively growing potato mash cultures. This observation suggested a carry-over effect of some kind. The property of pectin utilization was, therefore, investigated further, using a variety of media and inocula of washed spore suspensions. In this way, a considerable measure of standardization of the inocula was achieved and carry-over effects virtually eliminated.

Of the numerous media used from time to time in this study, the five following provided data of most value.

1. Basal medium (see above) + 0.2 per cent glucose + 0.5 per cent sodium pectate.
2. Basal medium + 0.5 per cent sodium pectate.
3. As for medium 2, but solidified with agar.
4. Basal medium solidified with agar.

(Media 1 to 4 were sterilized, complete, by autoclaving for 20 min at 115 C).

5. As for medium 2, but sterilized by Seitz filtration. The use of sodium pectate instead of pectin, itself, eliminated confusing acid reactions in the medium caused by pectin-methylesterase activity. In the liquid media, pectate hydrolysis was deemed to be complete when the typical precipitate could no longer be obtained upon addition of two volumes of 99 per cent ethanol. Acid and gas production were taken as evidence of fermentation of the products of pectate hydrolysis.

A summary of the results obtained with cultures of *C. flavum* and *C. laniganii* in the various media is given in table 1.

It will be seen from table 1 that neither of the two species was able to initiate growth from spores in media where pectate was the sole added carbon source and where thermal degradation of pectate was precluded (medium 5). Both organisms, however, were able to bring about active hydrolysis and fermentation of pectate in the presence of a small concentration of an assimilable energy source, glucose (medium 1). In the case of *C. flavum*, an adequate energy source appears to have been provided by thermal degradation products of pectate, formed during heat sterilization, since growth of this organism in medium 2 was indistinguishable from that in medium 1 and typical colony formation occurred on the pectate agar (medium 3). Absence of growth in medium 4 eliminates the possibility of the energy source being supplied by other constituents of the media. On the other hand, the postulated thermal degradation of pectate only led to slight growth of *C. laniganii* in pectate media without glucose (media 2 and 3). Some appreciable quantitative differences in growth in the pectate media were observed with the various strains of this organism.

Glucose metabolism. Table 2 presents a summary of analytical results from carbon balance experiments using two strains of *C. flavum* and three of *C. laniganii*. It should be noted that neither organism can grow in the basal medium employed in the absence of a fermentable carbon source; thus, carbon balance studies were not complicated by, for example, utilization of amino acids as a carbon source.

Carbon recoveries with strains of *C. laniganii* were uniformly high and oxidation-reduction ratios were close to unity. It is evident, therefore, that no product other than those recorded was formed in appreciable quantity. The general pattern of metabolic products for this organism is

TABLE 2

Carbon balances for fermentation of glucose by Clostridium laniganii and Clostridium flavum

Product	Product Recovery as g per 100 g and mmoles per 100 mmoles of Glucose Fermented									
	C. laniganii						*C. flavum*			
	Strain 11-2		Strain 11-4		Strain 11-9		Strain 13-1		Strain 13-3	
	g	*mmoles*	*g*	*mmoles*	*g*	*mmoles*	*g*	*mmoles*	*g*	*mmoles*
Hydrogen	2.68	257.0	1.88	167.6	2.47	220.7	2.52	225.1	2.95	263.0
Carbon dioxide	46.9	192.0	46.7	191.0	46.7	189.6	39.8	162.8	48.2	197.7
Acetic acid	18.7	56.0	14.5	43.5	17.1	51.3	19.9	59.8	15.9	47.8
Propionic acid	nil	nil	nil	nil	nil	nil	nil	nil	0.14	0.36
Butyric acid	28.1	57.5	15.3	31.3	19.5	39.9	25.9	53.0	25.2	51.4
Lactic acid	2.25	4.49	1.81	3.62	2.97	5.94	3.48	6.96	3.25	6.49
Ethanol	0.22	0.86	0.59	2.32	0.54	2.11	0.14	0.55	0.19	0.75
Isopropanol	nil	nil	0.018	0.053	0.004	0.009	nil	nil	0.051	0.15
Butanol	2.12	5.16	12.2	29.5	7.01	17.0	0.062	0.15	0.34	0.83
Acetone	0.65	2.03	3.57	11.0	2.16	6.73	0.080	0.25	0.095	0.29
Total acids	49.1	118.0	31.6	78.4	39.6	97.1	49.3	119.8	44.5	106.1
Total "solvents"	2.99	8.05	16.4	42.9	9.72	25.9	0.28	0.94	0.68	2.02
Final pH	4.0		4.1		4.0		3.85		3.85	
Glucose fermented	0.962 g/100 ml		1.39 g/100 ml		1.09 g/100 ml		0.910 g/100 ml		0.800 g/100 ml	
Carbon fermented	0.384 g/100 ml		0.557 g/100 ml		0.436 g/100 ml		0.364 g/100 ml		0.320 g/100 ml	
Carbon recovery	0.369 g/100 ml		0.529 g/100 ml		0.402 g/100 ml		0.314 g/100 ml		0.280 g/100 ml	
Per cent recovery	96.0%		95.0%		92.2%		86.3%		87.6%	
Oxidation-reduction ratio	1.04		0.98		1.02		1.02		0.94	

intermediate between those of the *Clostridium butyricum* and *C. acetobutylicum* species groups. There is to be seen in table 2 a considerable variation in the amounts of neutral volatile compounds formed by different strains of *C. laniganii*, the ratios of acidic to neutral compounds varying from about 16:1 to 2:1. Maximum solvent yield, in these experiments, was given by strain 11-4 and was equal to about one-half of that obtained with typical strains of *C. acetobutylicum*. The weight of glucose fermented per unit volume of medium by *C. laniganii* varied in direct proportion to the solvent yield and it would appear that the pH level of the medium was a factor in determining the amount of glucose consumed.

In the case of *C. flavum* strains, carbon recoveries were lower and, although the oxidation-reduction ratios were reasonably close to unity, it is possible that some product(s) may have been formed in addition to those recorded. One possible cause of the relatively low carbon recoveries may lie in the viscous nature of the growth of these organisms in some liquid media, since this is probably due to formation of an extracellular polysaccharide. It can be stated with assurance that no formic acid or acetylinethylcarbinol was produced by *C. flavum* but, on the other hand, the possible presence of higher volatile fatty acids and nonvolatile acids other than lactic, was not investigated. The yields of various fermentation products obtained in this study show that, metabolically, *C. flavum* closely resembles the *C. butyricum* group, since only a small amount of the glucose fermented, less than 1 per cent, was accounted for by nonacidic products. The final pH of the *C. flavum* cultures was lower than that attained with *C. laniganii*, and this is in keeping with the higher proportion of acidic products. The former organism also consumed less glucose than the latter. This may well be related to a limiting effect of low pH level.

DISCUSSION

Pectin fermentation. The observations made in the course of this study have demonstrated one important aspect of pectin utilization by two retting organisms, *C. flavum* and *C. laniganii*; namely, their requirement for an assimilable energy source such as glucose, before they can initiate fermentation of pectic substances from spore inocula. In their natural environment, this requirement does not present a problem, since plant tissues upon which they develop will contain an adequate supply of the simple carbohydrates. Moreover, their ability to decompose polyuronides, once a vegetative population has been established, together with their high tolerance for relatively low pH levels ensures their survival when other members of the initial flora have died. The observed prerequisite for pectin fermentation by these bacteria does, however, offer an explanation for the irregular results previously encountered by the author in studies of this property. The work described above has been limited to experiments with washed spore inocula and it is not possible, therefore, to say whether or not vegetative cells of the organisms studied would also require a readily available carbon source for the initiation of pectin fermentation. Nevertheless, the initial observation that rather large inocula from young cultures were necessary to ensure uniform results, and the carry-over effects implied by it, at least suggests that the same requirement holds in this case also.

No conclusive evidence has been obtained from this work in regard to another important question; namely, that of whether the pectin-fermenting enzymes of these retting bacteria are constitutive or inducible in nature. The lack of growth in media containing filter-sterilized pectate as sole carbon source may be indicative of the absence of constitutive enzymes but it may equally well be attributed to failure of the spore inocula to germinate in the absence of an assimilable carbon source. Further investigation, using washed vegetative inocula for both filter-sterilized and autoclaved pectate media may help to clarify this point. The limited growth of *C. laniganii* in media 2 and 3 compared to that in medium 1 does suggest that pectin fermentation is an induced property of this organism, the induction being less favored by thermal degradation products of pectin than by glucose. This contention is given some further support by the varying response of different strains of this organism in media 2 and 3. Moreover, it was evident from the relative numbers of colonies appearing on medium 3 and the control glucose yeast extract agar that comparable numbers of spores had germinated on both media, i. e., with and without adding glucose. Therefore, if pectin fermentation were due to constitutive enzymes, equally good growth of *C. laniganii* should have

occurred in media 1 and 2 and it might have been expected that full development of colonies would have occurred on the pectate agar (medium 3). There is, therefore, presumptive evidence that *C. laniganii* utilizes pectin by means of an induced enzyme system.

In the case of *C. flavum*, on the other hand, the results are quite inconclusive. Thus, although the growth of this organism in medium 3 and its absence in medium 5 shows that pectin degradation products constitute an adequate energy source for initiation of vegetative growth from spores, the available evidence does not show whether the enzymes permitting pectin utilization are induced or constitutive.

Taxonomic relationships. Detailed descriptions of the cultural, morphological, and physiological characteristics of *C. flavum* and *C. laniganii* were given by Lanigan (1951) where these organisms were referred to as retting clostridia types IV and II, respectively. It is sufficient for the purposes of the present paper to recall some of the outstanding features of these organisms; in particular, those which, together with the new information relating to metabolic products, serve to distinguish them from other recognized species of *Clostridium*.

Seven pigmented pectinolytic species of the genus *Clostridium* are described by Breed *et al.* (1957). They are listed below, in order of first isolation and with their allotted species numbers in parentheses:

(75) *Clostridium felsineum* (Carbone and Tombolato) Bergey *et al.*
(78) *C. haumannii* (Soriano) Prévot (1948)
(71) *C. roseum* McCoy and McClung (1935)
(73) *C. corallinum* Prévot and Raynaud (1944)
(70) *C. saturnirubrum* Prévot (1948)
(74) *C. aurantibutyricum* Hellinger (1947)
(76) *C. flavum* McClung and McCoy

All of the foregoing species, with the possible exception of *C. roseum*, have been shown by various authors to ret sterilized flax straw, with varying degrees of effectiveness. It is, therefore, necessary to establish how *C. flavum* differs from each of them, which it does in several important features. In the first place, *C. flavum* exhibits a characteristic sporangial morphology (Lanigan, 1951) which, in the author's experience is a diagnostic feature in itself. The spore is long, narrow, and indented, giving it a beanlike appearance, and most sporangia show a definite kink at the junction of the spore and the remainder of the cytoplasm. Secondly, the yellow pigment of this species appears to be unique among clostridia. Of the six species mentioned above, only three, *C. felsineum*, *C. haumanii*, and *C. saturnirubrum* are described as forming colonies which are yellow at any stage; but, in each case, this color is confined to young colonies, which darken to some shade of orange or red as they mature. Colonies of the other three species are never yellow. Another point of distinction between *C. flavum* and *C. haumanii* is that the pigment of the former is confined to the bacterial cells, whereas that of the latter diffuses into the medium. *C. flavum* brings about moderately rapid liquefaction of gelatin, containing a small amount of glucose. Of the other three "yellow" clostridia, only *C. felsineum* does this. Separation of these two species is, however, readily achieved on the basis of their respective products of glucose fermentation. Thus, *C. flavum* brings about a typical "butyric type" of fermentation, with negligible yields of neutral, volatile compounds.[2]

C. felsineum, on the other hand, produces solvent yields comparable with those of *C. acetobutylicum* (van der Lek, 1930 and Langlykke *et al.*, 1935). Moreover, in the present studies, it has been found that, whereas *C. felsineum* readily ferments at least 2 g of glucose per 100 ml of medium, *C. flavum* can utilize less than one half this amount. Finally, the ultimate pH levels in glucose yeast peptone water cultures of these two organisms are approximately 4.5 and 3.9, respectively. In the light of the foregoing discussion, *C. flavum* emerges as a new, pigmented, pectinolytic species with properties which clearly distinguish it from previously described bacteria of this type.

Reference to the current edition of *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1957) shows that, within the genus *Clostridium*, *C. laniganii* is most closely related to the *Clostridium butyricum* group, on the one hand and to *C. acetobutylicum* on the other. If either, it resembles the latter more than the former, in view of its mildly proteolytic properties and the production, by some strains at least, of sub-

[2] In the original description of this organism, the statement (Lanigan, 1951), that culture in various carbohydrate media had a distinct odor of butanol, was erroneous.

stantial amounts of neutral, volatile compounds from glucose. Certain of its characteristics, however, clearly separate *C. laniganii* from both of these previously established species. First, the cellular and colonial morphology of the new organism are distinctive features. Early vegetative growth is characterized by incomplete separation of cells leading to tangled chains and filaments which, however, give place to separate cells of "normal" dimensions within about 48 hr. The colonies on solid media are characteristically composed of a central, butyrous region surrounded by an effuse, filamentous part which is largely embedded in the agar. Like *C. butyricum* and *C. acetobutylicum*, *C. laniganii* is actively saccharolytic, fermenting a wide range of carbohydrates and allied substances; in fact, it will not grow in the absence of an assimilable carbohydrate. Its stormy fermentation of milk is common to several other clostridia and its active fermentation of potato mash is strongly reminiscent of *C. acetobutylicum*. Unlike this species, however, it fails to ferment either mannitol or inulin.

The carbon balance studies reported in the present communication have shown that, metabolically, *C. laniganii* occupies a position between those of *C. butyricum* and *C. acetobutylicum* and that different strains vary appreciably in the proportions of acidic to neutral compounds produced.

From a practical viewpoint, at least, the most important property of *C. laniganii* is its ability to ret flax in pure culture, a property due, primarily, to its capacity for hydrolysis of the polygalacturonide, pectin. Evidence has been presented which indicates that this property is dependent upon enzymes which are induced rather than constitutive. If this is true, then it does not seem to place *C. laniganii* at a disadvantage as a retting agent, since the induction occurs readily in the presence of pectin and a fermentable carbon compound, ample supplies of which are present in the aqueous extract of flax straw. Indeed, all strains of this organism isolated to date have proved to be quite effective retting agents for flax. Among the nonpigmented species of *Clostridium*, the only other retting organism known at present is *Clostridium pectinovorum* (Breed *et al.*, 1957). Differentiation of this from *C. laniganii* presents no difficulty, since the former is a plectridial type, with a terminal ovoid spore and is, moreover, oxygen tolerant. In addition, *C. pectinovorum* is an inferior retting agent, due to its poor pectin fermenting capacity.

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SUMMARY

A study has been made of some factors governing pectin fermentation by *Clostridium flavum* and *Clostridium laniganii*. Evidence is adduced which indicates that the pectin utilizing enzyme system of the latter is inductive rather than constitutive. In the case of *C. flavum*, however, the results are inconclusive.

Results of carbon balance fermentations with these two species show that, whereas *C. flavum* is a typical butyric type, *C. laniganii* produces appreciable amounts of neutral volatile compounds and represents a metabolic type intermediate between *Clostridium butyricum* and *Clostridium acetobutylicum*.

The taxonomic relationships of *C. flavum* and *C. laniganii* are discussed and features which serve to differentiate them from related species are indicated.

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