The Chemical Action of Bacillus cloacæ *(Jordan) on Glucose and Mannitol.*

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The close relationship of *B. cloacæ* (Jordan) to *B. lactis aërogenes* (Escherich) suggested the investigation of the chemical action of the former on glucose and mannitol. The two organisms are lactose-fermenting bacilli, allied to *B. coli communis*, and showing a close resemblance to each other in their biological characteristics. *B. lactis aërogenes* is a non-motile, Gram-negative, non-liquefying bacillus, a facultative anaërobe which produces acid and clotting in milk. *B. cloacæ* is a facultative anaërobic bacillus, actively motile, Gram-negative, slowly liquefying gelatine, and producing acid and clot in milk. The chief biological characters of the organisms will be clearly seen in the following table, in which + means acid and gas, — no action :—

	Glucose.	Lactose.	Cane sugar.	Dulcitol.	Dextrin.	Inulin.
B. cloacæ	+	+	+	—	+	—
B. lactis aërogenes ..	+	+	+	—	+	—

Harden and Walpole* have already fully investigated the products of the decomposition of glucose and mannitol by *B. lactis aërogenes*, and a comparison of their results with those to be obtained from *B. cloacæ* presented a problem of considerable interest, owing to the fact that both organisms give the Voges and Proskauer reaction. This reaction is due to the presence of acetylmethylcarbinol, which is closely related to butylene glycol, a substance which had been found as one of the products of the fermentation of glucose by *B. lactis aërogenes*. The organism was grown anaërobically in a medium containing 1 per cent. of Witte peptone and 2 per cent. of the sugar in the presence of chalk. The products were examined by the method outlined by Harden† in his investigation of the action of *B. coli communis* on glucose.

An alteration in the method of collecting the evolved gases was made, with the object of eliminating the error involved in collecting over saturated brine, in which carbon dioxide is slightly soluble. The collecting apparatus

* 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 399, and 1911, B, vol. 83, p. 272.
† 'Chem. Soc. Trans.,' 1901, p. 610.

used consists essentially of an evacuated flask of about 5 litres capacity, and has been fully described in a previous paper.*

A. *Action of* B. cloacæ *on Glucose.*

The substances produced by the action of *B. cloacæ* on glucose were found to be the same as those found in the case of *B. lactis aërogenes*, viz., acetic acid, lactic acid, succinic acid, formic acid, ethyl alcohol, carbon dioxide, and hydrogen. No trace of marsh gas was detected. As in the case of *B. lactis aërogenes* the culture medium at the end of the fermentation gave Voges and Proskauer's reaction, and there was also production of butylene glycol, amounting to 19 per cent. of the sugar. The relative proportions of the products of fermentation differed considerably from those of *B. coli communis* and, in a less degree, from those of *B. lactis aërogenes*. These variations are shown in the following tables. The actual percentages by weight of the products on the glucose fermented in two separate determinations are given in Table I, Columns 1 and 2. For comparison, in Column 3 are given the results of a typical fermentation of glucose by *B. lactis aërogenes*, and in Column 4 those of a similar fermentation by *B. coli communis*. Table II shows the number of carbon atoms per molecule of glucose represented by each product.

On comparing the results given in Table I it will be seen that the ratio of hydrogen to carbon dioxide by volume, viz. 0·3 : 1, in the gas evolved from glucose by *B. cloacæ* is somewhat smaller than in the case of *B. lactis aërogenes* (= 0·42 : 1), and markedly less than for *B. coli communis* (= 1·19 : 1). Theobald Smith† gives the characteristic ratio for *B. lactis aërogenes*

Glucose.

Table I.—Percentages.

	B. cloacæ.		*B. lactis aërogenes.*	*B. coli.*
	1.	2.	3.	4.
Alcohol	16·55	13·15	17·1	12·85
Acetic acid	1·17	3·04	5·1	18·84
Lactic acid	2·04	10·99	5·5	31·90
Succinic acid	2·03	1·74	2·4	5·20
Formic acid	4·31	3·20	1·0	0·0
Carbon dioxide	41·75	41·11	38·0	18·09
Carbon dioxide, c.c. per grm.	211·3	208·0	198·3	91·8
Hydrogen, c.c. per grm.	64·0	55·7	82·4	110·0
Ratio H_2/CO_2	0·3	0·27	0·42	1·19

* Harden, Thompson, and Young, 'Biochem. Journ.,' 1910, vol. 5, p. 230.

† Theobald Smith, 'Centralb. f. Bacteriol.,' 1895, vol. 18, pp. 1, 494, 589.

Glucose.

Table II.—Carbon Atoms.

	B. cloacæ.		*B. lactis aërogenes.*	*B. coli.*
	1.	2.	3.	4.
Alcohol	1·30	1·03	1·34	1·01
Acetic acid	0·07	0·18	0·31	1·13
Lactic acid	0·12	0·66	0·33	1·91
Succinic acid	0·12	0·11	0·15	0·32
Formic acid	0·17	0·13	0·04	0·00
Carbon dioxide	1·71	1·68	1·60	0·74
Total	3·49	3·79	3·77	5·11
Hydrogen, atoms per molecule	1·04	0·90	1·33	1·77

$H_2/CO_2 = 1:1$, but this result does not represent the actual ratio of the gases produced, owing to the solubility of the carbon dioxide in the liquid medium contained in the ordinary fermentation tubes which he employed. This source of error has been obviated, as already pointed out, by collecting the gases over mercury in an evacuated flask. Formic acid was found in the products obtained from *B. cloacæ* in far greater amount than in those from *B. lactis aërogenes,* while those given by *B. coli* are usually almost free from this substance. It is, however, probable that at least a portion of the carbon dioxide is derived from the decomposition of formate primarily formed as an intermediate product. A very marked difference in the relative proportions of alcohol and acetic acid produced by the three organisms will be noticed. While the molecular ratio alcohol/acetic acid for *B. coli communis** is 1, and for *B. lactis aërogenes* (average of three determinations) = 4, that for *B. cloacæ* was found in two experiments to be 18 and 6. The large difference between these results is due to the fact that only a very small amount of acetic acid is produced, and a small absolute difference in this produces a large change in the ratio. Succinic acid is produced by *B. cloacæ* in rather smaller amount than by *B. lactis aërogenes,* and in less than half the quantity given by *B. coli communis.* The amount of alcohol is approximately equal to that given by *B. lactis aërogenes.* A considerable deficiency of carbon in the fermentation of glucose by *B. cloacæ* was found, and, remembering the very similar biological characters of *B. cloacæ* and *B. lactis aërogenes,* butylene glycol was sought for.

* Harden, 'Journ. Hygiene,' 1905, vol. 5, p. 488.

Production of 2.3-*Butylene Glycol by* B. cloacæ.

A medium containing 50 grm. of glucose in 1 litre of a 1 per cent. solution of Witte's peptone, to which had been added 10 grm. of chalk, was inoculated with the bacillus. After six weeks' incubation at 37°, the liquid was distilled to dryness under reduced pressure at 40°. The dry residue was extracted with boiling absolute alcohol, and the alcoholic solution distilled at 40° under reduced pressure. The residue, weighing 10·6 grm., was fractionated at normal pressure. A fraction distilling between 178° and 184°, weighing 9·5 grm., which solidified completely in a freezing mixture, was obtained. That this substance was 2.3-butylene glycol was proved by converting a portion of it into diacetyl* by the action of bromine water under the influence of light. From 4·5 grm. butylene glycol was obtained 1 grm. diacetyl, from which was prepared the phenyl-osazone.† After recrystallisation from alcohol and water the latter was found to have a melting point 243°.

B. *Action of* B. cloacæ *on Mannitol.*

Considerable differences, while on the whole not so marked as in the case of glucose, are also found on comparing the results of the fermentation of mannitol by *B. cloacæ* with those of *B. coli communis* and *B. lactis aërogenes.* In Table III, Columns 1 and 2, are given the results of two separate determinations of the products resulting from the action of *B. cloacæ* on mannitol, and in Columns 3 and 4 are given for comparison the figures obtained by typical fermentations of mannitol by *B. lactis*

Mannitol.

Table III.—Percentages.

	B. cloacæ.		*B. lactis aërogenes.*	*B. coli.*
	1.	2.	3.	4.
Alcohol	27·45	26·48	32·5	28·1
Acetic acid	4·23	3·67	2·5	9·5
Lactic acid	2·64	2·24	8·6	18·6
Succinic acid	2·29	4·24	3·2	8·9
Formic acid	5·56	4·56	1·5	3·0
Carbon dioxide	29·02	31·20	35·5	28·44
Carbon dioxide, c.c. per grm.	146·8	157·8	180·3	143·0
Hydrogen, c.c. per grm.	110·2	116·9	138·3	167·0
Ratio H_2/CO_2	0·75	0·74	0·77	1·18

* v. Pechmann, 'Ber.,' 1890, vol. 23, p. 2427.

† v. Pechmann, 'Ber.,' 1888, vol. 21, p. 2754.

Mannitol.

Table IV.—Carbon Atoms per Molecule of Mannitol.

	B. cloacæ.		*B. lactis aërogenes.*	*B. coli.*
	1.	2.	3.	4.
Alcohol	2·17	2·10	2·57	2·22
Acetic acid	0·26	0·22	0·15	0·58
Lactic acid	0·16	0·14	0·52	1·13
Succinic acid	0·14	0·26	0·20	0·55
Formic acid	0·22	0·18	0·06	0·12
Carbon dioxide	1·20	1·29	1·47	1·16
Total	4·15	4·19	4·97	5·76
Hydrogen, atoms per molecule	1·80	1·91	2·26	2·7

aërogenes and *B. coli communis* respectively. Table IV shows the number of carbon atoms per molecule of mannitol represented by the various products. As in the case of glucose, a considerable deficiency in the carbon will be noticed; this is to be accounted for by the production of acetylmethylcarbinol and butylene glycol, a qualitative experiment having shown the presence of both these substances.

In the case of mannitol there is practically no difference in the ratios H_2/CO_2 for the two organisms *B. cloacæ* and *B. lactis aërogenes.* As with glucose, the amount of formic acid obtained from *B. cloacæ* is considerably greater than from either *B. lactis aërogenes* or *B. coli communis.* On the other hand, the opposite is to be observed in the figures given for succinic acid. A comparison of Tables I and III shows that *B. cloacæ* produces twice as much alcohol from mannitol as from glucose. This further confirms the suggestion previously made by Harden* that the formation of alcohol in these reactions is related to the presence of the terminal $CH_2(OH)\cdot CH(OH)$ group, which occurs twice in the molecule of mannitol, and only once in that of glucose.

* Harden, 'Chem. Soc. Trans.,' 1901, p. 610.