CLXXXI. CHEESE-RIPENING STUDIES¹.

II. THE NITROGEN REQUIREMENTS OF THE LACTIC ACID BACTERIA.

By WILFRID SADLER, BLYTHE ALFRED EAGLES AND
GLADYS PENDRAY.

From the University of British Columbia, Vancouver, Canada.

(Received August 12th, 1932.)

In a previous paper [1932] we have reported upon the nitrogen distribution in forty-three nitrogen sources and have shown the influence of each of thirty-seven of these defined sources on the fermentation of glucose, mannose and lactose by five strains of lactic acid streptococci. The work confirms the findings of Orla-Jensen [1919] as to the importance of assuring a suitable nitrogen source in fermentation studies on the lactic acid bacteria, draws attention to the significant differences in the nitrogen distribution in certain of the sources we have investigated, and indicates clearly that in fermentation studies on the lactic acid streptococci the "kind" and the "amount" of nitrogen are both critical [see Eagles and Sadler, 1932].

In the light of these results we have studied the influence of four of the more significant of these nitrogen sources on the sugar-fermenting ability of thirty-three strains of lactic acid streptococci.

EXPERIMENTAL.

We have employed nitrogen sources 1, 28, 30 and 34 (see Table I), the broth in each case being prepared to contain 1 %, or approximately 1 %, total nitrogen. The distribution of the nitrogen in the four sources is shown in Table I, quoted from our previous paper.

Table I.

Nitrogen source	Protein-N	Proteose-N	Peptone-N	Sub-peptone-N
1 Peptic caseinogen digest	4.76	57.69	20.36	17.19
28 Proteose peptone (Difco)	0.0	29.68	47.84	22.48
30 Hydrolysed caseinogen (Difco)	4.82	35.84	27.26	32.08
34 "Bacto yeast extract"	0.0	0.0	24.20	75.80

The thirty-three strains of streptococci had been isolated from a Kingston cheese two weeks old. At the time of isolating, all colonies from one plate of a specific dilution had been picked off. Of the 54 colonies then recovered in pure culture, 33 only were available after a lapse of two years. It is unfortunate that

¹ These studies are provided for by a Research Fund established jointly by the Empire Marketing Board and the University of British Columbia.

Table II. *Titratable acidity in g. lactic acid per litre.*

Culture No. EMB ₁	Xylose				Mannitol				Fructose				Glucose				Mannose				Galactose				Sucrose				Maltose				Lactose				Raffinose				Salicin																																																																																						
	1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34				1 28 30 34																																																																																		
	Nitrogen sources (see Table I)																																																																																																																														
172	0.7	0.5	2.3	1.6	0.7	4.1	3.2	0.9	0.2	6.8	0.7	1.1	0.2	6.1	7.0	2.5	0.2	5.6	5.9	1.4	5.9	0.7	3.4	1.8	0.0	0.0	0.2	2.1	2.7	0.2	0.7	1.6	6.8	5.9	0.5	0.2	0.5	0.0	0.5	0.0	0.0	0.2	0.0	0.9																																																																																			
173	0.7	0.5	1.6	1.1	2.7	4.3	4.5	0.2	7.4	7.2	8.1	3.4	8.6	7.0	7.4	2.0	7.4	6.5	6.8	2.5	7.2	5.2	4.5	1.8	1.1	0.5	0.2	2.5	1.1	0.5	0.7	1.4	7.9	6.5	7.2	2.0	1.1	0.2	0.9	0.0	6.5	6.3	7.7	0.9																																																																																			
174	0.9	0.0	1.4	0.7	0.7	0.2	0.2	0.2	7.2	6.1	6.5	3.4	7.2	5.6	5.4	1.8	6.3	5.2	5.4	1.8	5.9	4.5	3.4	1.1	0.9	0.0	0.2	1.1	0.7	0.0	0.5	1.4	6.8	5.4	6.1	1.4	0.5	0.2	0.5	0.0	5.0	3.6	0.0	0.7																																																																																			
175	1.4	0.7	2.9	0.0	0.5	0.7	0.5	1.4	7.9	6.3	7.2	1.6	9.2	7.0	7.4	0.9	8.6	7.0	7.0	0.7	7.2	5.2	4.3	0.0	0.9	0.0	0.0	0.0	1.1	0.2	1.1	0.2	7.9	6.8	7.0	2.0	0.9	0.0	0.5	0.2	7.7	6.3	0.5	0.5																																																																																			
176	1.1	0.2	1.4	1.4	1.1	0.5	0.5	1.1	8.1	6.3	8.3	4.1	8.6	6.8	6.8	2.5	7.9	6.3	6.1	1.1	6.5	4.7	3.2	1.8	0.9	0.0	0.0	0.5	1.1	2.0	0.5	1.8	7.2	6.1	6.5	3.2	0.9	0.0	0.9	0.5	7.0	6.3	5.0	1.6																																																																																			
178	1.4	0.9	2.5	0.5	0.2	0.5	0.7	0.5	8.1	6.8	8.1	0.2	9.5	7.9	7.9	2.7	8.6	7.0	7.4	0.7	5.4	0.9	0.9	1.4	0.7	0.7	0.2	0.2	0.7	0.7	—	1.1	1.1	0.5	1.0	0.7	0.2	0.7	0.5	0.5	1.8																																																																																						
179	1.4	0.0	0.9	1.6	0.5	0.5	0.9	0.2	7.9	6.5	8.3	3.4	8.1	5.9	5.9	2.5	7.2	6.1	6.5	1.1	0.9	4.7	5.0	2.0	0.9	0.0	0.7	0.7	0.9	0.0	—	1.8	7.4	5.6	6.8	3.4	0.7	0.0	0.9	0.5	0.0	0.4	0.7	1.1																																																																																			
180	0.9	0.5	1.6	1.4	0.5	0.7	0.7	0.0	8.6	6.5	7.9	1.1	9.5	7.4	7.9	2.0	8.3	6.8	7.4	0.7	7.2	5.0	4.3	1.8	0.9	0.9	0.5	0.0	0.9	0.7	—	1.6	7.7	6.8	7.4	2.0	0.9	0.2	0.5	0.0	0.9	0.5	0.2	0.5																																																																																			
181	0.0	0.0	1.1	0.9	0.0	0.0	0.2	0.2	0.5	0.5	0.5	1.8	6.5	5.2	5.9	2.0	0.5	0.5	0.7	0.5	2.0	5.2	4.1	1.6	0.5	0.2	0.0	0.5	0.7	0.5	0.2	1.6	2.5	5.9	5.9	0.2	0.5	0.0	0.2	0.2	0.0	0.0	0.2	0.5																																																																																			
182	0.2	0.5	0.5	0.0	0.5	0.5	0.9	0.0	0.5	0.7	0.9	0.5	0.2	0.2	0.0	0.9	0.2	0.5	0.5	0.5	0.9	0.7	0.0	0.7	0.5	0.2	0.5	0.2	0.5	0.2	0.5	0.0	0.5	0.5	0.0	0.9	0.2	0.7	0.0	0.5	0.7	0.7	1.4																																																																																				
183	0.7	0.2	0.9	1.1	0.0	0.2	0.0	1.1	7.7	6.8	8.1	3.2	9.0	7.2	7.0	2.0	8.1	7.0	7.2	1.1	6.8	5.4	4.5	0.2	0.9	0.0	0.0	0.9	1.1	1.6	0.2	1.6	8.1	6.5	6.1	3.6	0.5	0.2	0.2	1.1	6.8	5.0	0.2	1.6																																																																																			
184	1.8	0.0	0.5	0.9	2.5	4.3	1.1	1.8	8.1	6.8	7.4	1.8	8.6	6.8	6.8	0.5	8.6	7.2	6.5	0.9	7.7	5.2	4.1	0.7	1.1	0.0	0.7	0.0	0.0	0.5	1.4	1.6	8.1	6.5	6.5	3.2	0.9	0.0	0.9	0.7	6.8	5.0	4.1	1.6																																																																																			
185	1.1	0.5	2.0	1.1	0.5	0.5	0.7	1.1	7.7	6.8	7.2	3.4	8.1	6.3	6.1	3.4	7.2	6.1	6.5	3.6	6.5	4.7	4.1	0.9	0.7	0.2	0.2	0.9	0.9	0.2	0.5	2.5	7.2	5.9	6.1	3.4	0.7	0.0	0.2	0.9	6.1	3.2	5.2	1.8																																																																																			
188	0.9	0.2	1.6	1.8	0.2	0.0	0.2	1.4	0.2	0.5	0.7	1.8	7.2	6.3	7.0	2.5	0.5	0.2	0.9	1.6	2.0	2.3	0.5	1.6	0.5	0.0	0.2	1.4	0.5	0.5	0.5	2.0	1.8	6.1	5.2	0.5	0.2	0.2	1.1	0.2	0.2	0.0	1.6																																																																																				
189	0.9	0.0	1.1	1.6	0.5	0.9	0.7	1.6	8.6	7.2	7.4	1.6	8.1	6.3	5.9	2.5	7.4	6.1	6.1	1.8	6.5	3.6	1.1	1.1	0.7	0.7	0.5	1.1	4.5	1.1	4.5	2.9	7.7	6.5	7.0	3.8	0.5	0.5	0.0	0.9	2.3	1.1	0.5	1.1																																																																																			
190	0.0	0.0	1.6	1.4	0.0	0.9	2.3	0.0	7.7	6.1	5.4	2.3	7.7	5.4	6.5	1.8	7.2	3.4	5.0	0.2	4.1	0.0	0.5	0.2	4.1	4.3	7.2	3.6	4.3	5.6	5.6	2.3	0.0	0.0	0.0	0.0	0.2	0.0	0.5	0.0	0.0	1.8	0.0	0.0																																																																																			
191	0.9	0.2	1.6	0.7	0.7	0.5	0.7	1.1	8.1	6.1	7.7	3.8	8.8	6.8	6.8	1.6	8.6	6.5	6.3	1.6	7.4	5.2	3.6	1.6	0.9	0.0	0.0	1.6	1.1	0.0	—	2.3	7.7	6.1	7.0	2.7	0.9	0.0	0.5	2.0	7.7	4.5	0.2	1.4																																																																																			
192	0.7	0.0	1.4	0.0	0.5	0.5	0.7	0.9	8.3	7.0	7.2	1.6	9.5	7.4	7.0	2.5	8.6	7.0	6.3	0.9	6.3	1.4	0.5	1.4	1.1	0.7	0.7	0.9	0.7	0.9	0.5	1.1	1.1	1.6	1.6	0.9	0.7	0.7	1.1	0.5	0.9	0.7	0.9																																																																																				
193	1.4	0.7	1.6	2.9	0.7	0.7	0.7	1.1	8.3	6.8	8.1	2.9	9.5	7.4	8.1	2.3	8.8	7.4	7.9	0.0	8.3	5.6	5.9	0.2	1.1	0.0	0.5	6.3	1.4	0.0	1.8	0.5	8.6	7.0	7.2	3.4	0.7	0.0	0.5	1.1	7.0	5.4	4.1	1.8																																																																																			
195	0.7	0.0	1.1	1.6	3.8	3.8	3.6	0.7	8.1	7.4	8.3	2.7	8.3	7.0	6.5	0.5	7.9	6.3	6.1	1.4	6.8	4.5	3.6	1.4	1.1	0.2	0.7	0.0	1.1	0.0	2.0	1.6	7.7	6.5	6.3	2.3	1.1	0.0	0.7	0.0	6.5	—	5.0	0.7																																																																																			
196	1.1	0.0	1.6	0.7	0.5	0.0	0.2	0.2	7.9	6.3	7.4	1.1	9.0	7.4	7.4	2.3	8.1	5.0	—	1.1	6.8	5.4	0.9	1.1	2.9	0.7	0.5	0.0	0.7	0.7	0.2	1.4	0.7	0.7	0.9	0.2	0.5	0.0	0.0	1.8	0.0	0.0	0.0																																																																																				
197	1.4	0.5	2.3	1.6	0.7	0.5	0.7	1.4	10.1	8.1	9.2	1.1	9.9	7.7	7.9	2.3	8.8	7.0	7.9	1.4	8.6	5.0	6.1	1.4	1.1	0.2	0.5	0.9	1.1	0.7	0.7	2.3	9.0	7.0	8.1	4.1	0.9	0.2	0.5	1.4	0.9	0.7	0.5	2.0																																																																																			
200	1.4	0.2	1.4	0.5	0.5	0.2	0.5	0.7	8.6	6.3	7.2	1.0	9.2	7.0	7.4	2.0	8.1	6.5	7.2	1.1	7.4	4.3	3.6	0.9	0.9	0.2	0.7	1.1	0.9	0.5	0.5	1.6	8.3	1.8	6.3	4.1	0.9	0.0	0.5	1.6	3.2	0.7	0.2	1.6																																																																																			
201	1.1	0.0	0.9	1.6	0.7	0.5	0.7	0.7	9.0	7.7	8.6	2.7	9.2	7.4	5.9	1.8	8.1	6.3	7.2	0.7	7.0	4.7	5.9	0.0	0.9	0.7	0.5	0.5	1.6	0.7	0.9	2.5	8.6	6.8	7.7	2.7	0.7	0.2	0.5	1.1	0.5	0.7	0.5	0.7																																																																																			
203	0.9	0.2	1.1	2.3	0.2	0.2	0.2	1.6	0.5	0.2	0.7	2.0	6.8	5.6	6.3	2.3	0.5	0.5	0.2	1.8	4.7	4.7	4.3	1.6	0.7	0.5	0.2	1.6	0.5	0.5	0.2	2.3	6.1	5.2	5.4	0.7	0.2	0.2	0.5	0.2	0.2	0.2	1.1																																																																																				
204	1.4	0.7	2.3	0.7	0.7	0.2	0.7	0.0	8.8	6.5	7.7	1.6	9.2	7.0	7.9	1.1	8.6	6.8	7.0	0.7	7.4	5.4	5.0	0.9	0.9	0.5	0.7	0.5	0.9	0.7	—	1.1	8.3	6.8	7.7	3.4	0.9	0.2	0.9	0.2	0.4	0.5	0.2	1.1																																																																																			
205	0.9	0.2	2.3	0.9	0.2	0.2	0.0	0.0	8.3	8.1	7.4	0.5	9.0	8.1	7.7	1.4	8.1	7.9	7.0	0.0	7.4	6.3	5.0	0.5	0.7	0.2	0.0	0.0	0.9	0.7	0.5	1.4	7.9	7.9	7.0	0.7	0.7	0.2	0.2	0.7	3.2	4.3	0.2	0.7																																																																																			
206	1.6	0.7	4.1	0.9	3.2	4.1	1.6	0.0	8.1	7.0	7.2	4.3	7.9	6.5	6.3	2.3	7.2	5.9	6.1	0.9	6.8	4.5	4.1	0.9	1.1	0.5	0.2	0.5	1.1	0.2	1.1	1.8	7.2	5.6	6.3	2.5	0.0	0.2	0.5	1.1	5.0	3.2	0.7	1.6																																																																																			
208	0.9	0.0	0.9	0.2	0.2	0.2	0.0	0.0	7.7	6.1	7.0	0.7	8.6	7.0	7.0	0.2	8.3	6.3	6.1	0.0	7.0	4.7	3.4	1.4	0.9	0.2	0.2	0.2	0.7	0.5	0.5	1.1	7.9	6.8	7.7	0.2	0.5	0.0	0.2	0.0	0.5	0.2	0.5																																																																																				
209	1.4	0.9	2.3	1.8	0.5	0.5	0.7	2.0	8.6	7.7	8.3	2.7	8.3	6.5	7.0	2.3	7.7	6.5	7.0	1.6	6.3	3.2	0.7	0.0	0.9	0.2	0.5	1.6	4.3	1.1	2.3	2.7	7.9	6.3	7.7	3.8	0.7	0.0	0.5	0.2	3.6	4.5	0.2	1.4																																																																																			
210	1.4	1.1	1.8	1.4	0.5	0.2	0.2	1.1	8.3	7.7	8.1	0.0	8.6	5.6	7.0	1.6	7.9	6.1	6.8	0.7	6.3	3.6	1.6	1.1	0.7	0.7	0.2	0.7	5.0	1.6	1.6	2.3	7.9	6.1	7.0	3.2	0.2	0.2																																																																																									

among the strains lost were all those that responded appreciably to enrichment of media with yeast extract [Sadler, Eagles and Pendray, unpublished results]. The thirty-three cultures here reported upon are Gram-positive and appear in young milk culture as single cells, in pairs or as short or long chains. All fail to liquefy gelatin. The procedures followed in the fermentation study have been described in detail already [Orla-Jensen, 1919; Sadler, Eagles and Pendray, 1932].

The results of the determinations of the total titratable acidity—recorded as grams lactic acid per litre—produced by each of the thirty-three cultures from eleven carbohydrates when employing the nitrogen sources defined are given in Table II.

DISCUSSION.

On examining and comparing the total titratable acidity figures obtained by using the four nitrogen sources respectively, yeast extract is seen to be an unsuitable source for the fermentation by these streptococci of fructose, glucose, mannose, galactose and lactose. In the fermentation of raffinose, however, when yeast extract is the nitrogen source, a few strains produce from 1 to 2 g. acid per litre; whereas all the organisms fail to produce as much as 1 g. acid per litre when the nitrogen is provided as caseinogen digest, proteose (Difco) peptone or hydrolysed caseinogen (Difco).

In the fermentation of xylose by certain strains there seems to be a significant value in yeast extract as a nitrogen source when compared with proteose peptone; but the superiority of hydrolysed caseinogen over any of the nitrogen sources is pronounced—the two most suitable sources being those containing a comparatively high and a very high percentage of the sub-peptone nitrogen fraction.

Mannitol is fermented to some small extent by many of the streptococci when the nitrogen is supplied as yeast extract; when proteose peptone is the source of nitrogen fewer strains are active but those that are active produce as much as 4 g. acid per litre. The influence of hydrolysed caseinogen is, in a measure, comparable with that of proteose peptone; but caseinogen digest appears to be unsuitable.

Except one strain, EMB₁ 190, all the streptococci produce less than 1 g. acid per litre from sucrose when either hydrolysed caseinogen or proteose peptone is the nitrogen source. Caseinogen digest suits a few strains. When the nitrogen is supplied as yeast extract the total amount of acid produced is always low; but more of the cultures produce a titratable amount of acid if supplied with this source than is the case with any of the other nitrogen sources used.

In the fermentation of maltose, five strains produce over 2.7 g. acid per litre when the nitrogen is supplied as caseinogen digest and four strains produce over 2.0 g. when hydrolysed caseinogen is supplied. When the nitrogen source is yeast extract 18 strains produce from 1.0 to 2.0 g. acid per litre and

11 strains produce from 2.0 to 2.9 g. acid per litre. In the fermentation of maltose by these streptococci, yeast extract appears to have a significant value as a nitrogen source.

Few of the streptococci ferment salicin when the nitrogen is supplied as hydrolysed caseinogen. Many produce a small amount of acid when the nitrogen source is yeast extract. When the nitrogen is supplied as caseinogen digest a number of strains are inactive; but some twelve strains are vigorous—defining vigour and activity in terms of total titratable acidity produced. In lesser measure the influence of proteose peptone is comparable with that of caseinogen digest.

In considering the total titratable acidity produced by the streptococci from fructose, glucose, mannose, galactose and lactose, yeast extract as a source of nitrogen does not bear comparison with any of the other nitrogen sources employed. Almost all the strains produce a high total titratable acidity from these sugars when the nitrogen source is caseinogen digest, proteose peptone, or hydrolysed caseinogen. The activity of the organisms is most distinct, however, when the nitrogen is supplied as caseinogen digest, the superiority of this source being especially evident in the fermentation of galactose.

Considering the results as a whole, it is to be seen that when provided with yeast extract—a source containing 75 % sub-peptone-N, 25 % peptone-N and no proteose-N [Sadler, Eagles and Pendray, 1932]—many of the streptococci produce a small amount of acidity from most of the carbohydrates employed, the total titratable acidity never being high. In order to bring about active and vigorous fermentation of fructose, glucose, mannose, galactose and lactose these streptococci require a nitrogen source containing an appreciable amount of proteose-N, peptone-N and sub-peptone-N, and we have seen that these requirements are best met by using the peptic caseinogen digest.

Our results support Orla-Jensen [1919] in his insistence on the critical importance of the nitrogen source in fermentation studies on the lactic acid bacteria, emphasise the suitability of a peptic caseinogen digest—a suitability so clearly shown by Orla-Jensen and now shown by us in a still more specific sense—and confirm our previous findings.

The work would seem to suggest that the specific lactic acid streptococcus strains demand a certain moiety of a particular nitrogen fraction for the fermentation of a particular carbohydrate: that in some measure there is a protein-carbohydrate-streptococcus linking up. Furthermore, there is the suggestion that at least the variety of nitrogen fractions we have employed in this study might be used to advantage in studies on the sugar-fermenting abilities of the lactic acid streptococci.

REFERENCES.

- Eagles and Sadler (1932). *Nature*, **130**, 278.
Orla-Jensen (1919). The lactic acid bacteria. (Copenhagen.)
Sadler, Eagles and Pendray (1932). *Canadian J. Research*, **7**, No. 4.