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by

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Fixation of Atmospheric Nitrogen and Growth of *Azotobacter Vinelandii* in Acid Media

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Azotobacter vinelandii has been grown in acid media for about ten days, during which time both growth and nitrogen fixation has occurred. The pH of the medium rises slowly to 6.8 - 6.9, at which value the bacterial population slowly declines.

Experimental

Azotobacter vinelandii was grown in two culture media [1]; [2].

Medium 1		Medium 2	
Mannitol	15 g	Mannitol	50 g
K ₂ HPO ₄	1.6	KH ₂ PO ₄	0.4
KH ₂ PO ₄	0.4	MgSO ₄ ·7H ₂ O	4.0
MgSO ₄ ·7H ₂ O	0.2	NaCl	4.0
NaCl	0.2	CaCO ₃	2.0
CaSO ₄	0.1	FeSO ₄ ·7H ₂ O	0.3
FeSO ₄ ·7H ₂ O	0.015	Na ₂ MoO ₄ ·2H ₂ O	0.001
Na ₂ MoO ₄ ·2H ₂ O	0.001	Citric acid	0.16

Conc. hydrochloric acid 13.0 ml.

In each case, glass distilled water is made to 1 l. pH adjusted by addition of sodium hydroxide or hydrochloric acid, measured with a Pye pH meter and checked with BDH narrow range universal indicator paper.

* This work was performed when Turgut Gündüz was on leave from University of Ankara by a Nato grant in 1963.

The cultures were shaken continuously at 30°C, and 10-ml portions of each were removed at intervals and subjected to Kjeldahl digestion with 1 g of sodium sulphate, 0.1 g of mercury ion, and 3 ml of conc. sulphuric acid, for eight hours. The ammonia was distilled, using 10 ml. of 40 % sodium hydroxide and 10 ml of a mixture of 7 % Na₂SO₄ and 35 % NaOH, into 10 ml of 2 % boric acid, in the Micro-Kjeldahl still as modified by Hanif [3].

Simultaneously, samples were taken for counting, for staining, and plating ; on plating, the bacteria produced, a motile form and a yellowish pigment, soluble in the media. The results are tabulated below.

TABLE I (Medium-1)

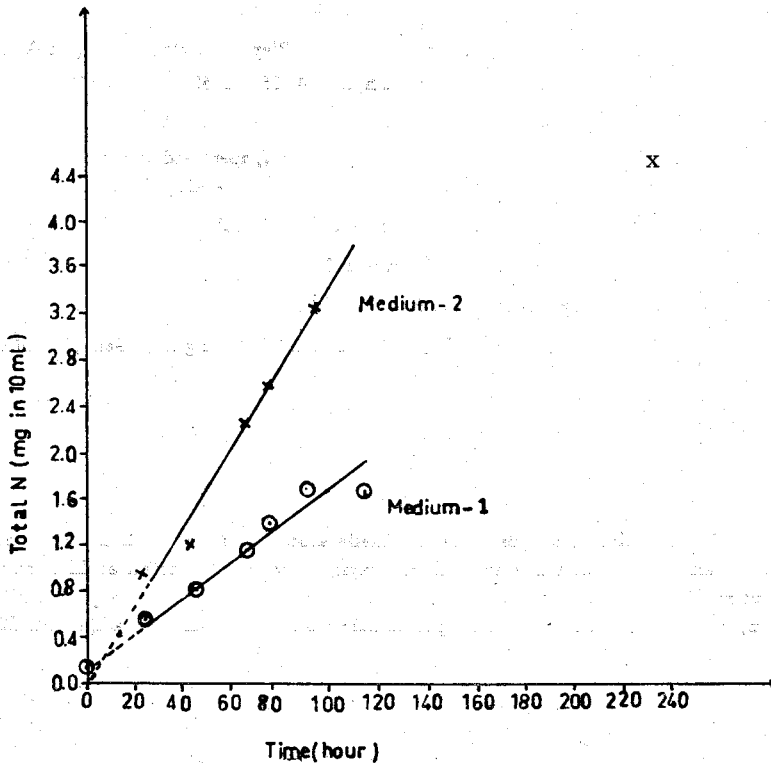
Time in hours	pH	Gram staining	Plating	Counting per ml	Total nitrogen (in 10 ml)
0	6.4	0	0	0	0.11 mg
23	6.4	-	+	1.3×10^{-9}	0.49 mg
43	6.4	-	+	1.7×10^{-9}	0.76 mg
65	6.7	-	+	1.7×10^{-9}	1.10 mg
76	6.7	-	+	-	1.34 mg
88	6.7	-	+	-	1.60 mg
106	6.7/6.9	-	+	1.3×10^1	1.53 mg
214	6.9	-	+	very few	-

Gram negative and motile. + Shows good growth.

TABLE II (Medium-2)

Time in hours	PH	Gram staining	Plating	Counting per ml	Total nitrogen in 10 ml
0	6.2	0	0	0	0.056 mg
23	6.4	-	+	1.1×10^{-9}	0.913 mg
43	6.8	-	+	1.0×10^{-9}	1.13 mg
65	6.7	-	+	1.16×10^{-9}	2.14 mg
76	-	-	+	-	2.48 mg
94	6.8	0	0	3.10×10^{-9}	3.17 mg
214	6.8	-	+	1.60×10^{-9}	- mg
238	6.8	-	+	1.8×10^{-9}	4.2 mg
257	6.8	-	+	1.7×10^{-9}	-

Total nitrogen has been plotted against time as shown in graph.



Conclusion

Thus it can be concluded that *Azotobacter vinelandii* do exhibit the power of fixing nitrogen under acidic conditions.

The relatively high concentrations of mannitol, magnesium, sodium, and ferrous in medium 2, seem to have maintained a steadier pH and to have allowed slightly higher density of culture to be reached, but over and above this effect, the amounts of nitrogen fixed in medium 2 are appreciably greater.

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Özet

Asidli ortamlarda, on gün kadar müddetle azotobakter vinelandii üretilmiş, bu zaman zarfında hem azotobakter vinelandii sayısında ve hem de tesbit ettikleri hava azotu miktarında artmalar gözlemlenmiştir. Bu arada ortamın pH ında da yükselmeler olmuş ve 6,8 - 6,9 yükselmiştir, ki bu pH da dahi bakteri üremesinin olmadığı zannedilmekteydi.

AVIS IMPORTANT

La Revue "Communications de la Faculté des Sciences de l'Université d'Ankara" sera publiée dorénavant sous forme de fascicules à l'arrivée de chaque article. Le tome 15 de la série A, commençant par ce fascicule, sera complété par le dernier fascicule à paraître avant le 31 décembre 1966.

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