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EFFECT OF METAL IONS ON THE PRODUCTION OF ACETIC ACID BY ACETOBACTER ACETI

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The metal ions requirement of *Acetobacter aceti* (Strain NRC-722) for acetic acid production were undertaken. All the metals were stimulatory at their optimum concentrations which varied from metal to metal. 3.7 % acetic and was produced in the presence of each of iron and calcium at a concentration of $10^{-3.4}$M and $10^{-3.6}$M, respectively, as against 0.6% in the absence of these metals. The optimum level of magnesium, manganese and copper, for maximum production of acetic acid was $10^{-3.8}$M. The yield was further raised to 6.5% when all the metals were present at their optimum concentration.

Studies dealing with the nutritional requirements of acetic acid bacteria have revealed the importance of certain minerals on their growth.[1,2] Fe, Mg, Mn and Na are the essential constituents of defined medium which is capable of satisfying the growth requirements of *Acetobacter* species. Apart from this, however, no systematic or detailed study has been carried out to determine the effect of minerals on the acetic acid fermentation by the bacteria. The present investigation describes the effect of five different metals on the metabolic steps leading to the fermentation of acetic acid from glucose and ethanol by a species of *Acetobacter aceti*.

Experimental

Source of Culture.—The culture of *Acetobacter aceti* NRC-722 was obtained from National Research Council, Ottawa, Canada.

Medium for Maintaining Stock Culture.—The culture was maintained on agar medium containing 1% glucose, 0.5% yeast extract, 0.25% calcium carbonate and 2.5% agar and was subcultured fortnightly.

Basal Medium.—Glucose 2.0 g, ethanol 4.0 ml (v/v), casein hydrolysate 0.5 g, pantothanic acid 100 mg, nicotinic acid 40 mg, p-aminobenzoic acid 40 mg, anurine hydrochloride 100 mg, metal ions, variable concentration, double-distilled water to make 100 ml, pH 6.2.

Preparation of Inocula.—Slants having luxurient cell growth after 48 hr of incubation were washed with sterile double-distilled water. The cell suspension was homogenised with glass beads and adjusted to 0.3 optical density at 660 mμ.

Methods

In the typical experiments all inorganic salts were omitted. The control in our experiments contained EDTA 10^{-4}M to bind the indigenous metal ions in media if any. The salts of metal ions were added separately to the medium in different concentrations. All the chemicals were of analytical grade (E. Merck). Solutions of glucose, casein hydrolysate, EDTA and salts of metal ions were sterilized separately at 15 psi for 15 min. Ethanol and vitamin solutions were sterilized by passing through Seitz filter.

The basal medium (19.8) ml was dispensed aseptically into 100-ml capacity sterilized Erlenmeyer flasks and inoculated with 0.2 ml of the inocula. The flasks were incubated at 30±0.5°C on an incubator-cum-shaker having 88 strokes/min. 1 ml broth was taken aseptically, for estimation of acidity, after 24-hr interval. The controls were run simultaneously.

Total acidity and the percentage of acetic acid was determined by titrimetric method (A.O.A.C.)[3] Vitamin-free casein hydrolysate was prepared by the method of Barton-Wright.[4] The optical density of the inocula was adjusted with Unicamp Spectrophotometer SP-600.

Results

The effect of different concentration of the metal ions, separately, on the accumulation of acetic acid by *A. aceti* are summarised in Tables 1–5 Figure 1 shows the combined effect of all the metal ions, at their respective optimum concentration on the fermentation of acetic acid.

Discussion

It is evident from the results (Tables 1–5) that the metals employed in the study were stimulatory for the enzymic reactions, leading to the fermentation of acetic acid. Less acetic acid was always formed wherever EDTA was added in the culture media to bind indigenous and free metal

Table 1.—Effect of Iron (Added as $FeSO_4.7H_2O$) on Production of Acetic Acid by *Acetobacter aceti*.

Concentration (M)	Percentage of CH_3COOH					
	1 day	2 days	3 days	4 days	5 days	6 days
Control	0.060	0.070	0.11	0.190	0.58	0.54
$10^{-4.0}$	0.072	0.070	0.13	0.375	1.54	2.10
$10^{-3.8}$	0.072	0.077	0.88	3.320	3.08	2.73
$10^{-3.6}$	0.075	0.080	0.94	3.560	3.26	2.94
$10^{-3.4}$	0.075	0.080	1.09	3.700	3.53	3.11
$10^{-3.2}$	0.080	0.080	1.09	3.300	3.08	2.81
$10^{-3.0}$	0.080	0.080	1.08	3.100	2.83	2.50

Table 2.—Effect of Calcium (Added as $CaCl_2.2H_2O$) on the Production of Acetic Acid by *Acetobacter aceti*.

Concentration (M)	Percentage of CH_3COOH					
	1 day	2 days	3 days	4 days	5 days	6 days
Control	0.072	0.15	0.25	0.39	0.62	0.67
$10^{-4.0}$	0.064	0.14	0.43	1.11	1.40	1.46
$10^{-3.8}$	0.064	0.20	0.64	2.50	2.96	2.64
$10^{-3.6}$	0.064	0.21	0.80	3.13	3.70	3.20
$10^{-3.4}$	0.064	0.25	0.40	1.95	2.35	1.94
$10^{-3.2}$	0.064	0.25	0.30	1.46	2.05	1.64
$10^{-3.0}$	0.064	0.21	0.24	1.50	1.77	1.46

Table 3.—Effect of Copper (Added as $CuSO_4.5H_2O$) on Production of Acetic Acid by *Acetobacter aceti*.

Concentration (M)	Percentage of CH_3COOH					
	1 day	2 days	3 days	4 days	5 day	6 days
Control	0.061	0.08	0.15	0.21	0.61	0.58
$10^{-4.0}$	0.051	0.47	0.61	0.62	0.64	0.62
$10^{-3.8}$	0.051	0.87	2.12	2.20	2.30	2.25
$10^{-3.6}$	0.054	0.45	0.67	0.73	0.75	0.67
$10^{-3.4}$	0.056	0.36	0.45	0.45	0.49	0.43
$10^{-3.2}$	0.061	0.30	0.38	0.41	0.41	0.38
$10^{-3.0}$	0.061	0.25	0.32	0.32	0.30	0.28

Table 4.—Effect of Manganese (Added as $MnSO_4.4H_2O$) on Production of Acetic Acid by *Acetobacter aceti*.

Concentration (M)	Percentage of CH_3COOH					
	1 day	2 days	3 days	4 days	5 days	6 days
Control	0.064	0.069	0.15	0.20	0.58	0.63
$10^{-4.0}$	0.043	0.470	0.64	0.67	0.67	0.64
$10^{-3.8}$	0.054	0.620	0.81	0.81	0.84	0.70
$10^{-3.6}$	0.056	0.610	0.77	0.77	0.76	0.67
$10^{-3.4}$	0.056	0.610	0.74	0.75	0.75	0.67
$10^{-3.2}$	0.056	0.600	0.72	0.72	0.73	0.64
$10^{-3.0}$	0.056	0.580	0.70	0.70	0.71	0.62

TABLE 5.—EFFECT OF MANGANESIUM (ADDED AS $MgSO_4.7H_2O$) ON PRODUCTION OF ACETIC ACID BY *Acetobacter aceti*

Concentration (M)	Percentage of CH_3COOH					
	1 day	2 days	3 days	4 days	5 days	6 days
Control	0.072	0.075	0.13	0.20	0.54	0.54
$10^{-4.0}$	0.064	0.064	0.12	0.21	0.49	0.90
$10^{-3.8}$	0.064	0.069	0.13	0.25	0.58	1.92
$10^{-3.6}$	0.064	0.064	0.12	0.23	0.47	1.50
$10^{-3.4}$	0.064	0.064	0.11	0.19	0.29	0.64
$10^{-3.2}$	0.064	0.064	0.11	0.21	0.27	0.51
$10^{-3.0}$	0.064	0.064	0.09	0.14	0.23	0.26

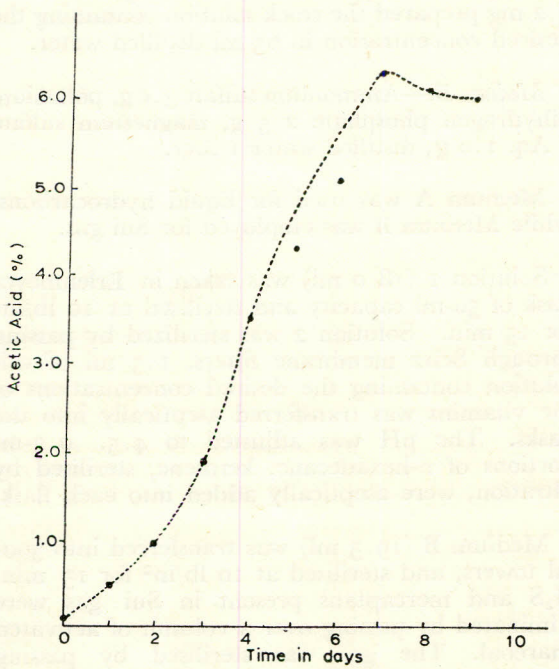

Fig. 1.—Production of acetic acid by *Acetobacter aceti*

ions, if any. The enzymic activity of the bacterium varied from metal to metal and was maximum only at certain concentration of particular metal. Maximum accumulation of acetic acid took place in the presence of Fe^{++} and Ca^{++} (3.7%) and was followed by Cu^{++} (2.3%), Mg^{++} (1.92%) and Mn^{++} (0.84%). The great difference in the amount of acetic acid produced in the absence and presence of the metals seems to suggest that metals, particularly Fe^{++}, Ca^{++} and Cu^{++} are important cofactors for the enzymes catalyzing the reactions. It is, however, difficult to ascertain from the present work which of the particular enzyme of the system requires the metals most for maximum activity.

Whereas Fe^{++} and Ca^{++} were stimulatory for the enzymes at all the concentrations between 10^{-4}M and 10^{-3}M (Tables 1 and 2), Cu^{++}, Mg^{++} and Ca^{++} brought about partial inactivation of the enzymes at higher levels of concentration. Optimum concentrations of Fe^{++} and Ca^{++} for maximum acetic acid production (3.7%) were $10^{-3.4}$M and $10^{-3.6}$M respectively. This amount of acetic acid was produced in presence of Fe^{++} in 4 days as against 5 days in case of Ca^{++}. Concentration beyond this optima retarded accumulation of acetic acid in both the cases, the effect being more pronounced in case of Ca^{++}.

Cu^{++}, Mg^{++} and Mn^{++} were most stimulatory at concentration $10^{-3.8}$M. These were, however, highly inhibitory at higher concentrations. Cu^{++} in particular brought about drastic reduction in accumulation of acetic acid at concentration beyond $10^{-3.8}$M. This is not unexpected as growth and metabolic activities of microorganisms are known to be retarded by higher doses of Cu^{++}.[5]

The combined effect of the metal ions is depicted in Fig. 1. Addition of all the minerals extended the fermentation period to 7 days. The production of acetic acid also increased from 3.7% in case of Ca^{++} and Fe^{++} to 6.3% when all the ions were present. These findings further support our assumption that these minerals are important cofactors of the enzymes catalyzing production of acetic acid.

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