

CHAPTER - V

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Production of organic acids by fungi in their medium of growth has been studied in 155 different isolates obtained from soils of Raipur. The property of production of organic acids seemed confined mainly to the members of the genus Aspergillus and in some of the Mucorales.

Aspergillus japonicus Saito (IMI-243984, isolate-AJ-IV) and Rhizopus oryzae Went & Geerlings (IMI-244011, isolate-RO-I) which were found as good producers of citric and fumaric acids, respectively, were selected for detailed studies. The production of tartaric acid by A. oryzae, discovered during the course of these investigations, has also been studied.

A. japonicus produced 3590 mg/100 ml of citric acid (CA) in presence of 0.5 ppm of zinc and with C/N = 9.0:0.4 of sucrose and potassium nitrate, in 9 days incubation time, at pH 7 and 30°C.

Similarly, maximum yields of fumaric acid (FA) (1100 mg/100 ml) and tartaric acid (TA) (1500 mg/100 ml) by A. oryzae were obtained in presence of 5 ppm of iron with glucose and ammonium tartrate, and in presence of

iron and zinc mixture (5 ppm each) with buffered medium containing glucose and NH_4Cl , respectively in 7 days incubation period, at pH 6 and 30°C .

For the accumulation of CA by A. japonicus, a certain minimal level of sugar in the medium of growth has been shown essential; below this critical level there may be good growth of the fungus but no production of CA. As this minimal requirement of sugar varied with the source of carbon taken i.e. 4-5% for glucose but only 3% for sucrose, it has been concluded that, it is not merely the quantity of sugar but also its quality that determines the yield of CA by this fungus.

As the lowering of the nutrient salt concentration resulted in an increase in the yield of CA alongwith a simultaneous decrease in the mold growth, it was concluded that a salt concentration that just restricts the mycelial growth would be more favourable for the yield of CA.

The studies made on the effect of incubation period suggested the existence of two clearly distinguishable phases in the fermentation of CA by A. japonicus; an 'initial growth phase' when the cells proliferate but do not accumulate CA, followed by 'acidogenic phase' when the organism accumulates CA in the apparent absence of growth or at least when the growth rate has been considerably reduced.

The occurrence of an inverse growth and CA production effects of pH on A. japonicus indicated an instrumental role of pH in setting the transition from the 'active growth' to the 'acidogenic phase' in the organism.

The studies on the effect of C/N showed that the fermentative production of CA in A. japonicus was primarily regulated by the concentration of carbohydrate as a gradual increase in the yield of CA was observed with the increase in the concentration of sucrose upto 9%.

(The organism showed specificity for the phosphorus source in its CA fermentation pathway as only the KH_2PO_4 induced this metabolism whereas other sources such as NaH_2PO_4 proved inhibitory.)

Relatively high activities of alkaline and acid phosphatases at the 6th day stage of growth, when the organism under went a growth stage transition, indicated existence of a positive relationship between the availability of phosphorus source and the initiation of the acidogenic phase.

(Amongst the various trace elements tried, individually or in combinations, zinc (0.5 ppm) singly was found to have the most significant effect on CA yield.) It was further observed that the yield/growth relationship effect of Zn in 0.5 ppm was higher than in 1 or 2 ppm.

Thus, a regulatory role of Zn on the growth and CA production was indicated.

Amongst the alcohols tried, methanol and ethanol stimulated the yield, while propanol showed an inhibitory effect. The studies made to observe the effect of methanol in relation to time of its adjunct showed much significance of this approach and suggested that, the use of methanol in the vicinity of the acidogenic phase may prove highly beneficial.

Though the raw carbohydrate sources, sweet potato, knol-khol and mango kernel oil mill residue could be made fermentable after certain treatments, they did not possess yield potential over basal medium. However, the cost economics of the two is to be worked out before any step of exploiting the raw material sources is taken.

The mutational studies showed that A. japonicus was capable of undergoing mutation under the influence of UV radiation and that strains superior to parent culture in respect of CA yield could be obtained.

The fact that R. oryzae can synthesize T₂ in substantial amounts has been reported for the first time.

The present isolate of R. oryzae was able to utilize sucrose directly and showed invertase capacity. Invertase has been reported lacking in rhizopus species in general. The isolate, therefore, may be used with a

great success over others for fermenting even sucrose containing crude carbohydrate sources like molasses etc.

The results obtained for the effects of N-sources showed that only ammonium tartrate and asparagine favoured FA formation. The ammonium salts of chlorine, nitrate and sulphate inhibited FA production but induced TA production simultaneously. A regulating role of nitrogen salt on the formation of FA and TA was thus observed here. This suggests that ammonium nitrogen sources (excepting ammonium tartrate) probably trigger TA metabolism and shunt FA formation.

The TA conversion coefficient value was maximum (57.59%) at one day stage of incubation in comparison to the 7 day stage when the maximum yield of TA was obtained. Therefore, replacement of the culture every day has been suggested for obtaining maximum yield of TA on this study.

For FA iron was found as the most effective trace ion whereas a mixture of iron and zinc proved best for TA production by R. oryzae.

None of the alcohols tried gave significant increase in the yield of FA or TA.

Studies on the utilization of crude sources of carbohydrate for FA and TA production showed that the pretreatment of these crude substrates was necessary

for obtaining any desirable yield. It was also found that maximum yields of both the FA and TA can be obtained from knol-khol and sweet potato substrates by treating with $K_4Fe(CN)_6$ and methanol, respectively.