OBJECT AND SCOPE OF THE PRESENT INVESTIGATION

Several secondary metabolites of diverse chemical nature are produced by different strains of the fungus *Aspergillus flavus*. These compounds play no obvious role in the economy of the organism. Whereas primary metabolism is basically the same for all living systems, secondary metabolism is restricted only to the lower forms of life and is often strain specific. Factors controlling the secondary metabolism of fungi are complex and not fully understood.

Kojic acid is generally considered as a secondary metabolite of fungal fermentation. The formation of secondary metabolites by fungal cells have gained a new momentum of interest recently. Several reports have appeared in the last few years regarding investigations on mycotoxins, antibiotics and other secondary metabolites, but the information available on kojic acid is scanty at present and not much is known about the mechanism of its biosynthesis and significance.

Some studies have been reported on the mechanism of kojic acid synthesis by different workers. However, these studies pertaining to the biosynthetic mechanism of kojic acid were mainly restricted to $^{14}$C incorporation studies and some probable mechanisms were tentatively suggested.\(^{(1,2)}\) It was therefore considered desirable to investigate the validity of proposed mechanisms on the basis of concrete experimental evidence. These investigations included studies on the
synthesis and degradation of kojic acid on different growth media, the development of a simple resuspended mycelial system, inhibition studies with specific inhibitors of key enzymes that may be involved in kojic acid synthesis employing growing as well as resuspended mycelia, relevant enzymatic studies at different periods of time during the growth of Aspergillus flavus and in resuspended mycelia under different conditions and chromatographic studies to identify the possible intermediates. Thus the information obtained from the proposed studies would help us to understand some of the metabolic pathways operative in A. flavus thus providing basic information in this area of microbial biochemistry.

Recently a lot of emphasis is being laid on proper utilization of by products of cane sugar industry viz. bagasse, molasses and pressmud. Out of these, greater attention has been paid to molasses on account of large amounts of carbohydrates present therein and available for fermentation by various microorganisms. In India cane molasses is mainly used for fermentative production of ethyl alcohol. However, its exploitation for production of industrially important products like food, pharmaceutical and bakers yeast, citric acid, lactic acid, vitamins and amino acids etc. has been markedly hampered on account of the deleterious effects of varied types of organic and inorganic impurities present in molasses on respective microorganisms. Preliminary studies
in our laboratory showed that this kojic acid producing fungus, \textit{A. flavus} had a comparatively greater tolerance to the impurities present in cane molasses than the citric acid producing organism, \textit{A. niger} and therefore cane molasses could be easily used for the production of kojic acid with little or no initial clarification or purification. It was felt that pretreatment of molasses and its supplementation with yeast extract and some salts may further enhance kojic acid production. Hence present studies were done using different concentrations of unclarified molasses as well as molasses clarified by different chemical and physical methods in the absence and presence of yeast extract and salts. It has also been found that resuspended mycelia of \textit{Aspergillus flavus} could produce large amounts of kojic acid in a simple resuspension medium comprising of phosphate buffer and sucrose or glucose. Such resuspension media not only economise on sugar consumption towards fungal growth but also help in investigating some fundamental metabolic pathways. It was therefore tempting to study if a similar fungal mycelium grown in yeast extract sucrose medium could be successfully resuspended in a molasses medium and still produce kojic acid and whether addition of 1\% yeast extract and some salts to the molasses resuspension medium exerted stimulatory effect on kojic acid production. Hence, the present investigation would also lead to the development of an economically viable process for the
manufacture of kojic acid from cane molasses. Kojic acid may be put to several uses in industry like in tanning industry, in the preparation of metal chelates, in resin formation, as an analytical reagent for iron gold, zinc and vanadium, in the preparation of derivatives for manufacturing insecticides, fungicides, dentifrice, bronchiodilators, and antibiotics, in the field of synthetic dyestuffs and drugs especially local anaesthetics particularly of the \( \beta \text{eucain} \) type.\(^6\text{ to }15\)