

SECTION - A

**INTRODUCTION**

## INTRODUCTION

Well over 150 species are known to belong to the genus Aspergillus of the family Moniliaceae, order Hyphomycetales in the class of Fungi Imperfecti (Deuteromycetes). Not all these species have been studied in detail. Those which have been the subject of intensive research are species of Aspergillus niger, A.oryzae, A.fumigatus, A.flavus, A.nidulans, A.parasiticus etc.

Aspergilli are capable of proliferating on almost any conceivable substrate. This has been exploited by man for converting unimportant and apparently useless substances into beneficial ones of his choice. They have thus played a pivotal role in the realm of biotechnology by providing man with a sophisticated and powerful tool. Its use as a source of gallic acid production records back to 1867 when Van Tieghem in his classical research first discovered the species of Aspergillus niger. Many species of Aspergillus are now being used for the biosynthesis of high quality fungal-mass, enzymes, organic acids, carbohydrates, alkaloids, vitamins etc. (Gray, 1959; Raper & Fennell, 1965). This fungus is of such great interest that hundreds of scientific papers are published every year on its various aspects.

Carter and Phillips (1944) discovered that enormous quantities of proteins of a nutritive value equivalent to animal protein can be produced by growing yeast on a source of sugar and inexpensive mineral salts. This triggered interest in the production of protein from cheap sources and Yin (1949) suggested 'microbial farming' to fill in the protein gap which then existed.

## 1. METABOLITES OF THE GENUS ASPERGILLUS

A number of metabolites have been isolated from this genus, and they include proteins, organic acids, toxins etc. A brief review of these metabolites is given below:-

### 1.1. PROTEINS

Proteins isolated from Aspergillus are broadly divided into:

1.1.1. Biologically active proteins.

1.1.2. Feed or food proteins.

**1.1.1. Biologically active proteins** - Among this group, enzymes are significant and play an important role. The enzymes isolated during the past six years have been recently reviewed (Ali & Zaidi, 1983) citing over 700 references. The enzymes synthesized by A.niger alone are well over 100. Most of these are of industrial importance and are used in bating of hides (Panneerselvam & Dhar 1980; Sharma & Sharma 1980), soy-sauce synthesis (Anon., 1982), fruit juice industries (Chung, 1978), dairy industries (Guenther & Buerger, 1982), brewing industries (Stentebjerg-Olesen, 1980) and meat/poultry products (Anon., 1980) etc.

A number of proteolytic enzymes have been isolated from this fungus. However only three are reported to be site specific e.g., aspartate protease, serine protease, leucine amino peptidase. Of the proteins, enzymes have been studied in great detail. The role of other proteins in biological process has not received much attention. Interesting studies reported recently are the isolation of glycoproteins from A.terreus and A.oryzae having high molecular weights that inhibit plaque formation of teeth (Akita 1982) and exhibit antitumour property (Anon., 1981) respectively. The anti-tumour glycoprotein from A.oryzae with a molecular weight

of 20,000-100,000 has been patented by a Japanese firm (Wakamoto Co.Ltd.) in 1981. Also reported in 1982 is the isolation of a haemagglutinin from A.flavus by a hot water extraction of mycelial cell, one of the agglutinins was found to be specific to blood group A (Zeringue et al.,1982).

**1.1.2. Food or Feed Proteins:** Fungal proteins produced by Aspergillus are found to have high nutritional values and are easily digested by animals. Different species of Aspergillus have been grown on various industrial and agricultural wastes for manufacturing fungal mass (Moo-Young & Robinson, 1981). Some of those successfully used are described below:

**Bagasse:** Srinivasan and Miller (1981) used bagasse as a substrate for the growth of Aspergillus terreus for producing biomass. Later Garg and Neela Kantan (1981,a) produced fungal mass from 1% alkali-treated bagasse for the same species at pH 4, and recovered 11.2% protein. By autoclaving bagasse with 40% alkali for 30 minutes, the protein content was raised to 19.9%. Garg and Neela-Kantan (1982) found that a 1% concentration of substrate resulted in a much higher yield of protein - as high as 60%. Whereas when A.sydowi was grown under similar conditions it gave 2.4% protein in untreated and 5.3% protein in 40% alkali treated bagasse.

**Molasses:** Karkalin (1966) used cane-molasses as a substrate for the growth of A.niger and reported a yield of 30% protein. Garcha et al., (1973) studied the growth of Aspergillus on molasses and obtained 33.7% yield of protein with high amount of some essential amino acids e.g. Trp, Lys, Met. Nandi and Mishra (1972) showed that de-ionised molasses was a better substrate for fungal-mass as A.niger converted sugar at a much faster rate.

**Straw, bran and husk:** Babirskaya and Stakheev (1981) used agricultural residues such as rye-straw (1.5%) supplemented by mineral salts for

production of protein rich fungal mass giving 20% yield. The protein contained major quantities of Asp, Glu, Cys and Val. Kobayashi & Koizumi (1982) studied in detail the growth of A.oryzae for the production of high protein fungal mass. The protein isolated can be used as animal feed. Kobayashi (1982) formulated another high protein feed from rice husk and bran.

Potato-processing-waste: Roger and Coleman (1974) utilized starch processing waste and whole potato waste for the growth of A.niger to produce fungal-mass having 37% content of protein. The amino acid composition and feeding assay showed it to be a high quality protein that could be used for animal feed. Senez (1979) used a mixture of potato waste, Cassava flour and banana refuse in solid state fermentation for A.niger and achieved 20% protein. Feeding experiments on rat and chick showed that the fungal-mass was highly nutritive.

Carob-waste: Imirie et al., (1975) have used coarsely ground carob-pod supplemented with minerals for A.niger to yield 45% fungal-mass with average protein content of 35%.

Palm-oil-processing-waste: Barker et al., (1981) reported the production of fungal mass of A.oryzae grown on palm oil processing waste. The protein obtained was 32.1% with sulphur containing amino acids as much as 2.8g/16gN and essential amino acid index of 87.4/100. High digestibility-0.96, NPU-0.65 and biological value of 0.68 were obtained for rats. Their later improvement increased the protein content to 40% having BOD and COD as 85 and 80% respectively.

Fish-processing-waste-water: Yoshida and Hoshii (1980) employed fish processing-waste-water, a bye product from fish processing industry, as a satisfactory substrate for the growth of A.tamarii.

**Cheese-whey-waste:** Foda (1981) has used whey from buffalo milk, sweet rennet and salted whey containing 15% NaCl for production of fungal protein using A.niger, A.ochraceus, A.flavus and A.terreus. The fungal-mass exhibited high proteolytic activity.

**Cassava-waste:** De-lamo & De-Menezes (1979) found that A.oryzae amongst a number of Aspergillus species was most successful for bioconversion of cassava waste and produced 41% fungal protein having a COD value of 80%.

**Soy-bean-waste:** Yoshida & Hoshii (1980) obtained a non-toxic biomass of high protein content by growing A.oryzae on cooked soy-bean waste.

**Mango-stone-waste:** Sethi and Grianger (1981) have demonstrated the growth of A.niger on steam gelatinized mineral supplemented mango-stone powder. The fungal mass was found to contain 17.3% protein and was suitable for animal feed.

**Banana-cabbage-waste:** Sethi and Grianger (1980) have used rejected banana in solid state fermentation by A.niger for the production of animal feed with high protein (250 mg/50 g) content. Lorbert-Stephen (1978) used banana pulp for growth of A.fumigatus. Cabbage waste together with organic waste from heavy industry also proved to be a good substrate for Aspergilli (Anon.,l. 1981).

## 1.2 ORGANIC ACIDS:

Many organic acids such as citric, gluconic, kojic, gallic, itaconic retronc etc., are produced by various species of Aspergillus. Some of these acids such as citric, kojic, gluconic, itaconic etc., are of great industrial importance and are produced on a large scale for use in pharmaceutical, food, plastic and glass industries. A few of these important organic acids are briefly described below:

**Citric acid:** Citric acid is produced by many species of Aspergillus. However, A.niger is the species most widely used for the manufacture of citric acid (Porges, 1932; Prescott & Dunn, 1940; Vaija et al., 1982). This acid is synthesized by many strains of A.niger (Thom & Curie, 1916; Macris, 1975; Pessoadé et al., 1982) and a number of patents have been filed for its industrial production (Leopold, 1967, 1981; Clark & Lentz 1963; Chaudhary et al., 1978; Gulova et al., 1982; Edourd, 1982).

**Gluconic acid:** Elnaghy (1981) has examined 79 species of Aspergillus for the production of gluconic acid of which 15 showed good production of the acid. A.carneus produced the highest amount. Gluconic acid has also been obtained from A.niger (Henrich, & Rehm, 1982; Takamatsu, 1981).

**Kojic acid:** The production of kojic acid has been reported by Bajpai et al., (1981, 1982), Challenger et al., (1929, 1931) etc. Elnaghy and Elkatny (1981) used many species of Aspergillus and found A.flavus, A.parasiticus and A.terricola to produce good yield of the acid. A.parasiticus gave the maximum yield with a sugar conversion rate of 64.7%. A good yield of kojic acid was produced by fermenting A.flavus on YES medium (yeast-extract-sucrose medium) with phosphate buffer of pH 6.5 for 10 days using 20% sucrose. It has been patented by Sensi Pharmaceutical Co. (1980) and others.

**Other acids:** Itaconic acid was reported to be produced by species of Aspergillus. A.terreus was found to give better yields (Rychtera, 1981; Luke et al., 1978). A.terreus is also known to convert cis-aconitic acid into itaconic acid (Ranzi et al., 1981) and to produce terrecyclic acid which is used as an antibiotic (Nakagawa et al., 1982). Gallic acid was obtained on industrial scale by culturing A.niger on plant raw material (Beridze et al., 1981). Zamir (1980) has recently reviewed penicillic acid synthesis by Aspergillus. Sulfa aminopenicillanic acid has been

patented by a Japanese Pharmaceutical Co. (Fuji Sawa, 1981) which is used as an antibiotic.

Anke et al., (1980) have isolated tetronic acid and its six derivatives from A.panamensis (CBS 120.45) grown on a medium having high carbon and low nitrogen content. They exhibited antibacterial activity towards Gram positive and negative organisms. Five out of six derivatives of the acid inhibited the synthesis of macro-molecules in cell of the ascitic form of Ehrlich's Carcinoma of mice. Yamamoto et al.,(1980) demonstrated the inhibitory effect of the acid on Ehrlich ascites carcinoma cell in-vitro and in-vivo.

### 1.3. TOXINS:

A large number of mycotoxins are produced by species of Aspergillus (Gallagher, 1979; Davis, 1981; Palten, 1981; Glinsukon, 1982). Singh and Sinha (1982) evaluated the toxins and showed that some species like A.ochraceus, A.flavus and A.parasiticus are poisonous whereas A.niger is mildly toxic and A.oryzae and A.candidus are non-toxic. Wood smoke, sorbic acid and cinnamic acid reduced the biosynthesis of toxins in food (Uraih, 1982; Chipley, 1981; Davis 1982). Yokotsuka (1981) has discussed the risks of these toxins in fermented food. Some of these mycotoxins recently reported from Aspergillus are given in Table I.

Table-1 Mycotoxins of *Aspergillus*

Name of the toxin	Name of the species	Toxic effect on	Related information	References
Aflatoxins	<u>A. flavus</u>	Cotton seed	Seeds show poor germination	Klich & Lee (1982)
Aflatoxin B1	<u>A. flavus</u>	rice-hull	dehulling prior to storage increases toxins	Linova et al., (1982)
Aflatoxin (overufin)	<u>A. flavus</u>	-	acetate as a C source, high rate of TCA cycle reduces toxin synthesis	Simpson et al., (1982) Shantha & Murthy (1981)
Aflatoxins	<u>A. flavus</u>	ground nut	peanut toxicity	Cohen (1980)
Aflatoxin	<u>A. flavus</u>	popcorn	greenish yellow sporulation	Mc-Million et al., (1982)
Aflatoxins	<u>A. flavus</u>	pea nut and melon	effect of Zn <sup>+2</sup> on toxin accumulation	Obidoa Ndubusi (1981)
Aflatoxin B1	<u>A. flavus</u> & <u>A. parasiticus</u>	brown rice	sporulation & toxin formation relationship	Ilag & Juliano (1982)
Aflatoxin B and G1	<u>A. parasiticus</u>	reduces biomass	effect of rubratoxin of penicillium	Moss & Badri (1982)
Aflatoxin B1	<u>A. flavus</u> & <u>A. parasiticus</u>	fruits (Mosambi)	effect of constituent of substrate	Singh & Sinha (1982)
Aflatoxins B & G	<u>A. parasiticus</u>	-	lignocellulytic fungi correlation	Utrej et al., (1982)
Versicolorne	<u>A. versicolor</u>	-	an anthra quinone derivative	Berger-Berger (1982)

Versicolorne	<u>A.ustus</u>	-	4 types of xanthenes, excluding versicolorine	Horak, et al., (1982)
Viomellein and xanthomegnin	<u>A.ochraceus</u>	-	quinones	Robbers et al., (1978)
Cytochalasins E.K.	<u>A.civatus</u>	toxic to animals	-	Steyn (1982)
Citrunin	<u>A.terreus</u>	toxic to animals	fungal quinone	Sankowa (1981)
Stellatin	<u>A.varicolor</u>	-	a dihydro isocoumarin	Simpson (1978)
Orlandin	<u>A.niger</u>	phytotoxic	non toxic to animal	Cutler et al., (1979).
Oxaline, neoxaline	<u>A.japonicus</u>	-	have similar effect	Konda et al., (1980)
Ochratoxin	<u>A.ochraceus</u>	feed	ammoniation detoxify the compd.	Chelkowski (1982)
Candidusin A,B	<u>A.candidus</u>	sea-urchin embryo	inhibit DNA, RNA synthesis, but not of protein	Kabayshi et al., (1982)
Azonalenin	<u>A.zonatus</u>	sea-urchin embryo	-	Kimura et al., (1982)
Mentilactone A.	<u>A.mentii</u>	LD 50 of 7 mg/kg on 1 day chick	a norditerpenoid lactone	Dorner et al., (1980)
Terretonin	<u>A.terreus</u>	-	polyketide-terpenoid	Meintyre & Simpson (1981)
Austalides A-E	<u>A.ustus</u>	animals	heterocyclic rings	Horak (1981)
Sterigmatocystin	<u>Aspergillus</u>	carcinogenic	-	Davic (1981)

#### 1.4 ALKALOIDS:

A number of alkaloids have been reported to be produced by Aspergillus (Krugiaya, 1981; Steyn et al., 1981). The one that have been studied in detail or where biosynthesis of this has been achieved is a solasonine which is produced by A.niger by converting solamargine (Regerat & Pourrat, 1981) and the other alkaloid (ergot) which has been produced by A.fumigatus (Narayan & Rao,1981;1982).

#### 1.5. MISCELLANEOUS

Species of Aspergillus are also known to produce other metabolites such a carbohydrates (Nordin & Bobbit, 1982; Bobbit & Nordin, 1978, 1982; Gomes et al., 1981; Barreto-Bergter, 1980; Matsuyama at el., 1982), steroids (Shapiro & Gealt, 1982), steroids (Mukerji et al., 1982), alcohols (Park & Rivera, 1982; Fogaty, Soni et al.,1982) and vitamins (Kole et al., 1979; Parry, and Naido, 1980; Suzuki, et al.,1973; Redchits, 1972). However these have not been studied in much detail.

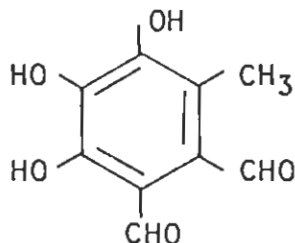
## 2. METABOLITES OF ASPERGILLUS FLAVIPES

Aspergillus flavipes grows in soils of tropical and humid areas (Raper & Fennel, 1965). From Pakistan it was isolated from the soil showing inhibitory properties against other soil-fungi (Ghaffar & Sheikh, 1982). The fungus produces colonies which are white or silvery white in color changing to pale to greyish buff in old cultures. The conidia are colorless, smooth, globose and 2-3  $\mu\text{m}$  in diameter. A review of literature indicates that the following metabolites are synthesised by this soil inhabiting fungus.

### 2.1. TOXINS:

As the great therapeutic value of certain toxins became apparent, one line of investigation was devoted to isolate, purify and identify them with the view of ultimately discovering the pathway for their biosynthesis. The following are some of the important toxins:

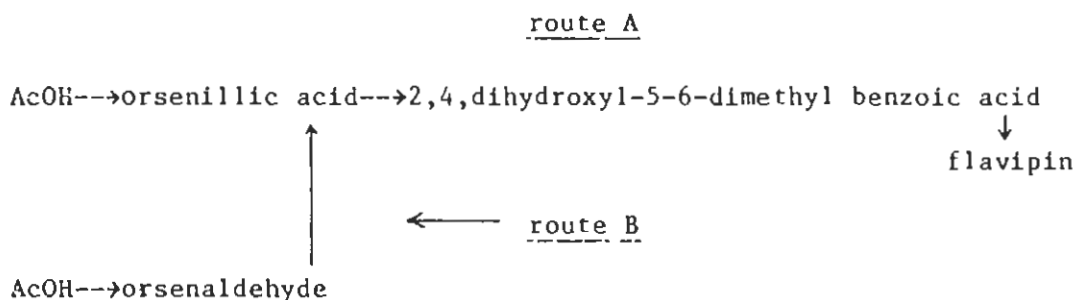
Flavipin: ( $\text{C}_9\text{H}_8\text{O}_5$ ) Raistrick and Rudman (1956) isolated flavipin from the filtrate of A. flavipes and A. terreus with ethyl acetate as yellow crystalline needles M.P. 223-4°C. It was identified as substituted pyrogallol and was found to be a powerful antifungal agent. Its structural formula is as follows:



The pathway of the biosynthesis of flavipin was studied in detail (Gosta, 1965) by using tracer techniques. It was demonstrated that the aromatic nucleus and the two formyl groups are derived from an acetate

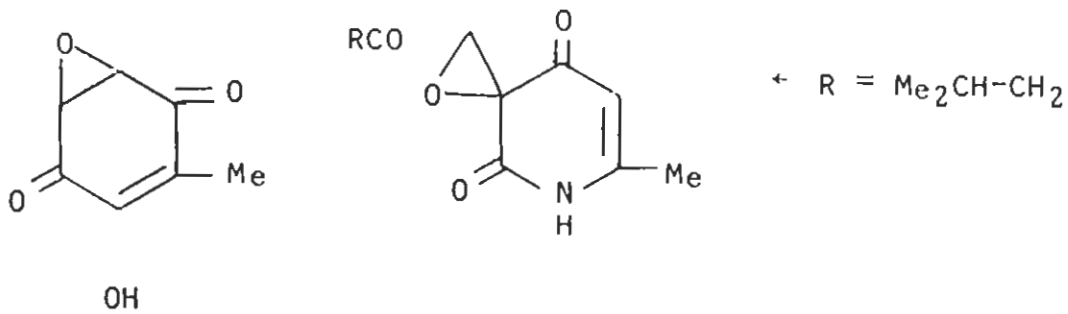
molecule, while the methyl group is transferred from  $C_1$  pool, the radioactive  $^{14}C$  of the methyl group of methionine being the sole source of  $CH_3$  group of flavipin.

The same year Gosta (1965) was able to demonstrate that A.flavipes culture had the enzyme system which could transfer a methyl group in the aromatic rings from  $C_1$  pool. He isolated the following four compounds from the A.flavipes fermentation: (i) orsenillic acid (ii) 2,4-dihydroxy-5,6-dimethyl benzoic acid (iii) orsenaldehyde and (iv) 2,4-dihydroxy-5,6-dimethyl benzaldehyde. He proposed the following two alternative pathways to flavipin using  $^{14}C$  in methyl and acetyl groups:

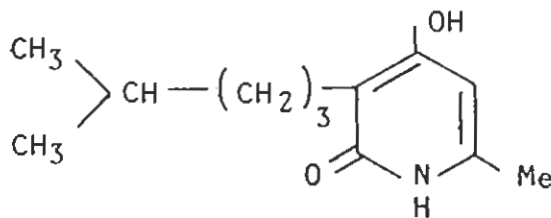


**C-methylation of aromatic compound:** After four years of discovery of transformation of  $CH_3$  group from  $C_1$  pool to benzene ring, Gatenbeck (1969) was able to isolate the enzyme fraction from cell-free-broth of A.flavipes which successfully produced 5-methylo-sellinic acid in the presence of 5-adenosyl-methionine, the later was the donor of methyl group. Thus the presence of methyl group was confirmed.

**Flavipucine:** Findlay and Radics (1972) isolated a pyridine base antibiotic, flavipucine and its isomer from A.flavipes and they identified the compound as [3'-isovaleryl-6-methyl-pyridine-3-spiro-2-oxirane-2(1H), 4(3H)-dione] or flavipucine. They also found terreic acid in A. flavipes cultures. All the three products exhibit antibacterial properties.



Findlay and Kuan (1972) further proved the structure of flavipucine by preparing a derivative 4-hydroxy-6-methyl-3-(4 methyl pentyl)-2-pyridone

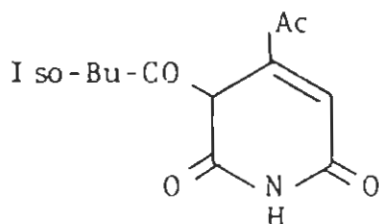


The compound was also a condensation product of di-ethyl (4,methyl-pentyl) malonate and ethyl-  $\beta$ -amino crotonate.

Girota, et al., (1976) gave a complete synthesis of  $\pm$  flavipucine by sequential condensation of pyridone and Me<sub>2</sub>-CH-CH<sub>2</sub>-CO-CHO. The product was acetylated and treated with HOAc to yield 55% of flavipucine.

**Glutamycin:** Casinovi et al., (1968,1969) while searching for new antibiotics, obtained glutamycin by growing A.flavipes on a synthetic fermentation medium at 22°C. The compound was extracted with CHCl<sub>3</sub> and purified by preparative chromatography on Al<sub>2</sub>O<sub>3</sub> using chloroform:ethyl acetate mixture (8:2) as the eluent. It was crystallised from benzene-

ligroin, gave MP 130-1°C and was assigned the following structure:



The structure was further confirmed by hydrogenating it over  $\text{PtO}_2$  in  $\text{AcOH}$  (MP 189-90°C). The acetylation in presence of  $\text{EtOH}$  and  $\text{EtOAc}$  gave a derivative having MP 175°C. Glutamycin was found to be highly active against gram +ve and -ve microorganisms.

Another glutamycine resembling antibiotic was obtained from A. flavipes grown on a synthetic medium containing sucrose, amino acids and inorganic salt in the following proportions (Casinovi et al., 1968).

Sucrose 100 g,  $\text{Ca}(\text{NO}_3)_2$  1 g,  $\text{KH}_2\text{PO}_4$  0.25 g,

L-asparagine 10 g,  $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$  .25 g,  $\text{KCl}$  0.125 g,

L-cystine  $\text{HCl}$  .01 g,  $\text{FeSO}_4$  .33 g,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  .027 g

Yeast extract .1 g and 3 drops of the solution containing

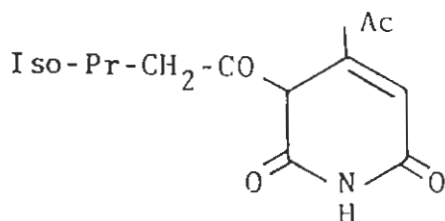
$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$  2 g,  $\text{KI}$  .5 g,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  .059 g,  $\text{CoCl}_2$

$\text{H}_2\text{O}$  0.05 g,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  .05 and  $\text{H}_3\text{BO}_3$  0.05g 1 ml of

$\text{H}_2\text{SO}_4$  and distilled water to 1 L.

The antibiotic had MP 130-1°C and  $[\alpha]_D^{21} = 71.8^\circ$  in 95% ethanol.

The following structural formula was suggested for it:



It also exhibited inhibitory activity against gram +ve and gram -ve bacteria. This antibiotic was, however, inactive against fungi.

**Geodine-like antibiotic:** Delmotte et al., (1956) obtained yellow colored crystalline hard needles having MP 229-30°C by absorbing the culture of A.flavipes on charcoal and eluting it with ethyl ether. It was purified by sublimation at 175°C (3 mm Hg). It was insoluble in water and highly soluble in chloroform,  $[\alpha]_D^{20} + 175^\circ$  (CHCl<sub>3</sub>) with MW of 198. Its biological activities differed from geodine as it exhibited no activity against E.coli whereas geodine was highly active. Geodine with Cl exhibits greater activity against Micrococcus pyogene and Bacillus subtilis in comparison to geodine.

**Nitrosamine precursors:** Using Gas chromatography and mass spectrometry Xin et al., (1979) isolated and identified four precursors of nitrosamines from corn bread inoculated with common fungi including A.terreus and A.flavipes in food. These precursors were produced after an incubation period of 8 days and identified to be dimethyl nitrosamine (DMNA), diethylnitrosamine (DENA), methyl benzyl nitrosamine (MBNA), and N-1-methyl-acetonyl-N-3-methylbutyl nitrosamine (MAMBNA), the later was a newly discovered derivative of nitrosamine.

## 2.2. RED-TOXIC PIGMENT:

A. flavipes when grown on Raulin-Thom medium produced a brownish red pigment in the culture filtrate (Jayaraman Shungasundaram, 1972). The pigment was purified by column chromatography and was toxic to insect larvae of rice (Corcyra cephalonica) even if present in minute quantity such as (500 µg/ml) in diet.

## 2.3. ALCOHOL AND ESTER:

Clarke et al. (1977) have reported the isolation of two novel metabolic products of A.flavipes from culture filtrate and mycelial cells.

They are: (A) N-benzoyl-L-phenyl-alanine (B) (S)-N-benzoyl-phenyl-alanine-S-2-benzamido-3-phenyl-propyl ester.

#### 2.4. ENZYMES:

Only two enzymes have been isolated from A.flavipes. Their biological activity has been studied:

(1) **Neutral proteinase:** Angilov et al.,(1977) have reported the isolation and purification of a neutral proteinase from A.flavipes with a molecular weight of  $29500 \pm 3200$  on sephadex G-200. The SDS gel electrophoresis showed it to be of  $27700 \pm 1700$ . They reported it to hydrolyse fibrin.

Recently Beshkova and Beshkova et al.,(1981) purified the enzyme by affinity chromatography. They studied the optimum conditions for coordination immobilization of the enzyme on a styrene-maleic acid copolymer using  $Cr^{+3}$ . The optimal ratio of the copolymer and enzyme was found to be 5:1 and optimum pH for immobilization was 7.4. It maintained 25% of its original activity.

(2) **L-galactosidase:** Suzuki et al.,(1975) have patented a preparation of L-galactosidase using a synthetic medium containing peptone, meat extract and inorganic salts. The preparation was reported to have a high enzymic activity of 4748 unit/g.

#### 2.5. OXIDATION OF ORGANIC COMPOUNDS BY A.FLAVIPES:

Certain microbes produce complex multiple enzyme systems which oxidise a variety of organic substances more quickly than does chemical oxidation. Such an enzyme system was found in A.flavipes also, though it has not been thoroughly studied. Oxidation reactions using A.flavipes cultures are listed below.

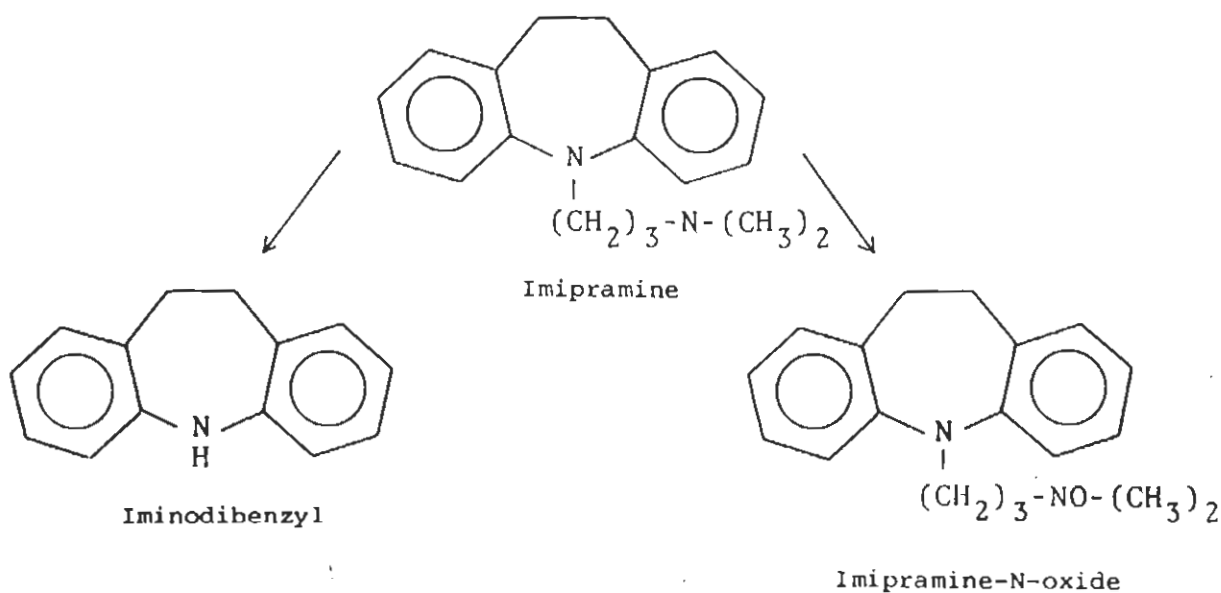
(a) **Oxidation of Steroids:** Joseph, et al., (1953) studied the capability of certain microorganisms for steroid oxidation. They found that the multiple enzyme complex in A.flavipes culture attacked the steroid ring

at three places (i) It degraded the side chain of the molecule, (ii) It cleaved the ring D and (iii) caused dehydrogenation of ring A. So it was responsible for the simultaneous oxidation, degradation and cleavage of the steroid ring.

(b) **Oxidation of Cyclo-penta phenanthrene:** Joseph, et al., (1956) then experimented by taking a different reactant such as cyclopentaphenanthrene and hydrogenated cyclopenta phenanthrenes instead of steroids and found that the two reactants were easily oxidised to lactones by A.flavipes.

(c) **Stereoselective oxidation of the (6aR)-(-) glaucine:** Davis and Talaat (1981) while studying the resolution of (R,S)-glaucine using microbiological systems found that A.flavipes catalysed the stereoselective oxidation of (6aR)-(-) glaucine and enantiomer of (6aS)+(-) glaucine to didehydroglaucine. Thus R and S organisms exist with regard to the oxidation of aporphines to didehydroaporphines.

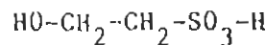
(d) **Oxidation and degradation of Imipramine side chain:** Hufford et al., (1981) have used A.flavipes for the study of biotransformations of drug imipramine, a widely used tricyclic antidepressant. The study of metabolism of imipramine using A.flavipes and other microbes was compared with human and mammalian metabolic studies. The two metabolites identified by HPLC normal system are given below:



**Taurine as sole source of sulfur:** Braun and Fromageot (1962) added taurine in the medium instead of any sulfate and found that A.flavipes possessed adaptive enzymes capable of biotransformation of taurine to isoethionic acid which later on was converted to sulfates and these were utilised further.

Pathway of microbial transformation of taurine is given below:

taurine----->isoethionic acid----->sulfate



### 2.6. Antifungal substance against A.flavipes:

Lawrence and Sui (1947) in an early study of A.flavipes demonstrated that the textile strength of the cotton cloth which was greatly decreased by fungal infection could be well protected if the fibers were impregnated with urea-formaldehyde or melamine-formaldehyde resins. His experiments showed that impregnated cloth retained 100% of the tensile strength during the 2 weeks test period, while untreated cloth lost all of its strength in less than a week. Kamal and Gupta (1973) found that Feroxone, if present in a small amount in the medium, restricted the growth of the fungus and sugar-nitrogen contents were also decreased along with the decrease in mycelial cells. Recently Tripathi and Tripathi (1980) reported that 4- $\mathcal{L}$ -naphthoquinones are toxic to A.flavipes and to many other fungal species.

### 3. Aims and objects of the present studies

Thousands of organic compounds synthesised by the members of the genus Aspergillus are the bases of numerous beneficial activities known to mankind. A review of literature dealing with Aspergilli revealed that about half of its species have yet to be investigated thoroughly. One

such species A.flavipes was, therefore, selected with a view to investigate the process of biosynthesis, isolation, purification and identification of proteins, peptides and amino acids. The biological activity of some of the proteins and peptides has also been determined.

Considerable amount of literature is available on the microbial transformation of agro-waste into fungal-mass by Aspergilli particularly with reference to their use in poultry feed etc. It therefore seemed reasonable to grow A.flavipes on a variety of waste materials. The rotten vegetable and fruits were chosen as substrates for two reasons: (i) they were not used as media by other workers in the past, (ii) waste vegetables and fruits in tropical and humid places like Karachi are available in bulk. These substrates are a constant source of environmental pollution and hazard to health with high BOD (biological-oxidation-demand) and COD (chemical-oxidation-demand) values. Therefore, if these materials could be put to profitable use, the pollution hazard will also be minimised.

The present study has the following aims and objects:

- A) to investigate proteins, peptides and amino acid etc. of A.flavipes.
- B) utilization of waste vegetables and fruits for economical production of fungal-mass.