INTRODUCTION

A large proportion of the Indian population is undernourished. The undernourishment may generally occur in respect of a number of nutritional elements but more important of these in the Indian context are calorie, iron, vitamin A and protein. Total national availability of calories, iron, vitamin A and protein from all normal natural sources, both animal and vegetable, currently entering the diets of Indian households, falls short of national requirements. The shortage on the basis of the population of 544 millions in 1970-71 and production of various food elements during this period amounts to about 400 calories per day per capita, 1535 tonnes of iron, 487902 billion of I.U. of vitamin A and 1 million tonnes of protein. This protein shortage is calculated after accounting for all exploitable potential conventional sources (1). The sufferance from malnutrition is most severe among the low income groups and in school children aged upto 6 years, constituting nearly 80 per cent of the total population.

By the year 2000, India's expected population is about 1200 millions. This will mean an increase of at least 220 per cent over the present level and the protein problem will become even more acute. The present consumption of high quality (animal) protein in India is only 0.2 million tonnes. In 2000 A.D., it will increase to 11.8 million tonnes (2). The problem is so immense that science and technology will have to be
geared to develop all possible sources of protein - conventional and non-conventional ones to save the people from acute protein malnutrition.

In India (3-7) and elsewhere research works are in progress to make the use of many "unconventional" sources like soyabean and other legumes, oil seed cakes, dried fish meal products, leaves and grasses and microorganisms, as sources of much wanted food supplements. Out of these, microorganisms appear to have distinct but not yet fully realized potentialities as future source of energy and protein for underfed people and as regenerative source of food in manned space vehicles or stations (8). The suitability of utilization of microorganisms as food material based on three advantageous factors, (i) rapid rate of proliferation of microbial cells, (ii) possibility of utilizing waste materials for cell growth and (iii) favourable economics of the fermentation technology which has undergone tremendous development during last twenty years in terms of time, labour and space (9).

MICROORGANISMS AS SOURCE OF PROTEIN

There has been considerable discussion in recent years on the possibility of using different microorganisms including algae, yeast, bacteria or molds, as food for animal and human nutrition. Many general reviews on this topic are published (9-13) in recent years.
Fresh water algae

The cultivation of autotrophic unicellular green algae as alternate food attracted popular attention long ago. The algae, particularly, chlorella could be grown in shallow tanks or ponds in liquid medium comprising of organic waste material. The yield of protein in the process may be quite high as when cultured under optimum conditions, chlorella may attain a protein content of about 50-60% on a dry weight basis. The proteins of chlorella are good sources of all essential amino acids, except that of methionine and cystine. The protein efficiency ratio (P.E.R.) of the chlorella-protein has been reported to be of same order as those of yeast and peanut (14). Studies conducted in Japan show that a considerable amount of dried chlorella could be added to green tea, soups, noodles and backed food products without affecting taste and appearances of foods thus supplemented. By feeding trials with human, it has been shown that a daily consumption of 100 gms of powdered algae was well tolerated, consumption of larger amounts, however, resulted in nausea, vomiting, abdominal distension, flatulence and bulky hard stools, these gastrointestinal symptoms disappeared shortly after the consumption of chlorella was discontinued (15). The large content of indigestable constituents limits the consumption of chlorella generally. Furthermore the cultivation of these species is restricted in the area which receives good amount of solar light and a suitable rapid and inexpensive method of harvesting the crop from large volume of culture medium is yet to be developed.
Marine algae

The enormous, self-replenishing supplies of marine algae (sea weeds) growing in oceans of the world have not yet been fully exploited directly as a source of human food. The common classification of marine algae is based on colour. Highly prolific brown (Phaeophyceae) and red (Rhodophyceae) species offer economic possibilities as food. Considerable quantities of sea weeds are consumed in the oriental countries, notably in Japan. The protein content of seaweeds ranges from 4 to 18% on dry weight basis. These proteins, however, are poorly digested and possess a low nutritive value (14, 16).

Food yeasts

This source of protein and vitamins is comparatively more acceptable than other food materials of microbial origin because of its high food value and relative freedom from toxicity. The potentialities of this source of protein and vitamins in human dietary of the developing countries have been well recognized. However production cost of food yeast is relatively high as yeasts generally require carbohydrate for growth, though it can assimilate inorganic nitrogen. According to different group of workers the presence of large amount of purine based compounds in yeast may be harmful on nutritional standpoint (17-18). Diets containing yeasts as the main source of protein were found to cause necrosis of the liver in experimental animals (19-20).
Microorganisms grown on hydrocarbons

The microbial conversion of petroleum hydrocarbons into edible proteins has been seriously considered for over 25 years. Experimental details and future developmental possibilities of this interesting plan for solving world food problem has been discussed by Champagnat (21-22). The yeasts, particularly several species of Candida readily utilize paraffinic hydrocarbons in the presence of inorganic nitrates, resulting in the formation of cells containing about 50% protein of good nutritional quality (23). Potentialities of hydrocarbon grown microbial cells as source of protein are not fully evaluated as yet. Petroleum as raw material for the production of "Petrobiochemicals" has certain advantages, the most noteworthy one is its low cost. This is particularly true for petroleum gases. Different bacteria and other microorganisms in addition to yeasts, utilize hydrocarbons and produce protein, fat or vitamins (24).

Micro-fungi and mushrooms

Like yeasts, mold too, particularly the members of the genera Aspergillus and Penicillium are capable of synthesizing proteins from inorganic salts as the source of medium nitrogen. But while these molds have been used on a large scale for obtaining fats from cheap carbohydrates, their use for the production of protein has not received much attention primarily due to inferior biological value of mold protein in comparison
to yeast proteins. However many mold species are capable of growing on cellulose or hemicellulose and they may be employed for the production of protein from mixture of fibrous waste-materials and inorganic salts (25).

Edible fungi of higher order have for long been greatly esteemed as food. The fleshy fungi, particularly mushrooms have received wide popularity due to their flavour as well as high protein, vitamins and mineral contents. Some of the most popular edible species are the common mushroom, _Agaricus campestris_; the shaggy mane, _Conrinus comatus_; the common ink cap, _C. atramentarius_; the glistening ink cap, _C. micaceus_; the oyster mushroom, _Pleurotus ostreatus_; the parasol mushroom, _Lepiota procera_; the honey agaric, _Armillaria mellea_; the velvet-stemmed mushroom, _Collybia velutipes_; the morel, _Morchella esculenta_; coral fungi and puffballs having pure white section (26).

CULTIVATION OF THE MUSHROOMS

The planned cultivation of the mushroom started in 17th century or little earlier in Europe. At initial stage the practice was to increase the production of _A. campestris_ or related fungi by the application of animal manures to the localities where the inoculating material had been strewn naturally. Subsequently the cultivation technique which involved plantation of "virgin spawn" (fungus seeds collected
in the wild) on horse manure beds (27) was developed. As the cultivation of fungi became economically very profitable, by latter half of 19th century and onwards, the large scale production was started by different industrial concern throughout the year under controlled conditions in green houses.

The composting of manure seemed to be very important in mushroom culture, so different techniques of composting started developing from 18th century onwards. The chemical and biological natures of composting have been studied in comparatively recent years. The knowledge gathered in such studies helped in developing compost of defined composition which may be supplemented with conventional manure for obtaining the products with improved properties. The most recent phase of commercial mushroom production was helped much by the study of mushroom genetics. New mushroom strains were developed from single spore isolates by mutation or selection. The strains so developed showed considerable improvement both in nutritional quality and crop yield.

PRODUCTION OF MUSHROOM MYCELIA BY SUBMERGED CULTURE

The idea of development of submerged culture methods for the production of mushroom mycelia was stimulated by the success of deep tank fermentation technique in the antibiotic field. It was Humfeld (28) who first showed that mycelial growth of certain strains of mushrooms could be achieved by this
method of cultivation. The method offered low cost production of mycelium having high nutritive value and acceptable flavour using easily available substrates. However before this, Lambert (27) and Treschew (29) had demonstrated that mycelium of at least one mushroom species i.e. A. campestris, could be grown submerged but Humfeld apparently was the first to recognize that the production of such mycelia on large scale might have commercial potentialities. It was soon realised that by similar process mycelia of many other edible species of higher fungi could be produced economically (30-45). However, it was found that not all fungi of the classes basidomycetes and ascomycetes could be cultivated in vitro (46). Curiously some of those strains which may be grown on solid media in vitro failed to grow under submerged condition. A comprehensive list of species which could be grown in submerged culture is compiled by Worgan (47). His list includes about 90 edible species, out of which Agaricus campestris, Tricholoma nudum and morels Morchella esculenta, M. crassipes and M. hortensis, are among the few which have been investigated in detail. Under submerged growth condition, on dry weight basis, highest protein containing mycelia was obtained from a culture of Tricholoma nudum (60.2%), though the total growth yield per litre of culture medium was low (47).
Characteristics of submerged growth of higher fungi

Behaviour of different species of higher fungi under submerged culture conditions are variable. Some mushrooms grow in small or large pellets while others give dispersed or milky type growth in liquid shake culture. The type and extent of agitation can influence the nature of growth and size of pellets formed. It has been suggested that in liquid medium, mycelia formed are more fine than those formed in natural fruit bodies. These fine mycelia are often fragmented into small pieces leading to the formation of secondary spores (30, 48-49). Block (30) obtained satisfactory yields of mycelium of Agaricus blazei under submerged condition only when the organism produced secondary spores and dispersed type of growth. Similar was the result of Sugihara and Humfeld (49) who showed that the yields were usually high when the growth occurred producing dispersed mycelia having secondary spores rather than when it occurred in the pellet form. The pellet forming strains were generally slow growing (50). On the other hand Szuecs (44) obtained better yield with pellet forming strain of Morchella esculenta. Addition of fine solids in the medium such as CaCO₃, CaSO₄ or cereal particles helped to increase yields. These solid particles could support the pellet formation by serving as nuclei for such process (44-45). Regarding the yield and efficiency of nutrient utilization, a wide variation was observed among different strains of same fungus, as for example, Sugihara and Humfeld (49) using a medium consisting of simple sugar, urea
and mineral salts for *A. campestris*, reported a yield of 60 gms of dried mycelium per 100 gms of sugar consumed. But Eddy (31) obtained only half of this yield from his strain even though he used nutrient-rich complex medium.

Neither Humfeld and Sugihara (50) nor Szuecs (51) could establish the growth of the fungus on conventional compost bed using the mycelial fragments obtained from submerged culture as spawns. These results indicate that the dispersed mycelia are in fact resulted from some physiological mutations. It may be mentioned here that such mutation could also be induced artificially in *A. campestris* and *Ustilago zeae* by the addition of \( \text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} \) to potato dextrose agar cultivation medium. The mutations of *A. campestris* so obtained may produce 5-7 times as much mycelium as the parent culture (52).

**NUTRITIONAL REQUIREMENTS OF HIGHER FUNGI GROWN UNDER SUBMERGED CONDITION**

**Oxygen**

The basidomycetes as a rule have highly aerobic growth habit. So *in vitro* culture of these fungi is depended very much on the oxygen supply. Different workers worked out the optimum aeration rate for the growth of mushroom mycelia under submerged conditions. Reusser, Spencer and Sallans (41) found that optimum aeration rate for the production of *T. nudum* mycelia is about 50 m moles \( \text{O}_2/\text{l}/\text{min} \). Litchfield (53) found that aeration
rate of 0.08 - 0.15 m M0₂/l/min is suitable for good flavour production by the morel mushroom mycelium, when the aeration rate exceeds 0.20 mM0₂/l/min, the growth becomes dispersed type and there is an impaired yield, with less development of flavour. Moustafa (40) obtained the best yields of A. campes-tris at an aeration rate of 13 mM0₂/l/hr.

Carbon source

Though apparently A. campestris and related species could efficiently be grown in different low cost medium formulations, they have got some definite nutritional requirements, and both yields and nutritive values of mycelial materials are dependent on medium composition. A wide variety of materials have been tried for the production of mushroom mycelium by submerged culture. These include simple carbohydrates (35, 37, 39) like glucose, galactose, mannose, fructose, maltose, xylose, arabinose, dextrin, mannitol, sucrose, lactose, soluble starch or complex one such as asparagus juice, beet and sugar cane molasses (33, 44), citrus wastes (30, 32, 34, 44), malt syrup (40), pear waste (34), pumpkin waste (38), and cheese whey (38). Litchfield, Overbeck (32) and Davidson (39) demonstrated that strains of Morchella hortensis and M. crassipes grew well in media containing glucose, lactose or maltose. The strain of M. esculenta used in these studies utilized glucose and maltose equally well but could utilize lactose with less efficiency. They also showed that yield of the mycelium depends on the
carbon to nitrogen ratio and the ratio of 8:1 in both cheese whey- and pumpkin waste-media gave better yields with all three organisms tested. Out of ten mushroom cultures investigated by Falanghe (54), only Agaricus campestris, Boletus indecisus and Tricholoma nudum grew in submerged culture in a medium containing vinasse, a waste product from the distillation of fermented cane juice, ammonium sulfate, and mineral salts. A. campestris gave better protein yield and B. indecisus greater mycelium growth. Another report from same laboratory (55) show that soybean whey medium is best for the growth and mycelial protein production by Tricholoma nudum and Boletus indecisus, whereas very little mycelial growth could be obtained in this medium from Morchella hybrida, Collybia velutipes, Cantharellus cibarius, Xylaria polymorpha and Agaricus campestris. Atacador-Ramos et al. (56) worked out the optimal conditions for the submerged growth of Volvaria volvacea, a banana mushroom which gave the highest yield of mycelia with an appreciably high protein and acceptable flavour content, amongst 5 species of mushrooms they studied. They found that sucrose, xylose, fructose and glucose were good carbon sources for this fungus.

Nitrogen source

Mushrooms are versatile in respect to their nitrogen assimilation. They can utilize different forms of nitrogen, ranging from inorganic nitrogen to complex nitrogenous compounds. Nitrogen sources utilized by mushrooms in submerged culture
included ammonium salts (39, 41, 44-45), nitrate (30, 41), urea (28, 32-35, 49), malt extract (40), corn steep liquor (39, 44-45) and yeast extract (45). The nature and amounts of nitrogen source of the medium greatly influence the yield and composition of the mycelium. Humfeld and Sugihara (50) showed that growth of A. campestris could occur even with very limited nitrogen concentration in the medium but this is reflected in the correspondingly small protein content of the mycelium produced. For the best growth, medium nitrogen necessary is 1000 mg/l. This requirement was 50-100% higher for maximum flavour development. Even in cultivation of mushroom fruit bodies on solid substrates, nitrogen sources play an important role. For example, the availability of either urea or ammonium sulphate as nitrogen compound in composting greatly affects the composition, namely the ash, urea and amino acid content of Agaricus bisporus fruit bodies (57). Aspartic acid and glutamic acid were found to be the best source of nitrogen for the cultivation of mushrooms, like Psalliota bispora, and Fomes albida, (58) nitrate was not utilised by these organisms. Similarly Hsu and Hu (59, 60) found no growth of Agaricus bisporus strain in medium containing nitrate or nitrite as the sole source of nitrogen. Ammonium nitrogen supported only limited growth but extensive growth was obtained in the synthetic medium containing urea as the sole nitrogen source.
Micronutrients

The requirements of micronutrients as growth promoters in mushroom cultivation have been worked out by different laboratories using growth substrates of defined compositions. For example, a mixture of B-vitamins when added to the horse manure substrate increased the production of fruit bodies of *Psalliota bispora*, *Fomes avellanea* and *F. albida* (58). Schisler and Patton (61) reported that ethyl linoleate could stimulate mushroom (*Agaricus bisporus*) yield. Wardle and Schisler (62) showed that addition of vegetable oils viz. linseed oil, corn oil, olive oil, peanut oil, cotton seed oil, soyabean oil, wheat germ oil, safflower oil and crude cotton seed oil; and beef and pork tallow stimulated the mycelial growth of *A. bisporus* to a considerable extent.

Detailed information regarding the micro-nutrient requirements for submerged mycelial culture of mushrooms are still lacking. Eddy (31) reported that the *Coprinus comatus* requires thiamine for its growth. Humfeld (35) did not find any increased in growth of *A. campestris* when a mixture of 12 vitamins were added to an agar medium. Coconut water appeared to contain a growth promoting factor that enhanced the yield of *Volvariella* (volvaria) *volvacea*, (banana mushroom) mycelia more than 2 folds (56) in submerged culture.
Use of Industrial waste products

The use of industrial waste materials and other available cheap sources of carbon and nitrogen for the production of edible fungus mycelia rich in protein and having acceptable flavour would be very helpful particularly to developing countries. This possibility was tested by different laboratories. The results obtained are very encouraging (Table I).

Table I

Use of industrial waste materials for submerged cultivation of mushroom mycelia

<table>
<thead>
<tr>
<th>Industrial waste used in the medium</th>
<th>Fungus grown in submerged culture</th>
<th>References</th>
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<tbody>
<tr>
<td>Citrus fruit waste</td>
<td><em>Agaricus blazei</em></td>
<td>30</td>
</tr>
<tr>
<td>Molasses</td>
<td><em>Collybia velutipes,</em></td>
<td>44, 63</td>
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<tr>
<td></td>
<td><em>Agaricus campestris,</em></td>
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<td></td>
<td><em>Xylaria polymorpha,</em></td>
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<td></td>
<td><em>Tricholoma nudum,</em></td>
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<tr>
<td></td>
<td><em>Boletus indecisus,</em></td>
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<tr>
<td></td>
<td><em>Morchella hybrida,</em></td>
<td></td>
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<tr>
<td></td>
<td><em>Cantharellus cibarius.</em></td>
<td></td>
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<tr>
<td>Sulfite waste from paper industry</td>
<td>-do-</td>
<td>41, 63</td>
</tr>
<tr>
<td>Vinassee</td>
<td><em>A. campestris,</em></td>
<td>54</td>
</tr>
<tr>
<td></td>
<td><em>B. indecisus,</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>T. nudum.</em></td>
<td></td>
</tr>
<tr>
<td>Food industry waste</td>
<td><em>Morchella hortensis,</em></td>
<td>39</td>
</tr>
<tr>
<td></td>
<td><em>M. crassipes,</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>M. esculenta.</em></td>
<td></td>
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</table>
Carbohydrates

The carbohydrate content of A. campestris fruit bodies was analysed by McConnell and Esselen (64) in 1947. Employing standard chemical method, they identified and quantitatively measured, mannitol, reducing sugars, glycogen, crude hemicellulose and furfural yielding substances in this fungus. An interesting characteristic in the composition of carbohydrate is the presence of large amount of mannitol. Hughes, Lynch and Somers (65), using chromatographic method identified following sugars and related compounds in the cultivated mushroom, A. campestris L. exFries: two pentoses (xylose and ribose), two methyl pentoses (rhamnose and fucose), three hexoses (glucose, galactose and mannose), two amino sugars (glucosamine and N-acetyl glucosamine), two sugars alcohol (mannitol and inositol), two sugar acids (galacturonic acid and glucuronic acid), an uronide of low Rf value and two unidentified methylated sugars. The constituents present in high quantities were galactose, mannitol and glucose followed by rhamnose, xylose, mannose, galacturonic acid and the unidentified uronides. Rast (66) detected trehalose in sporocaps and mannitol (which comprises 1-4% of fresh weight of the tissue) in sporulating fruit bodies of A. bisporus. Griffin, Brennan and Losel (67) demonstrated the presence of glucose, trehalose and mannitol in acetone dried powder of the mycelium of the cultivated mushroom A. bisporus. Trehalose has also been identified in Boletus edulis (68).
Besides this, D-arabitol was identified in *B. bovinus* (69) and D-threitol in *Armillaria mellea* (70).

**Proteins, amino acids and related compounds**

As it is already emphasised that mushrooms are generally consumed for food as they are rich in proteins. Robinson and Davidson (42), and Block (46) pointed out the potential value of mushroom mycelium as a source of protein for man and domestic animals. As early as 1912, Reuter (71) identified the following amino acids in *Boletus edulis* sporophores: alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, trimethyl histidine, leucine, lysine, proline, phenylalanine and valine. Fitzpatrick, Esselen and Weir (70) determined the amino acid content of mushroom *A. campestris* protein by microbiological assay and showed that it contain approximately 203, 458, 242, 144, 5 and 326 mg respectively of arginine, isoleucine, leucine, methionine, tryptophan and valine per 100 gms of fresh weight. They also showed that the total nitrogen content of the mushrooms mycelia was about 0.5% of fresh weight out of which 63% was in the form of protein. Purified mushroom protein had a nitrogen content of 11.79%. Hughes, Lynch and Somers (65) claimed that the cultivated mushroom, *A. campestris* L. exFries, contain all the eight amino acids known to be essential in human nutrition. These authors also showed that the composition of free pool amino acids in this fungus is similar to the protein amino acids.
Among the non-protein nitrogenous constituents urea is a major component of the fruiting bodies of the higher basidomycetes including those of Agaricaceae. The accumulation of urea may approach 6% of the sporophore dry weight (72-74). In fruit bodies of commercial mushroom, A. bisporus and in some Lycoperdon species urea is present in considerable amount. Reinbothe (75) suggested that urea serves as fungal nitrogen reserve and is consumed during spore formation.

Block et al. (30) performed qualitative paper chromatographic analysis of the amino acids present in mycelial proteins of Agaricus blazei grown in submerged culture which showed that these proteins contained all the essential amino acid for man but no quantitative values were recorded. Reusser, Spencer and Sallans (41) published the quantitative data on the essential amino acid composition of Tricholoma nudum cultured under submerged conditions. Litchfield, Vely and Overbeck (76) studied the amino acid composition of the protein of morel mushroom mycelium similarly cultivated. It was noted that the lysine, leucine, and tryptophan contents of M. esculenta mycelia were equivalent to or more than the contents of these amino acids prescribed for the reference protein of F.A.O. (77). The contents of isoleucine, phenylalanine plus tyrosine, threonine and valine in different morchella mycelia were slightly lower than those of F.A.O's reference protein. While the sulfur amino acid content was significantly less. Amino acid compositions of T. nudum grown by submerged culture were similar to
those of morel mycelia except for the fact that lysine, phenylalanine and tryptophan contents were somewhat higher. The deficiencies of sulfur amino acids in microbial protein is well known. To increase their biological value, microbial proteins could be supplemented with synthetic methionine, which hopefully, will be increasingly available at lower prices in the future.

**Lipid**

Very little information is at hand regarding the composition of lipids in higher fungi. Ramsbottom (78) reported that the fat contents of fresh sporophores of 10 different edible species may vary between 0.20 - 0.76 per cent. Angelo et al. found that maximum fat content of *A. bisporus* cultivated on urea and (NH₄)₂SO₄ integrated compost beds was 2.15 and 2.2% of dry weight respectively. Among the fatty acids determined, linoleic acid was most prevailing and with palmitic acid, it comprised about 90% of the total fatty acids (57). Holtz and Schisler (79) analysed the lipid components of 4 cultivated strains of *A. bisporus*. The sporophore extracts contained free sterols, free fatty acids, triglycerides, phosphatidyl choline and phosphatidyl ethanolamine. High amounts of linoleic acid were found in both neutral and polar lipid fractions. Mycelial extracts also contained large amounts of linoleic acid. Free fatty acids, triglycerides, phosphatidylcholine and phosphatidyl ethanolamine was present in mycelia but there was no free sterol. Ivanov and Bliznakova (80) showed lipid composition of
commercially grown *M. esculenta* and *Coprinus comatus* was similar to that of cotton seed oil, except for the fact that fungal lipid contained much less saturated fatty acids. The presence of heptadecanoic acid (C₁₇) in *morchella* lipid fraction is a noteworthy feature. The lipids may play an important role in flavour and aroma development in mushrooms. Leegwater, Craig and Spencer (81, 82) have shown that the triglycerides of *T. nudum* mycelium grown in submerged culture contain unsaturated fatty acids such as palmitic, palmitoleic, oleic, linoleic and linolenic acid which as they claim, may serve as precursors of flavouring constituents. Phospholipids such as phosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidic acid were identified in this fungus and it was assumed that these compounds may also serve as precursors of flavouring substances. The carbon and nitrogen ratio in the medium may influence fat production. Reduced medium nitrogen concentration induces increased fat formation in *T. nudum* (41).

**Vitamins**

Common edible mushrooms are good sources of vitamins and minerals. Mushrooms are particularly rich in B-vitamins, like riboflavin, nicotinic acid and folic acids but poor in vitamins A, D, or E (83). They also lack in vitamin B₁₂ (84). Nicotinic acid, riboflavin, pantothenic acid, biotin and thiamine of cultivated mushroom were well preserved during cooking, canning, drying and freezing operations (85). Similar results
were reported by Filios and Esselen (86) who showed that the concentration of riboflavin, nicotinic acid, pantothenic acid and biotin of canned mushrooms diminished only to small extent during storage of about 12 months. These authors concluded that commercial canned mushrooms preparations are excellent sources of B-vitamins.

Vitamin content of the mushroom mycelia including those of morel mycelia grown in submerged culture has been reported by different laboratories (30, 35, 44, 87-89). Results indicate that these mushroom mycelia are also good source of B-vitamins.

BIOLOGICAL VALUE OF MUSHROOM DIETS

Though the amount of protein present in mushrooms is high but it is biologically inferior to protein of animal origin. Reasons for this low biological value of mushroom proteins are low digestive quality and less balanced amino acid composition of these proteins on nutritional standpoints. In rat feeding experiment the digestibility of mushroom protein was found to be 56.6% (70). But by human feeding trials Lintzel (90) showed that the proteins of Psalliota campestris, Cantharellus cibarius, Boletus edulis and Morchella esculenta are approximately equivalent to muscle protein on nutritional qualities. He suggested as the digestibility of mushroom-protein was about 72-83%, 43-62 g of these or 100-200 g of dry mushroom tissue was necessary, when these were used as only dietary protein, to maintain nutritional
balance in a normal human subject weighing 70 kilograms. Pujol (91) found, using albino rat as experimental animal, that the biological value of A. campestris protein presented in the form of dried meal was about 80.4. Antal et al. (92) found significant lower growth of male rats which were maintained on mushroom diet in comparison to those maintained on the control diet containing animal proteins. They also found that total amount, protein level, and lipase and chymotrypsin activities of the pancreatic juice were decreased, whereas the serum lipase activity and the fat content of the liver were significantly increased in animals maintained on mushroom diet in comparison to those in control animals. A diffuse damage of liver was also noted in rats fed with mushroom mycelia for long time. This indicates that the rats were not able to utilize the mushroom protein effectively. Rafalski et al. (93) showed that biological value and digestibility of meadow mushroom protein is comparable to that of yeast but lower than that of gluten or beef.

FLAVOUR OF THE MUSHROOM MYCELIA

One of the formidable problems of deep-tank fermentative production of mushroom mycelium is development and maintenance of the flavour of the product similar to those of naturally occurring species. By feeding trials it has been established that mycelia obtained by submerged cultivation have the flavour of original strains but these favours are not equivalent to those of sporophore (46). The type of growth probably has some influence on flavour development as Block (46) found no characteristic
flavour development in dispersed grown mycelium of \textit{A. blazei} but the original unmutated strain which grew very slowly had an mushroom like aroma that was too detected only in the fermentation medium. Eddy (31) found that strong mushroom like flavour of \textit{C. comatus} was retained when the mycelia were harvested from the surface of a shallow liquid medium even after the growth of 3 weeks, but the same organism when grown in submerged way did not produce any flavour. Humfeld and Sugihara (50) showed that the inorganic salt concentrations of the growth media control the flavour development. For making the product acceptable to consumer, the control of flavour development in mycelia obtained by submerged culture is an important factor. Unfortunately so far little work has been done with regards to the chemical identification or elucidation of biosynthetic mechanism of flavouring constituents of mushrooms so that suitable precursors may be designed and incorporated into the fermentation medium for the enhancement of flavour of mycelia. All attempts for improving flavour by autolysis (30, 46, 49), salting (43) or cooking (30, 39, 42, 49) of mycelia obtained by submerged growth failed so far.
AIM OF THE PRESENT WORK

It is well recognized that diets consumed by the low-income groups in developing countries like India do not provide adequate amounts of protein of high nutritive value, and that consequently protein malnutrition is widely prevalent. The problem of utilization of available additional sources of proteins for overcoming protein deficiency in the diets of Indian people has engaged the attention of food scientists of this country during the past decade. The present study was undertaken to develop a suitable strain of *A. campestris* for submerged culture. The nutritional requirements of the strain so developed for maximum protein production were studied. The possibility of utilization of *A. campestris* mycelia as additional source of protein was also investigated.

The selection of the organism, *A. campestris* for development of fermentative protein production method is based mainly on two reasons: (i) A good amount of technical data is available regarding the submerged cultivation of this organism. The most of the work in this respect was carried out by Humfeld and Shugihara, the pioneers of this field. Unfortunately in India, no attempt was made so far to make use of this potentially useful source of protein. (ii) At least a section of Indian population is familiar with the taste and nutritive value of this fungus.

As indicated above the most of the works published on submerged production of *A. campestris* mycelia were based on the
strains developed by Humfeld. The strains were latter on designated as NRRL 2334, 2335 and 2336. However in 1962, Molitori's questioned the identity of these strains. According to his analysis, these strains were Beauveria tenella and not A. campestris (94-96). The controversies relating the basic identity of the strain cast doubts on the validity of previously published data relating the nutritional pattern of A. campestris and induced the need for a restudy of this subject by developing a new in vitro culture of the fungus after proper verification of its identity.

In this dissertation, however for sake of convenience, authors' identification of strains are retained while reviewing the literature.