SOIL MYCOBIOTA: ROLE AND IMPORTANCE

Garrett (1950) surveyed upon ecological groups of soil fungi and substrate relationships. Accordingly, an ecological group of soil fungi was defined as an assemblage of species characterized by some peculiar advantage for pioneer colonization of a particular substrate. Five such ecological groups have been indicated as examples, viz. saprophytic fungi, root inhabiting fungi, lignin-decomposing fungi, coprophilous fungi, and predaceous fungi. The saprophytic fungi, typically exemplified by the Phycomycetes, are pioneer colonizers of injured, moribund and dead plant and animal tissues, for which role they are ecologically equipped by an exceptionally high growth rate, and especially by a capacity for rapid germination of resting spores and mycelial cells. A rapid ‘flare up' in the presence of a suitable substrate, followed by a period of quiescence, appears to be characteristic of such fungi, and it is suggested that the substrate comes to the dormant fungus at least as often as the active fungus makes contact with fresh substrates by mycelial growth through the soil.

Coleman (2001) discussed on the soil biota, soil systems and major soil processes occurring in those ecosystems. The review describes how soils act as components of ecosystems, their role as organizing centers in ecosystems, various major soil processes and biodiversity in soils. It is apparent that a large proportion of the biota associated with soils are as yet undescribed, with the most extreme cases being the bacteria and fungi. Therefore, it is premature to give even a rough estimate of the total numbers of species that occur in many of these taxa, as such large percentages of the total number of organisms are still unknown. It is incumbent on the rising generation of ecologists and biologists to develop more innovative ways to describe, catalog, and understand the myriad patterns and processes in the biosphere, which are due in large part to the actions of the biota.

Cowan (2001) made a review on fungi as life support for ecosystems. Fungi are fundamental to the success and health of almost every ecosystem on the earth, both terrestrial and aquatic, and essential to the sustainability of biodiversity. Fungi are perhaps the most
unappreciated, undervalued and unexplained organisms on earth. They perform a crucial role in the transport, storage, release and recycling of nutrients. Despite their central role in ecosystems and their applications in biotechnology, knowledge about fungi remains at a low level. It has been estimated that only 5% of the World's fungi have so far been discovered, and for most of these, little is known about their biology. However, how often do we consider their existence within a habitat? Let alone how conditions could be improved by active encouragement and management of the fungal diversity?

Jenkins (2005) describes soil fungi as microscopic plant-like cells that grow in long threadlike structures or hyphae that make a mass called mycelium. The mycelium absorbs nutrients from the roots it has colonised, surface organic matter or the soil. It produces special hyphae that create the reproductive spores. Some fungi are single celled (e.g. yeast). Fungi have many different structures but they can act in similar ways and thus are not as plant specific in their needs as some soil bacteria such as Rhizobia. There are three functional groups of fungi: Pathogens, Mutualists and Decomposers that they perform important functions within the soil in relation to nutrient cycling, disease suppression and water dynamics, all of which help plants become healthier and more vigorous.

SOIL POLLUTION AND ITS IMPACT ON MICROBIOTA AND HUMAN HEALTH

The State of Environment (SoE) report of Tamilnadu (2005) provides a general picture of the state of the bio- physical and socio-economic condition and an understanding of how human activities affect the environmental conditions and its implications on human health and economic well being. The report helps in revealing the data regarding industrialization rates, solid waste disposal in major cities, sewage pollution, pollution load due to industries, etc. in Tamilnadu as well as the role of TNPCB in creating environmental awareness depicting various scenarios on waste management practices in the state.

Larsson et al (2007) reported that the effluent from drug manufactures contains extremely high levels of pharmaceuticals. The pharmaceuticals were analysed in the effluent from a wastewater treatment plant serving about 90 bulk drug manufacturers in Patancheru, near Hyderabad, India - a major production site of generic drugs for the world market. The samples contained by far the highest levels of pharmaceuticals reported in any effluent. The high levels of
several broad-spectrum antibiotics raise concerns about resistance development. The concentration of the most abundant drug, ciprofloxacin (up to 31,000 µg/l) exceeds levels toxic to some bacteria by over 1000-fold. The results from the present study call for an increased focus on the potential release of active pharmaceutical ingredients from production facilities in different regions.

Chandramohan and Bharathi (2009) investigated about Pallikaranai Marsh Land, an ecologically sensitive area with various natural habitats that has been gradually polluted and degraded due to dumping and burning of garbage and letting out of untreated or treated sewage water. This had led to deterioration of water quality causing eutrophication, in turn to water pollution affecting biological and chemical processes within wetlands. Also, an irreversible damage has been caused to the quality of subsurface water accompanied by pungent foul smell and colour change that had wiped out the vegetation, driven out small water fowls, local migrants, flora and microorganisms along the area. Burning of garbage has produced 27 toxic gases causing air pollution that altogether these pose major threats for human health leading to ill hazards likely of another Bhopal gas tragedy.

Saraswathy et al (2010) analysed the speciation and determination of heavy metals in polluted soils of Ranipet industrial area in India. Heavy metals are important environmental pollutants, particularly in areas with high anthropogenic pressure. Trace amounts of these in atmosphere, soil and water can cause serious problems to all organisms and heavy metal bioaccumulation in the food chain altering the normal biogeochemical cycling. Several chemical speciation and fractionation methods for heavy metal analysis in soils have been and are still being developed and applied. Total metal concentration is not sufficient to assess the environmental impact of polluted soils since heavy metals may have different chemical forms and only a fraction can be remobilized easily. The use of sequential extraction procedures of soil analysis may help in the prediction of trace elements mobility, bioavailability and fate of the metal contaminant. Their study has been discussed on the different fractions (Speciation) of the heavy metals in the soil sample, particularly, Cobalt (Co), Cadmium (Cd), Lead (Pb) and Zinc (Zn) which is a good compromise method that gives information on the environmental risk.

Rabah and Ibrahim (2010) observed changes in microbial community content as well as physico-chemical properties of soil contaminated with tannery effluents in Sokoto metropolis.
that were determined using standard procedures. The results showed that the soil sample contained a variety of microorganisms which include *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens, Escherichia coli, Streptococcus pyogenes, Klebsiella pneumoniae, A. niger, A. flavus, A. fumigatus, Penicillium notatum, Mucor pusillus* and *Fusarium sporotrichioides*. It also revealed high counts of bacteria and fungi in all the sampling sites. The viable count of bacteria was in the range of 8.60±1.80 – 8.70±0.52 ×105 cfu/g while that of fungi was 1.70±0.30 – 2.0±0.10 × 104 cfu/g. Similarly, it revealed high levels of Sulphide (0.35-0.44 mg/g), Ammonia (0.40-0.60 mg/g), and Chromium (Cr) (0.20-0.26 mg/g) in all the sampling sites. These levels exceeded the tolerable levels set by the Federal Ministry of Environment. The presence of these microorganisms and chemical substances pose a potential threat to the local inhabitants of these areas.

**ISOLATION OF SOIL SAPROPHYTIC FUNGI**

Ottow and Glathe (1968) presented rose bengal-malt extract-agar, a simple medium for the simultaneous isolation and enumeration of fungi and actinomycetes from soil. Studies were done on fungi and actinomycetes in periodically water-logged soils (gleys) for which a simple rose bengal-malt extract-agar was developed, suitable for the enumeration of both fungi and actinomycetes: Commercial malt-extract (Bio-Malz), 20 g; K₂HPO₄, 0.5 g; Fe²⁺, Mn, Cu, Zn, Mo, B, Co, 1 ppm each (added as soluble salts, not as nitrate); rose bengal, 1 part in 15,000; agar, 20 g; tap water, 1 liter; pH 6.0 to 6.2. The incorporation of rose bengal in the medium suppressed and reduced the development of bacterial colonies, which either remained white or were slightly dyed. Comparative results yielding highest counts of actinomycetes and fungi were obtained on von Plotho’s glycerol-glycine medium, closely followed by rose bengal-malt extract-agar with least colonies on oat meal agar.

Bills *et al* (2004) has elaborated the isolation techniques for filamentous fungi from soil along with the details of taxonomy, diversity, and distribution of soil saprobic fungi. Accordingly, explanations on taxonomic literature has been given describing how to evaluate the diversity of fungi with preliminary planning, scale, and distribution patterns. comprehensive details on isolation techniques of filamentous fungi initiating from collection of samples, characterization of sampling sites and soils, media selection, choosing principal isolation methods like warcup method, suspension plating method, particle filtration method, fractionating
the soil fungal community, documentation and preservation of isolates, cultural characters, taxonomic analysis, encouraging sporulation in sterile isolates, ending up with recommendations for quantitative and qualitative inventories of saprobic soil fungi.

Saravanakumar and Kaviyarasan (2010) examined the seasonal distribution of soil fungi and chemical properties of Montane wet temperate forest types of Tamil Nadu. Forty eight soil samples were collected from Wet evergreen forest of Tamil Nadu, Southern India and the fungi from these soil samples were isolated in (June – July) South West monsoon and (November – December) North East resting monsoon both seasons. About different species belonging to various groups viz., Ascomycotina, Zygomycotina and Deuteromycotina were identified with the help of relevant literatures. A total of 76 taxa belonging to 25 genera were isolated, these include one species of Acromycetes, one species of Coelomycetes five species of Zygomycetes and remaining species were Deuteromycetes. Twenty one species of Penicillium and 14 species of Aspergillus were also recorded from both seasons. The diversity indices of forest soil fungi over the two seasons were 2.953, 2.699 (Shannon-Weinner), 0.9033, 0.8491 (Simpson index) and 0.2219, 0.3485 (Fishers’s alpha), respectively. The soil nutrients were also analyzed for montane wet temperate forest. The macro nutrients such as N, P, and K content were rich in these soil samples after the raining season and organic content of natural soil was also increased.

Swer et al (2011) studied the population and diversity of soil fungi in organically amended agricultural soils of Meghalaya, India. Fungal populations were much higher in organically fertilised plots as compared to the control (CTRL). Altogether, 122 fungal species and two sterile mycelia were isolated from all the plots of which 25 fungal genera belonged to Deuteromycotina, seven to Ascomycotina, four to Zygomycotina and one to Mastigomycotina. The most common genera isolated from all the plots include Penicillium, Aspergillus, Acremonium, Fusarium, Mortierella, Mucor, Paecilomyces, Talaromyces, Trichoderma and Verticillium. Significant positive correlations between fungal populations and Corg were observed in all the organic amended plots. The organic matter level in the organically managed soil systems can play a pivotal role in fungal growth, sporulation and diversity.

Gaddeyya et al (2012) isolated a total of 15 species belonging to 6 genera of fungi were from agricultural fields at Salur Mandal during March 2011 to November 2011 in three intervals. The mycoflora were isolated by using soil dilution technique and soil plate technique on Potato
Dextrose Agar and Czapek Dox Agar medium supplemented by suitable antibiotics such as penicillin and streptomycin. Identification and characterization of the mycoflora were made with the help of authentic manuals of fungi. The most common among them viz., *A. flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus*, *Penicillium chrysogenum*, *Penicillium frequentans*, *Penicillium funiculosum*, *Trichoderma viride*, *Trichoderma harzianum*, *Fusarium oxysporum*, *Fusarium solani*, *Curvularia clavata*, *Curvularia lunata*, and *Rhizopus stolonifer* were isolated and characterized. The seasonal variation and percentage frequency of the mycoflora were statistically analyzed.

Khalil *et al* (2013) analysed the mycobiota composition of the mangrove soil in Egypt by collecting 24 soil samples. Almost all samples showed clay, sandy to sandy loam texture. pH of the soil samples ranged from 7.5 to 8.9 and water content ranged from 8% to 9%. The filamentous fungi of each soil sample were examined using two isolation methods. Fifteen fungal species belonging to nine genera were identified. Results showed that most of the genera detected belonged to the Ascomycotina with fewer proportions belonging to the Deuteromycotina and Zygomycotina. The frequent species were in decreasing order: *Aspergillus* sp, *Cladosporium* sp, *Alternaria* sp, *Penicillium* sp, *Rhizopus* sp, *Absidia* sp, *Acremonium* sp, and *Trichoderma* sp. One-way Analysis of Variance (ANOVA) test showed significant differences of richness and diversity of mycoflora between sites likewise pH and Na ion of soil analysis. In Egypt as well as in other developing countries, the information on diversity of fungi associated with mangrove soil is limited. Thus, this study was conduct to elucidate the distribution and diversity of fungal species associated with selected mangrove areas.

**ASPERGILLUS: DOMINANT SOIL GENERA**

Ahmad *et al* (2005) investigated an agricultural field soil of Aligarh city receiving long-term application sewage and industrial effluent as irrigant and recovered *Aspergillus* and *Rhizopus* to be predominantly occurring genera resistance to multi metals. Further study was done to check their biosorption potential evaluation of Cr and Cd. Pretreated, dead biomass of above fungi was used for bioadsorption experiment at pH value 4.5 with the biomass, 1-5 mg in a 100 ml metal solution of different concentration (2, 4, 6 and 8 mM) with a contact time of 18 hrs and agitation, 120 rpm. Bioadsorption of Cr ranged from 6.20- 9.5 mg/g of dry mass at one or other initial metal concentrations by *Aspergillus* and *Rhizopus* sp. The bioadsorption of Cd was
ranged from 2.3-8.21 mg/g. On the comparative basis Rhizopus sp. could bioadsorbed higher concentration of both metals as compared to Aspergillus sp. Bioadsorption of Cd and Cr was influenced by initial metal concentration and nature of organism. The findings revealed that fungi of metal polluted sites showed higher metal tolerance and bioadsorption capacity of chromium and cadmium.

Santos et al (2007) isolated four Aspergillus sp. strains from contaminated soil in Rio Grande, Southern Brazil. The biodegradation potential of these strains was evaluated using a simple method involving the determination of colony growth rates on plates containing a specific hydrocarbon or petroleum derivative as the only carbon source. The LEBM1 strain presented a high tolerance level to BTX. It was the only strain capable of growth on all the media, with growth rates varying from 1.3 to 2.2 mm/day. The LEBM2 strain presented the potential for phenol degradation, while the LEBM3 strain could be used for gasoline, diesel oil, hexane and chlorobenzene.

Okafor et al (2009) recovered twelve fungal isolates from oil-contaminated soils and screened for crude oil biodegradation activity in a shake-flask culture. Among the twelve fungal isolates, only eight showed potentials for biodegradation. Of these eight isolates, two of them identified as Aspergillus versicolor and A. niger which exhibited the fastest onset and highest extent of biodegradation were selected for further study on specific polycyclic aromatic hydrocarbon (PAH) biodegradation. Both isolates exhibited above 98% degradation efficiency for polycyclic aromatic hydrocarbon moieties when grown in a culture medium incorporated with 1% crude oil (hydrocarbon) and 0.1 %Tween 80 for 7 days.

Sharma (2010) explored the soil mycoflora from Katao, Gangtok by serial dilution method. In investigation period, 146 colonies of 21 fungal species were observed the maximum percentage contribution of A. fumigatus, A. niger (12.32%), was followed by A. flavus, A. luchensis, Mucor sp. (6.84%) and minimum percentage contribution of Cladosporium sp. (0.68%). The maximum fungal species belongs to the Anamorphic fungi (121 colonies), Zygomycotina (18 Colonies) and mycelia sterilia (white) (7 colonies) were observed.

Iram et al (2011) studied the micro-fungal flora of heavy metals contaminated peri-urban agricultural fields of Pakistan that were investigated in terms of their diversity by soil serial
dilution method. A total of 30 micro-fungi were isolated from 6 sampling sites. Of these isolates 24 belong to phylum Ascomycota, 3 to phylum Zygomycota, 2 to phylum Basidiomycota and 1 to phylum Deuteromycota. The most widespread genus was *Aspergillus* and common species *A. niger*. Frequency percentage showed that Kasur is rich in fungal population as compared to other peri urban areas while Wah Cantt showed maximum fungal Colony Forming Unit (CFU). The aim of present investigation was to see the diversity of fungi in heavy metal contaminated soils of peri-urban agricultural areas and study them in future for heavy metal tolerance and biosorption analysis in reference to bioremediation.

Wahegaonkar *et al* (2011) sampled twenty three soil samples of three ecosystems in and around the city of Aurangabad and were investigated for total number of organisms and the specific composition of hyphomycetous fungi. Total 45 genera distributed in 85 species were isolated, maximum being in agricultural soils. The relationship between the genera of fungi and different ecosystem type was analyzed. No obvious variation was observed in the different soil types. The dominant genera in all the ecosystem types were also studied. *Aspergillus* was dominant in all the three types of soils followed by *Alternaria, Cladosporium, Trichoderma, Gliocladium* and *Gloeosporium*. Species diversity and diversity indices of these soil types were calculated.

**SECONDARY METABOLITES OF SOIL FUNGI**

Wolf (1973) has discussed in detail on the synthesis of various products, especially pigments by fungi. Fungi are capable not only of making pigments for use in their nutritional and functional needs but also of synthesizing many other substances. They may utilize already prepared material as such available in living and non living animal and plant tissues, but they also employ such materials in the synthesis of diverse products needed to supplement nutritionally the already prepared substances. Such factors as oxygen, light, temperature and the associative influence of other organisms may modify the synthesizing processes. How pigments and other synthesized products modify the physiologic activities of fungi remains quite speculative.

Hajjaj *et al* (2001) studied lovastatin biosynthesis by *A. terreus* in a chemically defined medium to investigate the influence of carbon and nitrogen sources on lovastatin biosynthesis.
Lovastatin is a secondary metabolite produced by *A. terreus*. Among several organic and inorganic defined nitrogen sources metabolized by *A. terreus*, glutamate and histidine gave the highest lovastatin biosynthesis level. For cultures on glucose and glutamate, lovastatin synthesis initiated when glucose consumption levelled off. When *A. terreus* was grown on lactose, lovastatin production initiated in the presence of residual lactose. Experimental results showed that carbon source starvation is required in addition to relief of glucose repression, while glutamate did not repress biosynthesis. A threefold-higher specific productivity was found with the defined medium on glucose and glutamate, compared to growth on complex medium with glucose, peptonized milk, and yeast extract.

Petit *et al* (2009) discovered novel antimicrobial secondary metabolites from a *Penicillium* sp. Isolated from Brazilian cerrado soil. Chemical and biological investigation on the ethyl acetate extract of *Penicillium* isolate resulted in the isolation of three new naphthalenoids: a major metabolite, methyl 6-acetyl-4-methoxy-5, 7, 8-trihydroxynaphthalene-2-carboxylate and two minor ones, methyl 6-acetyl-4-methoxy-7,8- dihydroxynaphthalene-2-carboxylate and methyl 6-acetyl-4-methoxy-5, 8-dihydroxynaphthalene-2-carboxylate. Their structures were determined based on their mono and bidimensional nuclear magnetic resonance data. Antimicrobial activity of the major natural product and its semisynthetic derivatives was screened by macro dilution methodology and the corresponding minimum inhibitory concentrations were determined. Natural secondary metabolite methyl 6-acetyl-4-methoxy-5,7,8-trihydroxynaphthalene-2-carboxylate, isolated in a very high yield (0.3175 mg.L⁻¹) showed to be the most active compound, possessing expressive activity against *Candida albicans* (minimum Inhibitory Concentration (MIC) 32 μg/mL), *Listeria monocitogenes* and *B. cereus* (MIC 64 μg/mL for both).

Takahashi and Carvalho (2010) had written a review on nutritional potential of biomass and metabolites from filamentous fungi. Although, non basidiomycete filamentous fungi from several genera such as *Rhizopus* or *Penicillium* are commonly not associated with human diet, these have been reported to produce several metabolites useful to be added to food for preservation, coloring, flavoring or even aiming at a biological effect such as antioxidants and essential fatty acids. The review had focused on metabolites produced by filamentous fungi that have been showing potential for use by food industry such as sources of antioxidant agents.
(Rhizopus oligosporus, P. herquei), flavors (Ceratocystis fimbriata), color pigments (P. blakesleeanus, B. trispora), vitamins (Ashbya gossypii) and essential fatty acids (Syncephalastrum racemosum, M. cirinelloides). The study initiates the use of filamentous fungi biomasses for human nutrition as sources of proteins and minerals.

Klempova et al (2013) studied 28 Zygomycetes fungal soil isolates screening for their potential to synthesize these biologically active compounds. Although all fungi produced C18 Poly Unsaturated Fatty Acids (PUFA), only nine strains formed β - carotene. Although Actinomucor elegans CCF 3218 was the best producer of γ-linolenic acid (GLA) (251 mg/l), Umbelopsis isabellina CCF 2412 was found to be the most valuable fungus because of the dual production of GLA (217 mg/l) and β – carotene (40.7 mg/l). The calculated ratio of formed PUFAs provided new insight into activities of individual fatty acid desaturases involved in biosynthetic pathways for various types of PUFAs. The maximal activity of δ - 9 desaturase was accompanied by high accumulation of storage lipids in fungal cells. On the other hand, maximal activity of δ - 15 desaturase was found in strains synthesizing low amounts of oleic acid due to diminished δ - 9 desaturase. Activities of delta-6 desaturase showed competition for fatty acids engaged in n3, n6, and n9 biosynthetic pathways. Such knowledge about fatty acid desaturase activities provides new challenges for the regulation of biotechnological production of PUFAs by Zygomycetes fungi.

**Fungal Fatty Acids: Production, Analysis and Application**

Christie (1993) has reviewed in detail regarding the preparation of ester derivatives of fatty acids for chromatographic analysis. The technique of Gas Chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time. For this purpose, the fatty acid components of lipids are converted to the simplest convenient volatile derivative, usually methyl esters, although other esters may be preferred for specific purposes. There are certain considerations in deciding the procedure to adopt for preparing ester derivatives for GC like the lipid composition of the samples to be analysed. If these are free fatty acids alone, mixtures containing significant amounts of free acids or mixtures of unknown composition that might contain free acids, then acid-catalysed procedures are generally preferred. The newer quaternary ammonium reagents, which can be used both for esterification and transesterification, appear to be promising...
alternatives. On the other hand, alkaline transesterification procedures are so rapid and convenient that they must be considered for mixed lipid samples that contain no unesterified fatty acids or for single lipid classes containing ester-bound fatty acids.

Graham et al (1995) analysed the fatty acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizae. Arbuscule-forming fungi in the order Glomales form obligate endomycorrhizal associations with their host plants Fatty acid methyl ester (FAME) profiles were analyzed to assess the diversity and quantity of fatty acids in 53 isolates of 24 glomalean species. Spores and endomycorrhizal roots of Sudan grass (Sorghum sudanense) and the Citrus rootstock Carrizo citrange (Citrus sinensis) were examined. Spores yielded reproducible FAME profiles from replicate spore collections extracted from soil pot cultures. FAME profile comparisons provided a robust measure of similarity below the family level. FAME profiles in Sudan grass roots containing vesicles and/or spores of Glomus intraradices were more similar to spore profiles than to profiles from nonmycorrhizal roots. The FAME profiles for Gigaspora species, which do not form vesicles or spores in roots, were less distinct from nonmycorrhizal roots. Production in citrus roots of the fatty acid 16:1v5 cis by two Glomus species was correlated with the development of mycorrhizal colonization as measured by clearing and staining procedures and by estimates of total incidence and vesicle intensity. FAME analysis of roots not only provided a measure of colonization development but also served as an index of carbon allocated to intra radical fungal growth and lipid storage.

Pennanen et al (1996) analysed the phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in Scandinavian coniferous forests soils. One was near the Harjavalta smelter in Finland, and one was at Ronnskar in Sweden. Phospholipid fatty acid (PLFA) analysis revealed a gradual change in soil microbial communities along both pollution gradients, and most of the individual PLFAs changed similarly to metal pollution at both sites. The relative quantities of the PLFAs branched br18:0, br17:0, iso-branched i16:0, and i16:1 increased with increasing heavy metal concentration, while those of 20:4 and 18:2v6, which is a predominant PLFA in many fungi, decreased. The fungal part of the microbial biomass was found to be more sensitive to heavy metals. This resulted in a decreased fungal/bacterial biomass ratio along the pollution gradient towards the smelters. Both the PLFA pattern and the bacterial community tolerance were
affected at lower soil metal concentrations than were bacterial counts and bacterial activities. At Harjavalta the increased Cu tolerance of the bacteria and the change in the PLFA pattern of the microbial community were found at the same soil Cu concentrations. This indicated that the altered PLFA pattern was at least partly due to an altered, more metal-tolerant bacterial community. At Ronnskar, where the PLFA data varied more, a correlation between bacterial community tolerance and an altered PLFA pattern was found up to 10 to 15 km from the smelter. Farther away changes in the PLFA pattern could not be explained by an increased community tolerance to metals.

Bertran et al (1999) described a new procedure for determining free fatty acids (FFA) in olive oil based on spectroscopic Fourier transform infrared – attenuated total reflectance spectroscopy measurements was proposed. The range of FFA contents of samples was extended by adding oleic acid to several virgin and pure olive oils, from 0.1 to 2.1%. Calibration models were constructed using partial least-squares regression (PLSR). Two wavenumber ranges (1775–1689 cm\(^{-1}\) and 1480–1050 cm\(^{-1}\)) and several pretreatments [first and second derivative; standard normal variate (SNV)] were tested. To obtain good results, splitting of the calibration range into two concentration intervals (0.1 to 0.5% and 0.5 to 2.1%) was needed. The use of SNV as a pretreatment allows one to analyze samples of different origins. The best results were those obtained in the 1775–1689 cm\(^{-1}\) range, using 3 PLSR components. In both concentration ranges, at a confidence interval of \(\alpha = 0.05\), no significant differences between the reference values and the calculated values were observed. Reliability of the calibration versus stressed oil samples was tested, obtaining satisfactory results. The developed method was rapid, with a total analysis time of 5 min; it is environment-friendly, and it is applicable to samples of different categories.

Ahmed et al (2006) conducted studies on the fermentative production of GLA using seven strains belonging to Mucorales. An oleaginous fungal strain, isolated from the Western Ghats of Kerala produced GLA at a level of 8 % (by mass), when grown in a complex medium containing glucose as the sole carbon source. Effects of different culture conditions were investigated in shake flasks. Maximum dry biomass and total GLA obtained were 48.4 g/l and 636 mg/l, respectively, in the culture cultivated at 30°C and 200 rpm for 7 days. Among the organic nitrogen sources investigated, yeast extract, and combination of corn steep liquor and
baker’s yeast in 1:1 ratio were useful for enhancing the GLA production and the effects were comparable.

Karlinski et al (2007) investigated the whole cell fatty acid (WCFA) compositions of three different structures of ectomycorrhizal (ECM) fungi: sporocarps, pure culture mycelia and ectomycorrhizas were analysed to evaluate the potential use of fatty acid profiles as biomarkers for ECM fungi and ectomycorrhiza-associated bacteria. The results revealed species-specific composition of fatty acids of fungal sporocarps and pure culture mycelia. Ectomycorrhizal morphotypes distinguished and identified by morphological and molecular methods (PCR - RLFP and sequencing) created specific fatty acid profiles. The dominating fatty acids in pure cultures and sporocarps were 18:2ω6, 9, 18:1 ω 9 and 16:0, whereas ectomycorrhizas also contained plant and bacterial specific fatty acids. The results showed that fatty acid based methods can be useful in studies of ectomycorrhizal fungi, both as a quick method for differentiation of fungal species and also in studies of mycorrhiza associated microorganisms in the field.

Zain (2009) has determined the secondary metabolite and fatty acid profiles of A. flavus, A. parasiticus, A. ochraceus in addition to P. expansum and found that the olive oil affects the secondary metabolite and fatty acid profiles when added to the growth medium. Griseofulvin, 6-Methylsalicylic acid, and rubratoxin B were produced by P. expansum grown on olive oil-free medium and on medium supplemented with olive oil in concentration of 2%, 4%, and 6%. However, Epoxysuccinic acid, (-) flavoskyrin, 2-pyruvoylamino benzamide, Roquefortine B, Roquefortine C, and Stipitatic acid were produced by P. expansum only in presence of olive oil. On the other hand, the presence of olive oil in the growth medium prevented the terrein and xanthomegnin production. The fatty acids Butyric, caproic, caprylic, capric, undecylic, lauric, myristic, pentadecylic, and palmitoleic were detected in P. expansum grown on olive oil-free medium and not detected on medium supplemented with olive oil. Cyclopenol, desacetylpebrolide, (-) flavoskyrin, fumarprotocetraric acid, lobaric acid, protocetraric acid, and rubratoxin B were produced by A. flavus, A. parasiticus only in presence olive oil. Alectoronic acid, austdiol, cinnamic acid, a-collatolic acid, and fulvic acid were produced only by A. ochraceus grown on medium supplemented with olive oil. Caprylic, capric, undecylic, lauric,
and myristic were detected in both *A. flavus* and *A. ochraceus* grown on olive oil free medium and not detected in *A. parasiticus*.

Gayathri *et al* (2010) had studied ω-3 fatty acid production by soil microorganisms. Studies on the application of functional lipids such as PUFAs have proceeded in various fields in search of novel and rich sources for health and dietary requirements. Natural limitations favour a novel approach for the production of ω-3 fatty acids. A series of PUFAs including Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) have widespread nutritional and pharmaceutical values. This study investigated the potential production of these two economically important fatty acids from fungi. The microorganisms used were *Trichoderma* sp. and *A. niger*, isolated from soil. The use of *Trichoderma* sp. is preferred since it produced considerable amounts of EPA and DHA. This paper presents the results on the ratios of EPA and DHA produced by these microorganisms and recovery aspects.

Nelson (2010) examined a single-step, extractive reaction for extraction of lipids such as biodiesel components, ω-3 fatty acids, or other triglycerides from microbial cells. A simplified, single-step reactive extraction method was applied that combines the sequential extraction followed by transesterification using acidified alcohols – a process known as in situ transesterification. This was tested using a marine fungus, *Schizochytrium limacinum* SR21 whose growth resulted in biomass yields of 0.3g-biomass/g-glycerol and accumulated high amounts of palmitic acid (C16:0, 0.255g-FAME/g-biomass), docosahexaenoic acid (DHA, C22:6, 0.185g-FAME/g-biomass), myristic acid (C14:0, 0.017g-FAME/g-biomass), and pentadecanoic acid (C15:0, 0.012g-FAME/g-biomass). After FAME separation, the remaining methanol was recycled and used in subsequent in situ reactions that greater than 85% of product extraction and recovery was achieved.

**BETA CAROTENE PRODUCTION BY FUNGI**

Garton *et al* (1951) studied the general conditions governing β - carotene synthesis by the fungus *P. blakesleeanus burgeff*. The dry-weight, lipid (ether-soluble) and carotene production by the (+) and (-) strains of *Phycomyces* has been examined under a variety of conditions. On Schopfer's (1934) medium, maximal production of dry weight and lipid occurred after 5-6 days and of b- carotene after 5-9 days. β - carotene production decreased in ageing cultures. The (-)
strain produces twice as much β-carotene as does the (+) strain, although lipid and dry-weight production is the same in both. When the nitrogen source is kept constant (0-2 % L-asparagine), variation in the glucose concentration of the medium affects b-carotene synthesis to a much greater extent than it affects dry weight and lipid production. β-carotene production was normal with maltose, but reduced with xylose or fructose. Neither lactose nor glycerol supports mycelial growth.

Ciegler et al. (1958) analyzed the effect of various grains on production of β-carotene by *B. trispora*. The influence of various soybean products and grains, alone and in various combinations, on the synthesis of β-carotene by paired cultures of + and - strains of *B. trispora* was investigated. Hexane-extracted soybean oil meal gave the highest yields of carotene around 35 and 40 mg per 100 ml of fermentation medium. The ratios of the pigments present in the dried solids obtained from the fermentation of various other grains remained comparatively constant. Maximal yields of carotene were realized by the fifth day of incubation and microscopic observations indicated that mating, as defined by zygospore formation, did not occur in shaken-flask cultures but that the presence of the two opposite mating types was mutually stimulating for carotene synthesis.

Enrique et al. (2005) studied the Strain and Culture Conditions Improvement for β-Carotene production with *Mucor*. Several fungal species, particularly some included in the Mucorales, have been used to develop fermentation processes for the production of β-carotene. They described an approach to obtain *M. circinelloides* strains that could be useful in the industrial production of carotenoids and that, for commercial interests, specifically avoid the use of molecular genetic engineering. The method relies on classical genetic techniques to isolate and characterize β-carotene overproducing mutants and to build up strains that better fit the industrial production. *M. circinelloides* is a dimorphic fungus that grows either as yeast cells or in a mycelial form. This feature can be used to further develop strains with a better industrial potential by isolating monomorphic (yeast like) mutants or by controlling and modifying the morphology of the organism during batch cultivation.

Barrero et al. (2012) isolated a minor dihydropyran apocarotenoid from mated cultures of *B. trispora*. The heterocyclic C15 apocarotenoid 1 was isolated from mated cultures of the strains F986 (+) and F921 (-) of *B. trispora*. This new compound formed during sexual interaction is a minor constituent of the culture media and its structure was elucidated by spectroscopic data,
including 2D-NMR. A plausible biosynthetic pathway involving a double degradation of β-carotene, followed by several oxidations of the resulting monocyclofarnesane C15 fragment is proposed.

Nagy et al (2012) studied the carotenoid composition of some Mucorales fungi. Mucorales are known as β – carotene producing fungi and some species, namely B. trispora, P. blakesleeanus and M. circinelloides, are used as model organisms in carotenogenic studies. However, little is known about the carotenoid production and composition of other species belonging to this fungal group. In this study, carotenoid content of 21 isolates representing 11 species and five genera were determined. Carotenoid content of strains belonging to the same species showed high level of variability. Several promising producers were identified, of which carotenoid contents were comparable with those of the well characterized M. circinelloides and B. trispora. Effect of the temperature and several carbon sources on the carotenoid production of the tested strains was also examined.

**ANALYSIS AND PURIFICATION OF BETA CAROTENE**

Scott (2001) explains the detection and measurement of Carotenoids by UV/VIS Spectrophotometry. The majority of carotenoids exhibit absorption in the visible region of the spectrum, between 400 and 500 nm. Because they obey the Beer-Lambert law (i.e. absorbance is linearly proportional to the concentration), absorbance measurements can be used to quantify the concentration of a pure (standard) carotenoid or to estimate the total carotenoid concentration in a mixture or extract of carotenoids in a sample.

Rodriguez-Amaya and Kimura (2004) have described several procedures in detail to carry out carotenoid analysis in an efficient manner. Carotenoids are notable for their wide distribution, structural diversity, and various functions. More than 600 carotenoids, not including cis and trans isomers, have been isolated and characterized from natural sources. This impressive figure includes the enormous array of carotenoids in algae, bacteria, yeast, and fungi. To summarize, carotenoid analysis usually consists of (a) sampling and sample preparation, (b) extraction using water-miscible organic solvent (e.g., acetone, methanol, ethanol, or mixtures thereof) that allows better solvent penetration into biological samples, (c) partition to a solvent compatible with the subsequent chromatographic step such as hexane, petroleum ether, diethyl
ether, or dichloromethane, or mixtures of these (d) saponification and washing, (e) concentration or evaporation of solvent, (f) chromatographic separation using OCC, HPLC, etc (g) identification using TLC procedures, UV/visible absorption spectrum followed by quantification.

Ammawath and Man (2010) described a rapid method for the determination of commercial β-carotene in refined bleached and deodorized (RBD) palm olein using Fourier Transform Infra Red (FTIR) spectroscopy. The fifty RBD palm olein samples spiked by a known amount of commercial (30%) β-carotene to produce a wide range of concentrations up to 2000 ppm were used. Samples were separated into two groups for the calibration and validation models. The partial least squares (PLS) calibration models for predicting β-carotene was developed by using the FTIR spectral region at 980-915 cm⁻¹ which is associated with trans double bond CH absorption. The accuracy of the method was comparable to that of the HPLC method with a coefficient of determination ($R^2$) and standard error of calibration (SEC) for commercial β-carotene 0.9934 and 52.29, respectively. The FTIR method developed was shown to be efficient, accurate and suitable for routine quality control analysis for the food industry with results obtainable in about 2.5 min.

**TURBIDOMETRIC GROWTH STUDIES OF FUNGI**

Meletiadis et al (2001). A microbroth kinetic model based on turbidity measurements was developed in order to analyze the growth characteristics of three species of filamentous fungi (*Rhizopus microsporus, A. fumigatus* and *Scedosporium prolificans*) characterized by different growth rates in five nutrient media (antibiotic medium 3, yeast nitrogen base medium, Sabouraud broth, RPMI 1640 alone, and RPMI 1640 with 2% glucose). Among the different growth phases distinguished, the smallest variability in growth rates among the strains of each species was found during the log phase in all nutrient media. *R. microsporus* and *A. fumigatus* grew better in Sabouraud and yeast nitrogen base medium than in RPMI 1640. None of the media provided optimal growth of *S. prolificans*. The germination of *Rhizopus* spores and *Aspergillus* and *Scedosporium* conidia commenced after 2 and 5 h of incubation, respectively. In conclusion, the growth curves provide a useful tool to gain insight into the growth characteristics of filamentous fungi in different nutrient media and may help to optimize the methodology for antifungal susceptibility testing.
Meletiadis et al (2003). Turbidometric growth curves of different filamentous fungi in the presence of increasing concentrations of antifungal drugs were studied. 24 clinical mold isolates, including R. oryzae, A. fumigatus, A. flavus, and S. prolificans, were tested against itraconazole, terbinafine, and amphotericin B according to NCCLS guidelines. Exposure to increasing drug concentrations resulted in prolonged lag phases of the turbidimetric growth curves. The lag phases of the growth curves at drug concentrations which resulted in more than 50% growth (for itraconazole and terbinafine) and more than 75% growth (for amphotericin B) after 24 h of incubation for R. oryzae, 48 h for Aspergillus sp. and 72 h for S. prolificans. Using this system, itraconazole and terbinafine resistance (presence of >50% growth) as well as amphotericin B resistance (presence of >75% growth) was determined within incubation periods of 5.0 to 7.7 h for R. oryzae (for amphotericin B resistance incubation for up to 12 h was required), 8.8 to 11.4 h for A. fumigatus, 6.7 to 8.5 h for A. flavus, and 13 to 15.6 h for S. prolificans while awaiting formal MIC determination by the NCCLS reference method.

USE OF MUTAGENS AND METABOLITE PRODUCTION

Rasool and Mushtaq (1991) researched the genetic activity of certain chemicals including a mosquito repellent in salmonella-microsomal screening system. The Ames Salmonella typhimurium microsomal screening system was standardized and six alkaloids and a mosquito repellent were screened for their mutagenic potential. The results indicated that mosquito repellent and all the six (buxenone, ephedradine, protopine, saponin, pleiocarpamine and vindoline) alkaloids exhibited mutagenic activity (based on his– → his+ reversions, in TA98 tester strain. In addition, vindoline also induced reversion mutations in TA100.

Mehta et al (1997) developed new mutants of P. blakesleeanus for β-carotene production. The accumulation of b-carotene by the zygomycete P. blakesleeanus is increased by mutations in the carS gene. The treatment of spores of carS mutants with MNNG led to the isolation, at very low frequencies, of mutants that produced higher levels of β-carotene. Strain S556 produced about 9 mg of b-carotene per g of dry mass when it was grown on minimal agar. Crosses involving strain S556 separated the original carS mutation from a new, unlinked mutation, carF. The carF segregants produced approximately as much carotene as did carS mutants, but they were unique in their ability to produce zygospores on mating and in their response to agents that increase carotenogenesis in the wild type. The carotene contents of carF
segregants and *carF carS* double mutants were increased by sexual interaction and by dimethyl phthalate but were not increased by light or retinol. Mixed opposite-sex cultures of *carF carS* mutants contained up to 33 mg of b-carotene per g of dry mass. Another strain, S444, produced more b-carotene than did S556 but was marred by slow growth, defective morphology, and bizarre genetic behavior. In all the strains tested, the carotene concentration was minimal during the early growth phase and became higher and constant for several days in older mycelia.

Barbu *et al* (2006) described the modification of pigment composition in *Epicoccum nigrum* by chemical mutagenesis. *E. nigrum* MIUG 2.15 (dematiceous mould) produces in high concentration a combination of yellow food-grade pigments (i.e. carotenoids and flavonoids in a ratio of 20:1) by solid-state fermentation on maize and molasses based media. By exposing the parental strain to a chemical mutagen, MNNG, a mutant strain was obtained, that produces equal shares of flavonoids and carotenoids (i.e. 1:1) in a red pigment complex. UV-VIS spectrometry and HPLC analysis show a major content of glycosylated flavonoids and free phenolic acids, and also conjugated carotenoids, especially rhodoxanthin. The extracts may be used as red food colorants with functional properties.

Ueno *et al* (2010) showed the extent of variation in the composition and amount of carotenoids among a large number of phenotypic variants, induced from a single wild type parent by MNNG, has been compared between two strains of the carotenoid-rich, pink yeasts belonging to the order Sporidiobolales (*Rhodotorula dairenensis* Sag 17 and *Rhodosporidium diobovatum* Sea 2-11). Our data clearly showed that the choice of the parent with low level of relative β-carotene content is critical to improve these carotenogenic yeasts by mutagenesis treatment with a view to preferential synthesis of monocyclic pigments having potent antioxidant activities. We further examined the effect of substrate concentration on pigment production by the Sag 17 variants. Use of the variants exhibiting high level of cell mass in the stationary phase was found to be essential for carotenoid production under high substrate concentrations, although they need to be selected based primarily on the pigment contents.

Jaivel and Marimuthu (2010) conducted a research on strain improvement of *A. terreus* by physical and chemical mutagenesis for increased lovastatin production. The spore survival of *A. terreus* strain JPM3 after different periods of exposure to UV light and EMS was assessed. Four mutant clones were obtained by the mutagenesis program. In which two mutants were
derived by UV irradiation and another two mutants derived by EMS treatment. The yield of lovastatin varied from mutant to mutant and the mutant strain JPM3-UV1 produced the maximum lovastatin yield (1553.02 mg l-1) followed by strain JPM3-EMS2 (948.5 mg l-1).

Javed et al (2011) describes the improvement of a thermophilic fungal strain *Humicola insolens* using multi step mutagenesis for cellulase production. *H. insolens* TAS-13 has the potential to produce 1.00 IU/ml/min Carboxy Methyl Cellulase (CMCase), 0.43 IU/ml/min Filter Paperase (FPase) and 0.30 IU/ml/min β-glucosidase, which was improved through UV and chemical mutagenesis like MNNG, HNO₂, EMS or EtBr. Two alternative mutation steps were carried out that 5 best mutants (TASUV-4, TASNG-7, TASHN-4, TASEB-2 and TASEMS-1) from first step mutation were further screened on the basis of hypercellulolytic ability. When these mutants were further mutated alternatively with the same mutagens under the same conditions, a total of 33 mutants were picked up as second generation mutants. Among these, a mutant strain TAS-13UV-4 NG-5 proved to be the best mutant, which produced 43.19% CMCase, 60.15% FPase and 59.78% β-glucosidase more than the first generation mutant TAS-13UV-4. Furthermore, this mutant was highly stable up to many generations possessing cellulolytic ability.

Pradeep et al (2012) carried out studies on induction of chemical mutations involving treatment of EtBr in *A. niger* to enhance cellulase production. After mutagenesis, ten mutant strains (GNEB1 to GNEB10) were selected and their cellulase activities were assayed. Five of them (GNEB1, GNEB4, GNEB5, GNEB8, GNEB9) had significantly stronger ability to produce enzymes than that of normal wild type, and they were also very stable for a long period up to 8 generations to produce cellulase. Mutant Strain GNEB1 exhibited maximum Activities of cellulases, FPase-3.91 IU/ml, CMCase-3.15 IU/ml and β- glucosidase 0.87 IU/ml. These levels were, respectively, 2.3, 2.1 and 4.5 fold higher than those in parent strain (FPase-1.68 IU/ml, CMCase-1.48 IU/ml and β- glucosidase-0.87 IU/ml). The extracellular soluble protein content was also improved in the culture filtrate. The waste lignocellulosic material, ground nut shells was used as low-cost carbon source for cellulase production by mutant *A. niger* under submerged fermentation to make the process economically viable.
Mukherjee et al (2011) has reviewed the anticancer potential of vitamin A and β-carotene as a mechanistic approach addressing the present perspective and future possibilities of these micronutrients to control various forms of cancer. They enhance gap junctional communication and inhibit lipid peroxidation in chemically induced neoplastic transformed cells and also as a chain breaking anti-oxidant in the lipid phase by neutralizing peroxy radicals controlling cancer growth. This entails their possible mechanism of action where β-carotene is converted into 8/, 10/ and 12/-apo carotenals, further oxidized into isomers of retinoic acid that forms homo (RXR/RXR) or hetero (RXR/RAR) dimeric proteins binding with RXRE or RARE sites of DNA sequences, making it inaccessible thereby modulating the transcriptional machinery of regulatory proteins. This further leads to TR3/RXR-α heterodimer formation in the nucleus whose subsequent translocation in the cytoplasm down regulate antiapoptotic protein like Bcl-2 and Bcl-xl inducing apoptotic protein Bax resulting in ATRA induced apoptosis in the cancerous cells (Fig. 2).

Bumrela and Naik (2011) assayed the antimicrobial activity (disc diffusion method) and antioxidant activity by different in vitro methods (DPPH, hydrogen peroxide, nitric oxide radical scavenging and reducing power) of methanolic extract of Dipteracanthus patulus (MEDP) was evaluated. The qualitative and quantitative estimation of β-carotene and β-sitosterol in MEDP was carried out by High Performance Thin Layer Chromatography (HPTLC). The total phenolic content of was determined by Folin-Ciocalteu method. Experimental findings indicate promising antimicrobial activity (antibacterial and antifungal) and potent antioxidant activity of MEDP. In addition, phytochemical analysis and spectral studies of MEDP were also performed. It is presumed that antimicrobial and antioxidant activity observed with MEDP may largely be attributed to the presence of major phytoconstituents (β-carotene, β-sitosterol and iridoid glycosides) and other minor components may participate as promoters.

Darvin et al (2011) reviewed upon the role of carotenoids as antioxidants in human skin. The human skin is under the constant influence of Free Radicals (FR) that carotenoids are known to be powerful antioxidant substances playing an essential role in the reactions of neutralization of FR (mainly Reactive Oxygen Species - ROS). Carotenoid molecules present in the tissue are capable of neutralizing several attacks of FR, especially ROS, and are then destroyed. Human
Fig. 2 Mechanism of β-carotene regulating anticancer and apoptotic pathways
skin contains carotenoids, such as α-, γ-, β-carotene, lutein, zeaxanthin, lycopene and their isomers, which serve the living cells as a protection against oxidation. Results obtained from *in-vivo* studies on human skin have shown that carotenoids are vital components of the antioxidative protective system of the human skin and could serve as marker substances for the overall antioxidative status. Reflecting the nutritional and stress situation of volunteers,

Velayos *et al.*, (2000) reported a bifunctional enzyme with lycopene cyclase and phytoene synthase activities is encoded by the carRP gene of *M. circinelloides*. Using functional analyses in *E. coli* and *M. circinelloides*, it has been shown that a single *M. circinelloides* gene (carRP) codes for a protein with two different enzymatic activities, lycopene cyclase and phytoene synthase, which are encoded by independent genes in organisms other than fungi. This gene was identified using complementation tests among different classes of carotenoid mutants of *M. circinelloides*. The carRP gene product contains two domains: the R domain is located at the N-terminus and determines lycopene cyclase activity; the P domain is located at the C-terminus and displays phytoene synthase activity. The R domain is functional even in the absence of the P domain, while the latter needs the proper R domain conformation to carry out its function. The carRP gene is closely linked to the phytoene dehydrogenase (carB) gene, and the promoter regions of both genes are located within only 446 bp. Northern analyses show a co-ordinated regulation of the expression of both genes by blue light. Several motifs found in this promoter region suggest a bi-directional mode of transcription control.

Verwaal *et al.* (2007) studied to determine whether *S. cerevisiae* can serve as a host for efficient carotenoid and especially β - carotene production. Carotenogenic genes from the carotenoid-producing yeast *Xanthophyllomyces dendrorhous* were introduced and over expressed in *S. cerevisiae*. Furthermore, carotenoid production levels were higher in strains containing integrated carotenogenic genes. Over expression of *crtYB* (which encodes a bifunctional phytoene synthase and lycopene cyclase) and *crtI* (phytoene desaturase) from *X. dendrorhous* was sufficient to enable carotenoid production. Combined over expression of *crtE* (heterologous GGPP synthase) from *X. dendrorhous* with *crtYB* and *crtI* and introduction of an additional copy of a truncated 3-hydroxy-3-methylglutaryl–coenzyme A reductase gene (*tHMG1*) into carotenoid-producing cells resulted in a successive increase in carotenoid production levels. The strains mentioned produced high levels of intermediates of the carotenogenic pathway and
comparable low levels of the preferred end product β-carotene, as determined by high-performance liquid chromatography. High levels of β-carotene, up to 5.9 mg/g (dry weight) producing strain were accomplished by the introduction of an additional copy of *crtI* and *tHMG1* into carotenoid-producing yeast cells.