The cocultivation of the two sexual types plus and minus of the mold *Blakeslea trispora* is known to result in a series of biochemical and physiological events regulated by trisporic acid, a hormone-like compound (95). The most significant mating event in *B. trispora* is the strong stimulation of the biosynthesis of $\beta$-carotene, which is produced by the mold as a secondary metabolite (94). The mating phenomenon thus presents an interesting model of pheromone-mediated differentiation associated with secondary metabolism in this fungus (97).

In the present study, the various biochemical and physiological changes occurring in the two mating types during mating were investigated in each culture separately, under mating-simulating conditions (125). The patterns of biosynthesis of $\beta$-carotene in the two mating-simulated single cultures were elucidated by the relative rates and extent of $[2-^{14}C]$ acetate and $[2-^{14}C]$ mevalonic acid incorporation into $\beta$-carotene. The mating-simulated minus culture showed increased rates of incorporation of these radiolabeled precursors, concomitant with the increased synthesis of $\beta$-carotene, as compared to the plus cultures. Furthermore, the mating-simulated minus cultures showed a marked dilution of the radiolabel from $[2-^{14}C]$ acetate in $\beta$-carotene by unlabeled mevalonic acid, which was comparable with the increased dilution observed in the mated cultures. On the other hand, the mating-simulated plus culture showed much lower rates of radiopreursor incorporation, and almost no dilution of the $[2-^{14}C]$ acetate label by mevalonic acid. A strong analogy was also observed between
the mating-simulated minus culture and the mated cultures, with respect to the extent of incorporation of \([2-^{14}\text{C}]\) acetate into the various terpenoid intermediates of the \(\beta\)-carotene synthetic pathway.

These observations led to the conclusion that \(\beta\)-carotene synthesis is stimulated chiefly in the minus culture during mating, and that the stimulation affects all the biosynthetic reactions of the pathway equally in this culture. The stimulation of \(\beta\)-carotene synthesis by leucine and valine prompted an investigation of the possible role of leucine as a \(\beta\)-carotene precursor in \(B.\) trispora. From the \([U-^{14}\text{C}]\) leucine incorporation studies it was concluded that the alternate pathway of \(\beta\)-carotene synthesis (59) is operative in the mating-simulated minus culture and the mated cultures, as signified by the extent of radioactive leucine incorporation into \(\beta\)-carotene. However, the acetate pathway for \(\beta\)-carotene synthesis appears to be predominant.

The differential metabolic profiles of the mating-simulated single cultures were elucidated by monitoring the parameters like respiration rates, and the relative distribution of the radiolabel from \([2-^{14}\text{C}]\) acetate in the tricarboxylic acid cycle intermediates and \(\beta\)-carotene, during growth and carotenogenesis respectively. At the physiological level, the selective induction of asexual sporulation in the plus cultures gave a strong indication that the cellular differentiation processes as related to secondary metabolite synthesis are regulated by
molecular and/or biochemical factors intrinsic to each mating type. However, the characterization of such factors is required to be done through further studies.

β-carotene has been ascribed an antioxidant property, following extensive in vitro and in vivo studies on its interaction with oxyradicals (111,112). The photoprotection afforded by β-carotene to living systems is well documented (32, 33). The function of β-carotene as a precursor of trisporic acid, which regulates sexual differentiation in the Mucorales is also well established (94). Therefore, attempts were made to assign definite biological roles to β-carotene in B. trispora, in the present study. The studies conducted with mycelia blocked in β-carotene synthesis by specific inhibitors like DPA or pyridine showed that these carotene-deficient mycelia were more sensitive to photodynamic inactivation by UV-light. This was supported by the considerable reduction of [U-14C] leucine incorporation into cellular proteins, and the whole cell respiration rates. The increased lipid peroxidation levels in these mycelia possibly signified damage to the membranes and/or lipids. Moreover, since the effects of UV-light were evident shortly after irradiation, it was probable that the damage ensued at the level of membranes and/or protein synthetic apparatus.

An investigation of the possible role of β-carotene in asexual and/or sexual reproduction in B. trispora was made, by studying these phenomena in cultures grown on solid media.
containing DPA or pyridine. The deficiency of β-carotene did not appear to have any influence on the induction or degree of asexual sporulation in the plus or the minus cultures. However, the mated cultures grown in the presence of DPA or pyridine showed no mating-specific zygoaphore structures, or accumulation of β-carotene in the mating-zone. The pyridine treated mated cultures only showed extensive accumulation of lycopene in the mating-zone. These observation led to the conclusion that β-carotene has a definite regulatory or precursor function in the sexual reproduction but does not influence asexual reproduction in B. trispora.

β-carotene contained in fermentation biomass has been successfully used as a vitamin A supplement in animal feed (180). In the present study, β-carotene produced fermentatively by B. trispora from whey was tested for its biopotency in replacing the vitamin A supplement in rat feed. Albino rats (Rattus norvegicus) which were given oral feed containing the β-carotene rich B. trispora mycelia did not show any visible toxicity effects. Moreover, no degenerative changes were seen in the internal organs. An assessment of the vitamin A levels in the liver tissue of the rats given different vitamin A sources showed that mycelial β-carotene was efficiently absorbed and transformed in the animal system. Mycelial β-carotene also proved to be the nutritionally most effective vitamin A source, by virtue of its highly favourable effects on body weight gain, which is generally considered to be an index of the normal growth and development of animals. From these
observations, it was concluded that natural β-carotene afforded some kind of protection to the rats, resulting in the improved development of the animals. The definite mechanism of this effect, however, remains to be investigated.

β-carotene is a known scavenger of singlet oxygen (111), but has not been shown to interact appreciably with other oxyradicals (117). Since the assessment of the biochemical defense mechanisms against the highly reactive superoxide radicals is difficult, the levels of superoxide dismutase activities were assessed in B. trispora. SOD, known to function in most aerobic organisms against the toxicity of superoxide radicals (113) was found to be a major protein in B. trispora, with two or more distinct isoenzyme activities. The SOD activity was found to be inducible in the presence of extracellularly generated superoxide radicals. It was also observed that in the carotene-deficient mycelia, the levels of SOD, catalase and peroxidase were raised. This led to the conclusion that a synergistic association exists between β-carotene and these enzymes involved in scavenging oxygen free radicals. The partially purified major SOD isoenzyme from B. trispora had a molecular weight of 36,000 d, and was remarkably stable at high temperatures and highly alkaline pH values. The loss of activity observed on incubation of the enzyme preparation with 1 mM cyanide confirmed that it was a copper-zinc containing SOD.
In many parts of the less industrialized world, dietary β-carotene is the primary source of vitamin A (31). Owing to this, and the build-up of information relating natural carotenoids to preventive medicine (122, 175, 177), interest in the natural sources of carotenoids has increased. A study was therefore undertaken to design a fermentative process for large scale β-carotene production by *B. trispora* from a low cost dairy waste, whey. Whey was found to be an excellent medium for both, cell mass development and β-carotene production in this fungus.

The scale-up of the process to pilot level was conducted on the basis of volumetric oxygen transfer coefficient ($K_La$), and nutrient supplementation studies in shaken flasks and a 20 litre fermentor. These studies revealed that the enhanced β-carotene production from whey required very little nutrient supplementation, and a much shorter time of fermentation, as compared to the processes reported earlier (77). The increase in $K_La$, owing to the scaling up of aeration and agitation on the higher scales of fermentation improved the β-carotene yield remarkably. Extensive pelletization of the culture at high aeration and agitation rates also appeared to influence β-carotene synthesis favourably. These observations led to the conclusion that the whey fermentation process for pilot scale production of β-carotene has a definite potential for industrial application.