DISCUSSION
Until recently, thermophilic Bacilli were classified as either *Bacillus coagulans* or *Bacillus stearothermophilus*, but it is becoming increasingly evident that they comprise a diverse group of organisms. In addition to these two, there are at least eight other groups which appear to merit the rank of species (Sharp et al., 1989). The first systematic study of aerobic thermophilic sporeformers was made by Gordon and Smith (1949) in which they examined 216 cultures from a variety of sources. Walker and Wolf (1971) reported an extensive study of physiological, biochemical and serological properties of 230 strains of *B. stearothermophilus*. Heinen (1971) reported the isolation of three thermophilic bacilli from Yellowstone National Park in the USA which were named *B. caldotenax*, *B. caldovelox* and *B. caldolyticus*. Darland and Brock (1971) reported the isolation of *B. acidocaldarius* an acidophilic, thermophilic *Bacillus* capable of growing at pH 2.0 with a maximum growth temperature of 70°C. Golovacheva et al. (1975) described a strain initially considered to be a thermophilic strain of *B. megaterium*, which had some unusual characteristics. Subsequently the same isolate was confirmed to be a new species and named as *Bacillus catenulatus*. This isolate produced yellowish colonies, reduced nitrate to gas, its G+C content was 69 % and maximum growth temperature of 78°C.

Schenck and Aragno (1979) described an obligate thermophile which was strictly aerobic, oxidised hydrogen in the presence of O₂ and CO₂ and grew heterotrophically, producing spherical spores within a swollen sporangium. It had
an optimum growth temperature of 70°C and G+C content of 67-68%. Suzuki et al. (1983) reported the isolation of some obligate thermophilic aerobic sporeformers from soil in Japan, which they named *Bacillus thermoglucosidasins*. The main characteristics were sensitivity to azide and production of glucosidase. Growth occurred from 42-60°C with optimum at 45-46°C. The G+C content was estimated by thermal denaturation to be 61-63%. Scholz et al. (1987) described nine thermophilic *Bacillus* strains isolated from thermally treated waste water. The group of strains named *B. pallidus* had a growth range of 30-70°C with optimum at 60-65°C. The G+C content of the DNA ranged from 39-40 mole %. Zarilla and Perry (1987) reported the isolation of 10 obligate thermophilic bacilli able to utilize n-alkanes as growth substrates. The organisms were isolated following enrichment of mud and water samples on a medium with n-heptadecane added as substrate. The strains were named *B. thermoleovorans* and had G+C content ranging from 52-58%.

A number of thermophilic bacilli isolated from wide range of geographical locations exhibit a large variation in colony morphology and a wide variety of hydrolytic enzyme production. They show capability of fermenting number of carbohydrates and the isolates differ from each other in these properties (Sharp et al., 1989). These observations indeed substantiate heterogeneity among thermophilic bacilli as was proposed earlier. Thermophilic bacterial strains isolated from different habitats as reported by Kambourova and Emanuilova (1989) were mainly Gram positive bacilli and were able to hydrolyse different substrates such as pullulans, starch, skimmed milk and also Tween 80.

On this background isolation of *Bacillus* sp. *tap*, from compost, exhibiting unique features regarding its morphology, pH and temperature requirements for
growth, constitutive production of alkalophilic amylase and inducible alkaline protease does not seem to be very unusual.

On analysis of various properties of thermophilic bacilli, one could observe a common feature that most of them exhibit additional phenotype(s) conferring adaptations to other unusual or stress producing conditions, e.g. growth at higher temperature and capability to withstand complex chemical reagents such as pesticides, drugs and also unusual carbon sources like aromatic and aliphatic hydrocarbons. One wonders whether there is some relationship between the biochemical reactions and the genetic regulation of these phenotypes in terms of coevolution of mechanisms to encounter the stress conditions. It is also likely that such mechanism underlying cross resistance or tolerance to different agents might be differently regulated under various stress conditions. However, specific biochemical reactions involved in development of adaptation or tolerance to individual stress agents are shown to be different. The *Bacillus* sp. tap isolated from compost containing dimethoate exhibited along with thermophily, presence of growth in much higher concentrations of azide (upto 1 % w/v) and also Tween 80 (1.5 % v/v).

Microbes which are natural components of soil and water environments are potential agents for biological transformations of organic compounds that enter the ecosystem. Microbes are able to degrade a wide variety of chemicals, from simple polysaccharides, amino acids, proteins, lipids, etc. to more complex material such as plant residues, waxes and rubbers. Microorganisms belonging to families Pseudomonadaceae, Azotobacteraceae, Enterobacteriaceae and Bacillaceae are some important degradative bacteria that occur in water and soil
environments (Cork and Kruger, 1991). Some of these show the ability to grow on such compounds as sole carbon sources (Ribbons and Williams, 1982).

Organophosphorus insecticides have many structural similarities with naturally occurring compounds. Hence, it is possible that organophosphorus insecticides may be acted upon by the existing enzymes in microorganisms. The enzymes involved in pesticide metabolism may be constitutive (Rup Lal, 1982) or may require induction by either pesticide or an alternate chemical inducer (Alexander, 1965). Many microorganisms are able to adapt to one of a variety of substances added to the culture medium by forming an enzyme system that is not already present when the organism is grown in the absence of the added substance. Audus (1960) suggested that microorganisms can develop the ability to degrade pesticide either by enzyme induction or by chance mutation. Such microbes are important in safe waste disposal technology. In addition, the enzymatic detoxification process is many times faster and complete than chemical methods (Munnecke, 1979; Munnecke, 1980).

Thus, Bacillus sp. tap exhibits a number of varied desirable features, as temperature and pH conditions for growth, production of a number of hydrolytic extracellular thermostable enzymes. The isolate Bacillus sp. tap has rapid growth at 55°C in complex medium. Therefore, it has a great potential in environmental and industrial biotechnology.

Amylases are widely used in textile, confectionery, paper, brewing and alcohol industries. Bechima et al. (1983) reported investigations of 237 strains of thermophilic Bacillus, actinomycetes and fungi for the production of amylase. Thermostable α-amylase is now widely used in industries. Thermostable actinomycetes like Thermomonospora (Glymph and Stutzenberger, 1977, Upton
and Fogarty, 1977). *Thermoactinomyces* (Kuo and Hartman, 1966) and several *Bacillus* species are versatile producers of this enzyme. Out of 48 species of *Bacillus* described by Buchanan and Gibbons (1974), about 32 are reported to produce α-amylase but only a few of them are capable of secreting the enzyme active at high temperature. Two thermostable α-amylase producing strains of *Bacillus licheniformis* and *B. coagulans* have been isolated and characterised by Medda and Chandra (1980). Various reports have appeared on thermophilic and hyperthermophilic bacteria and archaebacteria producing thermostable α-glucosidase (Costantino *et al.*, 1990; Giblin *et al.*, 1987; and Saha and Zeikus, 1991). Campbell (1954), Boyer and Ingle (1972), Morgan and Priest (1981), Saito (1973) and Shih-Heng *et al.* (1995) have described different *Bacillus* strains capable of producing thermostable α-amylase at high temperature of growth.

The production of α-amylase from *B. stearothermophilus* is generally growth linked such that in batch culture, production occurs in the exponential phase of growth (Davels *et al.*, 1980). Similar results have been reported for other thermophilic *Bacillus* isolated from sewage sludge (Grueninger *et al.*, 1984) and *Bacillus caldolyticus* (Emanuelova and Toda, 1984) where production is constitutive throughout the exponential and stationary phases. This is unlike α-amylase production from *B. subtilis* and *B. licheniformis* which occurs between late exponential and stationary phases, prior to the onset of sporulation (Priest, 1977).

In case of *Bacillus* sp. *tap*, though initially stationary flask culture was used for amylase production, other conditions such as submerged fermentation and solid state fermentation were also used for obtaining higher yields of the enzyme. Decrease in extracellular amylase activity after initial production of the enzyme
could be related to appearance of protease activity in the culture filtrate. Proteases produced by *Bacillus* species are generally inactivated at alkaline condition or above pH 7.4 (Stein and Fischer, 1955; Windish and Mhatre, 1965). However, the protease produced by our isolate is both alkali- and thermo-stable. Subsequent increase in the net amylase activity could simply be due to higher levels of amylase production thus compensating for protease mediated inactivation. Thus, if one could segregate synthesis of these two enzymes completely, higher yields of amylase could be achieved. Production of amylase at much earlier time within 12 hours was yet another desirable feature of the *Bacillus* sp. *tap*. In most other cases, significant detection of amylase activity requires 24 hours growth. Fast multiplication of *Bacillus* sp. *tap* at 55°C could be one of the reasons for early appearance of amylase. The enzyme production was neither inhibited by presence of glucose nor was it stimulated by the presence of starch which is the case in most of amylase production. Amylase production normally requires presence of starch as inducer in the medium. However, *Bacillus* sp. *tap* produces amylase constitutively. Similarly in case of *Bacillus thuringiensis*, starch independent production of amylase even in presence of glucose has been reported by Tobey and Yousten (1977).

Starch independent amylase production has been found in case of *Bacillus* and *Pseudomonas* species (Bajpai et al., 1990; Fogarty et al., 1994; Tinkova et al., 1993). Synthesis of amylase on glucose is also not very unusual (Bajpai et al., 1990). Constitutive expression and level of production were not affected by various carbon sources used. However, unlike in most of the amylase producing strains, in case of *Bacillus* sp. *tap*, the amylase production corresponded with the log phase of growth.
Growth of *Bacillus* sp. *tap* on wheat bran and rice bran yielded apparently higher levels of amylase in the medium. This increase in the extracellular amylase level could be due to selective adsorption of protease on the bran and thus leading to protection of amylase from protease. This approach could be useful in other cases where starch induced amylases require separation or protection from coupled protease action. Addition of rice bran could thus be used as trap to remove protease by selective adsorption.

Production of amylase under submerged fermentation conditions in 1.5 L fermenter with controlled aeration, pH and temperature conditions resulted in a substantially increased yield in much shorter time compared to the production under stationary growth condition. Productivity of enzyme in fermenter was 3.5 fold more than the stationary flask culture. In an experiment where growth medium used (Nutrient broth + Rice Bran) under non-sterile conditions in a fermenter at 55°C, efficiency of enzyme production was unaltered. This observation could be effectively used for reducing both the energy requirement as well as total production cost of the industrially important alkalophilic and thermostable amylase using *Bacillus* sp. *tap*.

The SSF has been used to enhance the yields of amylase activity from *Bacillus* strain, wherein Lonsane and Ramesh (1990) have reported 33,000 DUN/g in bran in 48 hours. Compared to this, the productivity of amylase was 7-fold more in case of *Bacillus* sp. *tap* wherein 36,000 DUN/g were obtained within 8 hours. These observations indicate very high potential of *Bacillus* sp. *tap* for industrial enzyme production.

As mentioned earlier in the general introduction, search for microbes with many desirable features which can be beneficial under different requirements is
continuous. *Bacillus* sp. *tap* fulfils one such requirement particularly in the area of textile industry and could help decreasing not only the cost of treatment of the sized cloth but also would significantly improve the effluent qualities. The eco-friendly process thus would have a long range desirable impact. The same organism has an equally important role to play due to its thermostable and alkalophilic amylase in other industrial processes involving malting process in brewing as well as in detergent industry. In addition to such applications, this organism also provides a very interesting system for studying molecular features of thermostability of its enzymes. Indeed, it could present a wide variety of molecular interactions of interest to basic biologists.