diabetic mice leads to total regeneration of the pancreas within four weeks, leading to reversal of the diabetic state. The authors have further shown that repeated injection of cytosolic extract in the diabetic mice leads to the complete reversal of diabetes[13]. In view of the above information, it is likely that regenerating mahseer fin might be harbouring several growth factors within themselves, which stimulate *in vitro* growth of explants as seen in our study. The present work also suggests that the serum from the regenerating fin at specific time might have growth-promoting activity. This feature is of great significance in developing primary cultures within a short period.

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Effect of synthetic zeolites on xylanase production from an alkalophilic *Bacillus* sp.

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The present study pertains to stimulation of enzyme secretion in a xylanolytic alkalophilic *Bacillus* in submerged culture in media supplemented with different zeolites. Among different zeolites possessing various cations, only calcium-containing zeolites exhibited two-fold enhancement of extracellular xylanase activity. 0.5% calcium zeolite enhanced the level of activity to the same extent as 0.5% Tween 80 supplementation. The possibility of supplementing zeolite as an alternative to Tween 80 in large-scale, aerated–agitated fermentations is discussed.

IN the course of our studies to evaluate the effect of zeolites on bio-processes, we had reported enhanced ethanol production by *Saccharomyces cerevisiae* in the presence of ZSM 5 (ref. 1). In the present study we report the effect of zeolite catalysts on xylanase production by an alkalophilic *Bacillus* strain isolated in our laboratory. Recent interest in xylanases, which are cellulase-free and active at high alkaline pH, has emanated from the realization that such xylanases could be extremely useful in the pulp and paper industries for biobleaching and also in the manufacture of dissolving pulp. Besides being specific in their reactions at ambient temperatures, the use of xylanase also minimizes the use of toxic chemicals such as chlorine and chlorine dioxide which are environmentally hazardous[2]. The search for cellulase-free, alkali-stable and active xylanases of microbial origin has resulted in the discovery of several xylanases from bacteria, especially the genus *Bacillus,* actinomycetes and fungi[3–9]. Xylanases are induced enzymes secreted into the medium when grown on either pure xylan or xylan-rich agricultural residues. Addition of adjuncts such as amino acids and surfactants was shown to improve enzyme yields in production[10–12]. More recently, addition of zeolites has been shown to improve yields in amylase production[13].

Zeolites are crystalline hydrated aluminosilicates of group-I and group-II elements, in particular sodium, potassium, magnesium, calcium, strontium and barium. Zeolite structures are composed of a three-dimensional network of SiO_4 and AlO_4 tetrahedra in which a unit negative charge is associated with each AlO_4 tetrahedron. This charge must be counterbalanced by a positive inorganic or organic ion. Depending on the type of connections between tetrahedral building blocks, linear or pseudolinear channels may be joined with diameters ranging from 4.2 to 7.2 Å.

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Table 1. Effect of different zeolites on xylanase production by alkalophilic *Bacillus* (NCL 87-6-10)

Zeolite (0.5%)	Xylanase (IU/ml)
Control	46
CaA	93
MgA	47
BaA	38
NaA	50

Maximum activity was at 48 h; Medium used: Wheat bran yeast-extract.

Table 2. Effect of calcium zeolite (CaA) on xylanase production by alkalophilic *Bacillus* (NCL 87-6-10) in the presence and absence of Tween-80

Medium used	Xylanase (IU/ml)
Control	59
Control + CaA	144
Control + Tween 80	92
Control + Tween 80 + CaA	146

Maximum activity was at 48 h; Medium used: Wheat bran yeast-extract.

Sodium silicate and sodium aluminate were obtained from Rita Corporation, Mumbai. All other analytical grade chemicals for the preparation of zeolites were from Qualigens, Mumbai. All analytical and media chemicals were purchased from Hi-Media, Mumbai. Xylan (oatspelts) and 3,5-dinitrosalicylic acid (DNS) were purchased from Sigma Chemical Co, USA. Wheat bran was procured from the local market.

Alkalophilic *Bacillus* sp. (NCL 87-6-10) grown on alkaline nutrient agar xylan slants for 7 days was used as the starter culture. Culture media were dispensed in Erlenmeyer flasks and sterile sodium carbonate was added prior to inoculation to raise the pH to 9.5–10. Appropriate concentrations of zeolites were incorporated into the experimental flasks before sterilization. The vegetative inoculum was developed for 24 h in a medium containing 1% wheat bran and 1% yeast extract at pH 10. Ten per cent (v/v) inoculum was transferred to the experimental flasks containing 3% wheat bran and 1% yeast extract at pH 10 and the fermentation was carried out at 28 to 30°C at 180–200 rpm for 48 h.

Zeolite NaA (SiO_2/Al_2O_3 = 1.96) was prepared by using a standard procedure[14]. The cationic form of zeolite was prepared by using salt solutions of the respective cations (Ca, Mg or Ba), taking 5% solution (solid : solution ratio 1 : 10) and heating at 90–95°C for 8 to 10 h. The procedure was repeated three times after which the material was filtered, washed with deionized water and dried at 100–110°C. These zeolites were designated as CaA, MgA and BaA, respectively and were used in further studies.

Xylanase activity was estimated by the dinitrosalicylic acid method as described by Balakrishnan *et al.*[3] using oatspelts xylan as substrate. One unit of xylanase activity is defined as the amount of enzyme that produced one micromole xylose equivalent per minute under assay conditions (pH 8 and 50°C, 30 min).

The effect of supplementation of different zeolites at a concentration of 0.5% on xylanase production is given in Table 1. Among the different zeolites evaluated, only calcium-containing zeolite (CaA) showed approximately two-fold enhancement in xylanase activity. When the concentration of calcium zeolite was varied from 0.1 to 1.5%, it was found that a concentration of 0.5% was optimum for maximum production of xylanase and increasing its concentration further did not make any significant difference. Other zeolites did not show any increase in xylanase production. Tween 80 when added at a concentration of 0.5% was shown to enhance xylanase production in this *Bacillus*[3] and hence the effect of Tween 80 was studied in the presence and absence of calcium zeolite. Table 2 indicates that the addition of CaA produced the same level of activity enhancement as obtained with the addition of Tween 80 in the medium. Normally in submerged deep-tank fermenters, addition of Tween 80 causes serious foaming problems, leading to overflow and contamination. It appears that supplementing the medium with calcium zeolites could be a worthwhile approach to replace Tween 80.

In order to determine if the enhancement in xylanase production was due to the molecular sieving property of the zeolites, matrices such as Biogel P4 and P10 having different pore sizes were incorporated in the medium at (0.5%) levels. Since no enhancement of enzyme activity was observed, it was concluded that the molecular sieving property is not likely to be a contributing factor in the enhanced production of xylanase with zeolites. Moreover, since the channel dimensions (pore sizes) do not vary significantly with change in metal ions, the enhancement in enzyme activity due to molecular sieve effect could be ruled out. It is possible that the enhancement of enzyme activity could be due to the removal of specific ionic inhibitors from the medium. Further work is required to elucidate the exact mode of action of zeolites in enhancing xylanase production.

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Vestigial photoperiodic response in subarctic *Myrmica* ants

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Studies carried out on the effects of day-length on the development of the arctic insects are probably less because the Polar day does not provide strong photoperiodic cues. In arthropods living in the subarctic zone, where photoperiodic signals are relatively more distinct, the role of day-length in the control of seasonal life cycles has been studied only by a few workers. We performed experiments on two species of *Myrmica* ants from two populations in the Polar Circle region, which revealed a clear-cut photoperiodic response that appeared to be 'vestigial', i.e. non-adaptive and unfit to induce diapause in proper time under natural photoperiodic conditions.

IN spite of the very extensive studies on insect dormancy[1–3] relatively less is known about the control of seasonal life cycles in arctic and subarctic arthropods[4–6]. The effects of day-length on the development of insects living in the high arctic have not been studied probably because the photoperiodic cues are virtually absent during most of the summer season[5,6]. Nevertheless, such effects might exist, especially in species and populations from the subarctic zone where photoperiodic cues are a little more pronounced in summer. Ferenz[7] and Thiele[8] found that the photoperiodic response in the carabid beetle *Pterostichus nigrita* from a subarctic population differed from that of a temperate population and was clearly adaptive, i.e. had critical photoperiod corresponding to natural day-lengths characteristic of the proper season in the subarctic.

We have done experiments on two species of *Myrmica* ants from the subarctic regions. *Myrmica* colonies have 'split' brood cycles: larvae from eggs laid in summer either pupate and eclose the same summer or enter diapause, hibernate and complete their development during the following spring[9]. It has been shown earlier[10–13] that temperate populations of *Myrmica* use external ecological factors such as photoperiods and temperature together with an 'endogenous clock' to regulate the onset of diapause in larvae and the cessation of oviposition by the queens. Our preliminary experiments on subarctic *Myrmica* populations[14] showed that the development of their larvae is not influenced by photoperiods. The main aim of the present study was to test whether the day-length could influence the duration of the queen oviposition and whether onset of the queen diapause could be really controlled by natural photoperiods in subarctic populations.

Colonies of *M. ruginodis* and *M. scabrinodis* were collected in June on the Western coast of the White Sea just on the Polar Circle, near Poyakonda (66°33′N) and near Chupa (66°15′N). The cultures, each consisting of 150 workers, one queen and 30–50 overwintered larvae, were maintained in photothermostatic chambers at an optimal temperature (22.5 ± 1°C or 12-h daily thermoperiod 15/25°C) and long-day photoperiods (23 or 24 h light per day depending on the experimental set-up) until the middle of July. Then they were transferred to three photoperiodic regimes (under the same temperature): *Mid-summer day-length* (23 or 24 h), simulating the situation in nature during the Polar day, *August day-length* (18 or 17 h), corresponding to natural conditions in the middle or at the end of August, and *Late-autumn day-length* (12 h) observed in the Polar Circle region at the beginning of October.

Almost all overwintered larvae pupated regardless of the photoperiod. However, no new larvae hatching from the eggs laid by the queens completed their development – all of them entered diapause. Thus, photoperiods control neither the development of overwintered larvae nor the induction of diapause in new ones. In fact diapause appears to be obligatory for all larvae originating from eggs deposited during summer. This is in accordance with our preliminary results[14] and might be one important trait distinguishing subarctic *Myrmica* populations from those living in more southern regions.

The day-length exerted a distinct effect upon the duration of oviposition and the time of onset of diapause in the queens of both species (see examples in Figure 1). Late-

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