There are two models of mutations associated with influenza virus, namely antigen shift and antigen drift[4].

The H5N1 genome has eight segments (1–8), each segment codes for a protein: polymerase* (PB2), polymerase (PB1), polymerase (none), hemagglutanin (none), nucleocapsid (none), neuraminidase (NA), matrix protein 2(M) and nonstructural protein respectively.

Lysine (K) at position 627 of H1N1 (1918) has mutated to glutamic acid (E) in H5N1 (K627E), which is known to be pandemic for humans. This finding has resulted in a new approach of vaccine treatment for bird flu.

We notice one more mutation at position 628 which changes glutamine(Q) in H1N1 to proline(P) in H5N1 (Q627P), but this mutation has no effect on the protein function.

H1N1 *PB2* gene 'A(623)APPK(627)Q-(628)S' changes into 'A(623)APPE(627)-P(628)S'.

There are two domains present in segment of H5N1 genome, whose Prodom Domain ID is PD001667 and PD217887. However, position 627 comes under domain PD001667.

Secondary structure of segment 1 of H5N1 and H1N1 is almost similar according to the SOPMA secondary structure prediction. There are minor changes found in the extended strands and random coils, where the alpha helix is the same in H5N1 and H1N1.

Results have shown that the protein sequence of H5N1 is close to H1N1, but there are minor changes found due to a single mutation. Deletion of one amino acid at 627 is mainly responsible for the mutation. This leads to the new approach of vaccine treatment for bird flu. There are two domains found in segment 1 of H5N1 genome. The result of SOPMA shows that there are 284 alpha helices, 176 extended strands, 66 beta turns and 233 random coils present in the secondary structure.

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Isolation of halotolerant *Penicillium* species from mangroves and salterns and their resistance to heavy metals

Though it was believed that microbial communities at high salinities are dominated exclusively by archaea and bacteria and the eukaryotic species *Dunaliella salina*, studies on the microbial diversity in hypersaline environments revealed the presence of melanized fungi, 'considered as a new group of eukaryotic halophiles'[1], halotolerant black yeast and several other filamentous fungi[2–4], including *Penicillium* spp.[5].

Water bodies often become sinks for disposal of waste from effluent-treatment plants or run-offs from landfills, particularly during the rainy season. Much attention is given to heavy metal pollution because metals cannot be decomposed by *in situ* biological means[6]. Micro-organisms have been used at low costs to remove metals from effluents[7], with fungi known to be more tolerant to metals than bacteria or actinomycetes[8], and the *Penicillium* spp. being prominent[8–12] among these.

Although isolates of halophilic penicillia are reported from hypersaline environments, there has been little work done on this group of fungi from the coastal waters of Goa, India. Further, although the genus *Penicillium* has been studied and used for metal tolerance/removal, it has not been examined with respect to halophilic/halotolerant species possessing metal resistance. Here we report the isolation of extremely halotolerant penicillia from the mangroves and salterns of Goa; these species have the characteristic of being resistant to heavy metals such as Pb^{2+}, Cu^{2+} and Cd^{2+}.

Water samples were collected from a well (W) close to a copper-smelting plant and to the mouth of a river, from mangroves (M) and from solar salterns (S). These were filtered through a 0.45 µm filter, which was then placed over Czapek Dox Agar (CDA) containing 2% salt (S-CDA) for well-water and mangrove samples, while saltern samples were grown on CDA with 5% salt; observations were carried out for growth at room temperature (RT), i.e. 30°C. *Penicillium* species were selected and purified on isolation media containing 1 mM lead nitrate and maintained on S-CDA with lead.

The isolates were spot-inoculated onto CDA containing NaCl concentrations of 0, 2.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0% (w/v) and assessed for growth in terms of colony diameter at RT up to 7 days for three sub-cultures.

The isolates were then spot-inoculated on S-CDA containing 0–10 mM Pb^{2+} as $Pb(NO_3)_2$, or 0–5.0 mM Cu^{2+} as $CuSO_4 \cdot 5H_2O$, or Cd^{2+} as $3CdSO_4 \cdot 8H_2O/Cd(NO_3)_2 \cdot 4H_2O$ and monitored for growth as above.

A total of forty-eight isolates were obtained from the samples screened, among which 12 belonged to the genus *Penicillium*: one from well-water, denoted as WP1, three from mangroves: MP2–MP4, and eight from salterns: SP5–SP12. SP10 and SP11 were monoverticillate; MP1, SP5–SP8 were biverticillate symmetric; MP2, MP3, SP9 and SP12 were biverticillate asymmetric and MP4 was triverticillate.

All penicillia showed extreme halotolerance: WP1, MP2–MP4, SP5–SP7 had maximum tolerance at 17.5% NaCl, SP8 15% NaCl, and SP9–SP12 10% NaCl, and all cultures were able to grow even in the absence of salt (Figure 1). Optimal growth of WP1 and SP5–SP12 was obtained at 0–2% salt, while that of WP1 and SP10 was at 2% salt, and MP2–MP4 at 2–5% salt, displaying halophilic nature. Decrease in growth with respect to increasing concentrations of saline up to 17.5% NaCl was gradual in WP1, MP2–MP4 and SP5–SP7, while a rapid 50% biomass reduction was observed in the remaining isolates from the salterns (SP8–SP12) with every 2.5% increase in salt concentration. There was also a lag in growth as well as a decrease in sporulation in all isolates with an increase in salt concentration.

All cultures were resistant to Pb^{2+} at a concentration of 7.5 mM; most could tolerate either Cu^{2+} or Cd^{2+} as sulphate or as nitrate salt, with MP3 and MP4 showing resistance to all the heavy metals tested (Figure 2). WP1, MP2–MP4 and SP6 tolerated up to 3 mM Cu^{2+} and MP4 up to 5 mM Cu^{2+}. WP1, SP5 and SP11 could resist Cd^{2+} as $CdNO_3$ but not as $CdSO_4$, while MP2, MP4 and SP10 could resist only $CdSO_4$; MP3 and SP7 tolerated both $CdNO_3$ and $CdSO_4$; SP8, SP9 and SP12 could not tolerate either Cu^{2+} or Cd^{2+}. Lag in growth and decrease in sporulation were also seen with respect to increasing metal concentration.

Penicillium spp. were seen to be one of the predominant groups amongst the halotolerant fungi from the mangroves and salterns of Goa, with all twelve *Penicillium* isolates obtained showing high halotolerance. Tolerance to 25% NaCl by *Penicillium* spp.[13] and the presence of halotolerant black yeast rather than halophilic cultures from saltpans[5], have also been reported. Further, some of the cultures showed halophilic nature, distinguished by the requirement of hypersaline conditions for growth[14,15], while even minimal addition of NaCl slowed down the growth rate of halotolerant species[15]. Optimal growth of some isolates at salt concentration of 2% or more indicated these isolates to be of marine origin.

The isolates demonstrated resistance to the heavy metals tested, with resistance to lead at a high concentration of 7.5 mM being common to all. Those from mangroves showed higher resistance to copper and cadmium sulphate than those from
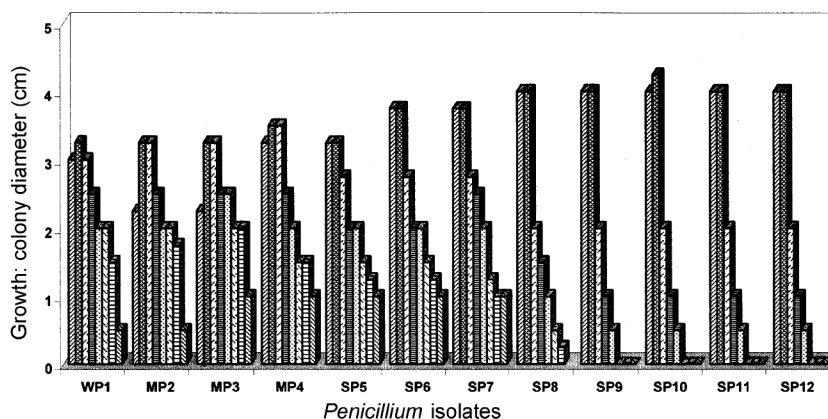

Figure 1. Growth of cultures in the presence of NaCl at: □, 0; ■, 2; □, 5; ■, 7.5; □, 10; □, 12.5; □, 15 and □, 17.5% concentration.
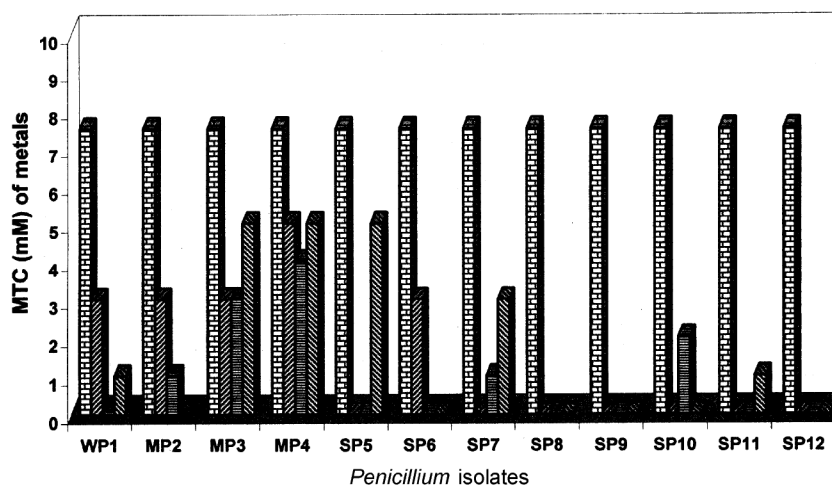

Figure 2. Maximum tolerance concentrations (MTC) of metals on cultures: ■, Pb^{2+} [$Pb(NO_3)_2$]; ■, Cu^{2+} [$CuSO_4$]; ■, Cd^{2+} [$CdSO_4$], and □, Cd^{2+} [$Cd(NO_3)_2$].

salterns, while resistance to cadmium nitrate varied amongst the isolates. Tolerance to metals differed for each of the *Penicillium* species involved, indicating a difference in the resistance level among species of the same genus. Interestingly, the triverticillate species MP4 was not only resistant to all the heavy metals tested, but also showed highest resistance levels amongst all the *Penicillium* species screened, especially to copper and cadmium which are more toxic metals.

The diversity of micro-organisms in hypersaline environments is of growing interest. Organisms able to grow under extreme environments offer good potential as indicators of pollution and as biosorbents[14], and in applications for bioremedial measures. Many natural geological formations, such as petroleum reserves, are associated with hypersaline brines.

Industrial processes also use salts and frequently release brine-effluent into the environment[14]. These extremely halotolerant *Penicillium* species that are able to grow at high concentrations of salt as well as in its absence, while also possessing high resistance to heavy metals, could be used as agents for abatement of metal pollution in hypersaline conditions or in waters of fluctuating salinity, as well as in non-saline environments.

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Capers: A food for Upper Cretaceous dinosaurs of Pisdura, India

Pisdura, a well-known Upper Cretaceous dinosaur locality in Warora district, Maharashtra is known for reptilian coprolites. Based on their external morphology, coprolites can be grouped under four main types (A, B, Ba, C)[1]. Coprolites described by various workers[1–3] from this area contain exclusively vegetal matter and their association with titanosaurid sauropod skeletal remains reflects that these animals were the producers[1,4–9]. Though recovery of plant remains from these coprolites is scarce and fragmentary, a few records of megaflora (dicots and monocots, including pollen and cuticles) have also been described[5,6,8]. Presence of monocotylednous seeds belonging to the family Arecaceae was reported earlier[10]. Recovery of grass phytoliths from all known categories of coprolites mentioned above suggests that Late Cretaceous titanosaurid sauropods of Pisdura were grass-eaters and grass was present during the Late Cretaceous[9]. Occurrence of chelonians has been reported from the Lameta Formation of Pisdura[11,12]. It is likely that other carnivorous reptiles also must have been thriving in the same phytozones.

The present fossil coprolite described here was recovered from Pisdura, 3 km east of Temurda village in Maharashtra (Figure 1). The locality falls under the Dongargaon–Pisdura area where the sediments are best exposed at Pisdura for recovery of coprolites. The formation is overlain by Deccan volcanic rocks associated with thin intertrappean beds. The Lameta Formation chiefly comprises red and green silty clays, planar and cross-bedded sandstones, grey marls and yellow laminated clays and shales interbedded with marlites and limestones. The formation rests over Precambrian rocks[13,14]. The coprolite which is a broken part of some bigger specimen (probably type-A)
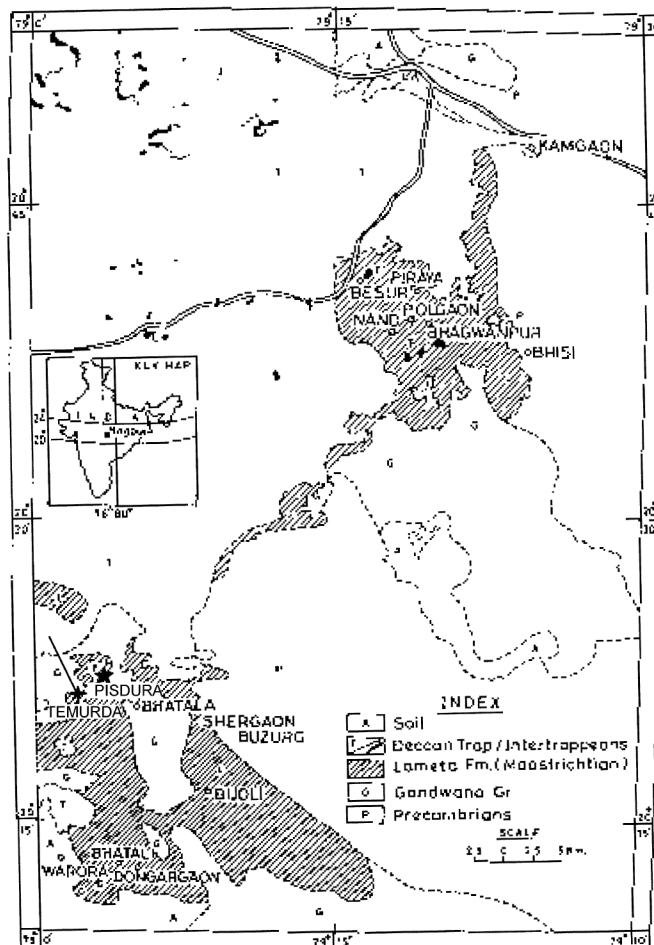

Figure 1. Map showing location and lithology of the area from where coprolites were collected (after Mohabey[13]).