Discussion

The Phosphate Solubilizing Microorganisms were isolated from rice rhizosphere soil of Mannachanalur area, Trichy district, Tamilnadu. by using Pikovskaya agar. Zone formation around the colony in Pikovskaya agar is the basic criteria for the selection of Phosphate Solubilizing Microorganisms. The earlier of reports were available for screening of Phosphate Solubilizing Microorganisms, using Pikovskaya agar. (Hardy et al., 1998; Puente et al., 2004) showed that the bacteria isolated from the rhizoplane of desert plants, was very effective in dissolving Calcium phosphates on solid medium.

Phosphate Solubilization

In the present study, the phosphate solubilization efficacy of isolated fungal strains was higher when compared to the bacterial strains in general. These results were in coincidence with the earlier findings of Arora and Gaur (1979);Kucey (1984). Among the fungal strains isolated in the present study, the *Aspergillus niger* was found to be predominant phosphate solubilizer followed by *Penicillium* sp., *Pseudomonas* sp. and *Aspergillus fumigatus*. This may be due to the production of more organic acid in culture medium by the Phosphate Solubilizing Microorganism. The more organic acid produced in the culture medium decrease the pH which is the key indication for more phosphate solubilization. The Ammonium Sulphate is a nitrogen source of Pikavskaya’s medium, the acid production in response to the assimilation of cations such as ammonium is well known fungal phenomenon. The uptake of ammonium by fungi in a liquid medium commonly leads to a rapid drop in pH of the medium. Whitelaw et al. (1999) reported that the soluble phosphate concentrations in the culture medium was directly proportional to organic acid concentration and inversely related to pH. The main mechanism for phosphate solubilisation by
Penicillium radicum was acid production leading to a decrease in pH. Halder et al. (1991) reported the phosphate solubilization was related to pH decrease caused by the Bradyrhizobium strains. Cerezine et al. (1988) reported that the soluble phosphate levels were correlated with pH of the culture medium, probably due to metabolic activity of Aspergillus niger resulting from consumption of sugar in the culture medium. Pradhan and Sukla. (2005) described that the Aspergillus sp. solubilized higher phosphate comparing Penicillium sp from Tricalcium Phosphate. Drop in pH during growth was more prominent in absence of Tricalcium Phosphate in the liquid medium. This indicates that absence of soluble phosphate in media induces the acid production.

Illmer et al. (1995) stated that the production of detectable amount of organic acids by A. niger is an important factor for phosphate solubilization mechanism. Nevertheless, organic acids alone are involved, although they are less effective compared to biotic leaching. Narsian and Patel (2000) explained that the acidic pH was accompanied with phosphate solubilization Aspergillus aculeatus in all the experiments and concluded no specific correlation could be established between maximum phosphate solubilization, growth or pH. Seshadri et al. (2004) also stated that the release of phosphate by Aspergillus niger in the liquid culture was associated with reduction in pH of culture medium. Barroso et al. (2006); Achal et al. (2007) studied the relationship between phosphate solubilization, extensive growth, acid production, and decrease in pH. They concluded the phosphate solubilization was related to acid production, pH drop and Aspergillus thubingensis growth in the culture medium.

Sperber (1957), Das (1963), Sethi and Subba Rao (1968) and Ahmed and Jha (1968) were reported there is no significant relationship could be established between the quantity of phosphate solubilized and drop in pH. The results of pH
drop also support the data of phosphate solubilization by *Pseudomonas, Bacillus* and *Rhizobium*, which is associated with acidification of the culture medium, but the extent of phosphate solubilization and pH drop are not proportionally correlated (Rodriguez and Fraga, 1999).

Bolan *et al.* (1994), Sagoe *et al.* (1997), Alam *et al.* (2002), and Kumari *et al.* (2008) have reported that Malic, Tartaric, Oxalic, and Citric acids have high capability to release soluble phosphate from insoluble Tricalcium Phosphate as well as Rock Phosphate. Kang *et al.* (2008) suggested that the solubilizing ability is not related to organic acid produced but to the nature of organic acid produced by *Aspergillus* sp. Kapri and Tewari (2010) highlighted the comparative phosphate solubilizing potential of *Trichoderma* sp. isolated from Pinus, Deoder, Bamboo and Oak rhizospheres. It was found that all the isolates were capable of differentially utilizing Tricalcium Phosphate from liquid medium. This was indicated by the soluble phosphate concentrations and increase in acidity of the growth medium.

In the present study, the phosphate solubilization efficacy of the wild strains was observed in the descending order of *Aspergillus niger* > *Penicillium* sp. > *Aspergillus fumigatus*. Among the UV irradiated fungal strains, ANuv60 was found as the predominant phosphate solubilizing strain followed by ANuv50, PEuv60, ANuv30, ANuv40, PEuv50 and AFuv50. In chemical mutagenesis of phosphate solubilizing strains, ANems120 showed the highest phosphate solubilization activity followed by ANsa120, PEsa150, PEems150, ANsa90, ANems90, PEsa120, ANsa60 and AFsa120. Achal *et al.* (2007) reported a significant increase in soluble phosphate level was observed in case of UV induced mutants of *Aspergillus* strains compared with wild strains. The present study also revealed that UV induced mutant enhanced the phosphate
solubilization compared with wild strains. There might be a possibility of alteration at genetic levels in case of mutants. Tripura et al. (2007) reported that the Ethyl Methane Sulphonate treated microbial strain *Serratia marcescens* have increased phosphate solubilization efficiency compared to the wild strains. Reyes et al. (2001) studied that phosphate solubilization of two UV induced *Penicillium rugulosam* along with wild strain. Among the studied strains, MP++ strain solubilized high amount of phosphate and they stated that the solubilization depends on the type of phosphate source.

In the present study, UV induced *Penicillium* sp. showed less solubilization when compared to UV induced *Aspergillus niger* and the same showed high solubilization when compared to wild *Penicillium* sp. This may be due to more production of organic acid, Acid Phosphatase and Phytase enzyme by mutant strains, which was supported by Achal et al. (2007). The mechanisms of action of mineral phosphate solubilization (MPS) were studied in the wild-type Mps+ *Penicillium rugulosum* strain and in negative (Mps−) and superpositive (Mps++) mutants derived from it. MPS activities were measured in liquid media using Sucrose as carbon source and nitrite, ammonium plus nitrate, ammonium and arginine as nitrogen sources. Ammonium significantly decreased phosphate solubilization, and this activity was higher in the Mps++ mutant than in the wild-type depending on the N sources used. The Mps+ phenotype was strongly associated with the production of gluconic or citric acids. The results also suggest for the MPS− mutant the involvement of the H+ pump mechanism in the solubilization of small amounts of phosphates. (Reyes et al. 1999). Vassileve et al. (1998) reported the encapsulated spores of *Aspergillus niger* solubilized Rock Phosphate in the culture medium due to the production of organic acids.
In the present study, *Pseudomonas* sp. was found to be the lowest phosphate solubilizer, when compared to the fungal strains. The UV and chemical mutated *Pseudomonas* strains were showed decreased phosphate solubilizing capacity when compared to wild strains. These results were in acceptable with the findings of Miller *et al.* (2010), who stated that mutants with showed calcium phosphate solubilization ability. Mutations in the *gcd* and *pqqE* genes greatly reduced the solubilization ability, whereas mutations in the *pqqB* gene only moderately reduced this ability. The combination of biochemical analysis and genomic comparisons revealed that alterations in the *pqq* biosynthetic genes, and the presence/absence of the gluconate dehydrogenase (*gad*) gene, fundamentally affect phosphate solubilization in strains of *P. fluorescens*. Goldstein and Liu (1987) have shown that mineral phosphate solubilizing activity by *Erwinia herbicola* is genetically coded in a gene cluster on the plasmids of microorganisms possessing this activity. It was also reported that gene expression and mineral phosphate solubilization of bacteria is affected by the presence of soluble phosphate due to feedback regulation. Kumar *et al.* (2001) suggested that it is possible to select physiologically efficient strains of *A. chroococcum* through mutagenesis starting from soil isolates and that microbial inoculants can be used as an economic input to increase crop productivity with lower fertilizer levels.

The present study indicates that the solubilization efficacy of the fungal strain *Aspergillus niger* treated with Sodium Azide (ANsa120) and *Penicillium* sp. treated with Ethyl Methane Sulphonate (PEems150) were about 1.5-fold higher when compared to wild strain. The chemical mutagenic agents such as Sodium Azide, Ethyl Methane Sulphonate may alter the gene sequence by altering the base pairs. The azide ion alters the structure of cytosine such that it forms hydrogen bonds with adenine, rather than guanine. This produces a
cytosine to thymine transition. Ethyl Methane Sulphonate is a strong mutagenic agent. It alkylates N7 of Guanine and severely alters the base pairing.

The phosphate solubilization efficacy of mutant fungal strains (ANems120, ANsa120 and PEsa150) were demonstrated in the presence of various carbon and nitrogen sources. The effect of different carbon sources on phosphate solubilization efficiency of fungal strains was found to be in the ascending order Mannitol < Lactose < Sucrose < Glucose. This is in disagreement with the findings of Seshadri et al. (2004) who reported that the Mannitol was the best carbon source utilized by *Aspergillus niger* preferred for higher phosphate solubilization. The present study revealed that Mannitol was found to be the poorest carbon source and the Urea as one of the predominant nitrogen sources for phosphate solubilization by *Aspergillus niger*. This in confirmation with the findings of Seshadri et al. (2004) who reported the nitrogen in the form nitrate was very effective and Urea was the poorest source of nitrogen by *Aspergillus niger*. This may due to the genetic alteration in the fungus due to chemical mutation. Reyes et al. (1999) studied that the Sucrose appeared to be the best carbon source for phosphate solubilization by UV induced *Penicillium rugulosum*. Nautiyal et al. (2000) reported that the Glucose and Lactose were the best carbon source and Sucrose, Sorbitol were identified as poor carbon source for phosphate solubilization, ammonium and nitrate source to be equally effective for phosphate solubilization. This is in contrary with the earlier report of Halder *et al* (1991) who stated that Glucose decreased the medium pH most and caused highest solubilization of phosphate followed by arabinose, sodium acetate, sodium gluconate and sodium succinate. Mohamed *et al* (1985); Abd-Alla (1994) reported that ammonium salts induced phosphate solubilization whereas the nitrate salts repress the phosphate solubilization activity.
Similarly, in the present study, the addition of various nitrogen sources in the medium to determine their effect on phosphate solubilization efficiency of the inoculated strains (ANems120, ANsa120 and PEsa150) gave the following results in the ascending order: Sodium Nitrate < Potassium Nitrate < Urea < Ammonium Sulphate. Whitelaw et al. (1999) reported that the Penicillium strain solubilized the insoluble phosphate in the culture medium containing ammonium or nitrate as sole source of nitrogen. Vassileve et al. (2007) compared the phosphate solubilization efficacy of Aspergillus niger by using corn steep liquor, Ammonium Sulphate and yeast extract as nitrogen source. The phosphate solubilization activity of Xanthomonas campestris was measured in both the wild type and mutant strains using various carbon and nitrogen sources. Glucose was found to be the best in both (wild 39.9%; mutant 67.1%) strains followed by Sucrose (46.8%) in the mutant and molasses (36.0%) in the wild type. Ammonium Sulphate was the best nitrogen source for both the strains, followed by Urea. (Sharan et al., 2008). Son et al. (2006) investigated the effect of carbon sources on the insoluble phosphate solubilization of Pseudomonas species. It solubilized insoluble phosphate with all carbon sources except Adonitol and Lactose. They also studied all nitrogen sources except Sodium Nitrite, increased the level of soluble phosphate production. Among the carbon and nitrogen sources used, Glucose and Ammonium Nitrate was the best carbon and nitrogen source respectively for the insoluble phosphate solubilization by Pseudomonas species. In all cases insoluble phosphate solubilization was accompanied by a distinct pH decrease.

Cerezine et al. (1988) reported the soluble phosphate levels were correlated with pH of the culture medium probably due to metabolic activity of fungus resulting from consumption of sugar in the Aspergillus niger culture medium. Fructose, Glucose, xylose and Sucrose were the carbohydrates that
favored ‘P’ solubilization when compared to Galactose and Maltose. Among the nitrogen sources tested ammonium salts favored the production of larger amounts of soluble phosphate than peptone, Urea and Nitrate. The results were positively correlated with the present study. Phosphate solubilization was decreased with decreasing Glucose concentration; Solubilization was increased when the nitrogen source was Nitrate by the soil fungus *Aspergillus niger* (Hardy *et al*. 1998). The present study revealed that the carbon source Glucose and nitrogen source Ammonium Sulphate were the best for phosphate solubilization, which confirm results of earlier reports. Goenadi *et al*. (2000) have reported that the accumulation of more insoluble phosphate using *Aspergillus niger* and Glucose as the carbon source. Illmer and Schinner (1992) have shown that the accumulation of high insoluble phosphate using Glucose utilizing *Penicillium* sp. and *Pseudomonas* sp. respectively. According to Srividya *et al*. (2009) the *Aspergillus niger* utilized the nitrogen salts having either ammonium group or nitrate group or both as nitrogen source. Ammonium Sulphate was found to be best in reducing the medium pH and simultaneous solubilization of phosphate, out of the entire nitrogen sources used and observed that *Aspergillus niger* was able to utilize Ammonium Sulphate most efficiently to decrease the pH of the medium for phosphate solubilization. Fasim *et al*. (2002) have reported that the bacterial isolates from the air environments of tannery solubilize the Zinc Phosphate only in presence of Glucose. Disimmine, (1998) reported solubilization of Zinc Phosphate by *Pseudomonas fluorescens* only in presence of Glucose and slight solubilization in presence of mannose while no solubilization was detected in presence of Gluconate, Galactose, Glycerol, Sorbitol and Fructose.

The present study reported that the poorest carbon source and nitrogen source were Mannitol and Sodium Nitrate respectively for phosphate
solubilization. The results of present study were negatively correlated with Barroso et al. (2006) who stated that solubilization of CaHPO₄ was enhanced by Aspergillus niger when the carbon source was Mannitol, Maltose, Galactose and Glucose (in that order) and this was positively correlated with the present study when evaluating with nitrogen sources. The solubilization of CaHPO₄ or AlPO₄ decreased in the following order: Glycine > NH₄Cl > NaNO₃ and NH₄NO₃ > Urea > (NH₄)₂SO₄, respectively. Ammonical nitrogen (NH₄⁺-N) sources were the most effective in the production of acids and in lowering of the pH.

Previous reports on phosphorus solubilizing microorganisms like Ectomycorrhizal fungi by Lapeyrie et al., (1991) who attributed the differences in phosphate solubilization (when ammonium and nitrate were used) to the use of different mechanisms for the generation of acidity in the culture. Production of organic acids is an important mechanism, but is not the only possibility for phosphate solubilization. The other mechanism involved in solubilization of insoluble phosphates is extrusion of H⁺ ions during ammonium ions assimilation by Parks et al. (1990) for Penicillium like fungus, Illmer and Schinner (1995) for Pseudomonas sp. and Penicillium aurantiogriseum Illmer et al. (1995) for Aspergillus niger, Penicillium simplicissimum, Pseudomonas sp. and Penicillium aurantiogriseum. In the present study, the phosphate solubilization using ammonium salts as the nitrogen source was substantially higher than with other nitrogen sources. Thus, the results suggested the second mechanism which acidification of the medium by proton extrusion during ammonium ions was involved in phosphate solubilization by these isolates.

Phosphatase

The Phosphatase production capacity of isolated bacterial and fungal cultures were analysed by inoculating the bacterial and fungal isolates into
pikovskaya broth. In the present study, para nitro phenyl phosphate was used as a substrate for Acid Phosphatase assay. The earlier reports were available for the utilization of para nitro phenyl phosphate as a substrate for Acid Phosphatase assay (Prada et al. 1996; Puruchothaman, 1994).

In the present study, the Phosphatase production efficacy of the wild strains were observed in the descending order of *Aspergillus niger* > *Pseudomonas* sp > *Penicillium* sp. > *Aspergillus fumigatus*. The UV irradiated and chemical treated bacterial strains in the present study showed that decrease in Phosphatase production, when compared to wild strains. Among the UV irradiated fungal strains, ANuv60 was found the best Phosphatase producing strain followed by ANuv50, ANuv30, ANuv40, AFuv50, PEuv50, AFuv60, PEuv40, PEuv60, PEuv30, AFuv40 and AFuv30.

Among the chemical treated fungal strains, ANsa120 was the predominant fungal strain followed by ANems120, PEems150, ANsa90, PEsa150, ANems90, PEsa120, AFsa120, PEems120, PEems60, PEems90, ANems60, AFsa120, AFsa90, PEsa90, ANsa30, AFems120, ANems120, ANems30, AFems90, PEsa60, AFsa60, AFems60, AFems30 and AFsa30. The result of the present study was in agreement with Relwani et al. (2008) who reported that the enzyme activities such as Acid Phosphatase and Phytase was increased significantly in mutants of *A. tubingensis* when compared to the wild type. Achal et al. (2007) reported that Acid Phosphatase production by UV induced mutants of *Aspergillus tubingensis*, might be the reason for highest phosphate solubilization by mutant strains. The production of the Acid Phosphatase was favoured by low pH values.
Among all physical and chemical treated fungal strains, the best Phosphatase producing strains such as ANsa120, ANems120 and PEems150 were demonstrated in the presence of various carbon and nitrogen sources in the present study. The effect of different carbon sources on Phosphatase production efficacy of fungal strains were found to be in the ascending order of Mannitol < Lactose < Sucrose < Glucose. Andrea et al. (1990) reported that *Pseudomonas aeruginosa* and *Rhizobium meliloti*, utilized several choline derivatives as the sole carbon and nitrogen source and increased the production of Acid Phosphatase activity. Spicers and McGill (1979) demonstrated an increase in Phosphatase activity during incubation of soil amended with Glucose and Ammonium Sulphate.

The effect of different nitrogen sources on Phosphatase production efficacy of fungal strains were found to be in the ascending order of Sodium Nitrate < Potassium Nitrate < Urea < Ammonium Sulphate in the present study. The results were in agreement with Semenova et al. (1986) who stated that the synthesis of the secreted enzyme depended on the sources of carbon and nitrogen nutrition. The enzyme yield was highest in a medium with Sucrose as a carbon source and Ammonium salt as a nitrogen source. The secretion of Acid Phosphatase is stimulated by an increase in the sugar content and a deficiency of the nitrogen source in the medium.

Semenova *et al.* (1986) studied that Acid Phosphatase production from *Saccharomyces cerevisiae* in presence of phosphate concentration of 10 mM which showed repression of the Phosphatase production. Sudha and Purushothaman (2000) reported that *Pseudomonas* strain isolated from the freshwater pond system showed repression. Casida (1959) isolated several species of *Aspergillus* sp. capable of producing Acid Phosphatase active against
organic phosphate in soils. Smith et al. (1973) reported the addition of gradient amount of Potassium phosphate to Phosphatase test broth was the least inhibitory to Phosphatase activity by Candida tropicalis. Sarapatka et al. (2004) stated that the increasing amount of available phosphate increasing the Phosphatase activity in cereal roots. Pedregosa et al. (1991) reported that Aspergillus strains produce non repressible, repressible and phosphate inducible Phosphotases. It is accepted that culture condition may cause fluctuations and multiplicity of fungal Phosphatases. Ponmurugan and Gopi (2006) stated that there was a positive correlation between phosphate solubilizing capacity and Phosphatase enzyme activity. These earlier reports were in agreement with present results which showed that the increase in inorganic phosphate concentration due to phosphate solubilization were not repressed the Phosphatase production by all strains used in this study. But disagreement with the earlier reports of Semenova et al. (1986) who studied that Acid Phosphatase production from Saccharomyces cerevisiae in presence of phosphate concentration of 10 mM which showed repression of the Phosphatase production. Ohta et al. (1968) stated that the production of Phosphatase by the black koji fungus Aspergillus awamori is a repressible enzyme.

The earlier reports explained that there are two systems of regulation in Phosphatase induction in microorganisms. This may be the reason for repressible and non repressible nature of Phosphatases by inorganic phosphate. Singleton and Sainsberry (1988) explained that about the phosphate controlled genes or pho genes, which is the gene cluster, consist of Pho A encodes Phosphatase, Pho S encodes inorganic phosphate binding site, PhoE encodes porin and Pho B encodes the regulator gene as like the operon model. Wagner et al. (1995) reported that Synechococcus strain system of phosphate irrepressible Acid Phosphatase in addition to the normal repressible system.
Lipase

The Lipase production capacity of isolated bacterial and fungal cultures were analysed by inoculating the isolates in Czapek’s dox broth enriched with 1% Olive oil as an inducer and the same were inoculated in Czapek’s dox broth replaced carbon source as 10% Olive oil for Lipase production. The number of reports were available for the addition of inducer to induce the production of Lipases. Salihu et al. (2011) utilized Olive oil and Palm oil as an inducer. Olive oil by Rifaat et al. (2010); Hosseinpour et al. (2011); Coconut oil by Rani and Panneerselvam (2009) and different lipid sources namely Olive oil, Coconut oil, Groundnut oil, Triacetin, Tributyrin and Surfactants namely Tween 20, Tween 40, Triton –X was used for acceleration of Lipase by Pogaku et al. (2010). It was suggested that the Lipase formed might be derived from carbohydrates in the medium with small quantities of lipid material as inducer (Minoda and Ota, 1980; Iwai et al., 1973).

In the present study, the Lipase activity of isolated bacterial strains were higher compared to the fungal strains in the present study. Rani and Panneerselvam (2009) showed that Aspergillus fumigatus, A. terreus, Penicillium chrysogenum, P. funiculosum and Fusarium moniliforme were selected as the highest Lipase producers. In the present study, the Lipase production efficacy of the wild strains was observed in the descending order of Pseudomonas sp.>Aspergillus niger >Penicillium sp. > Aspergillus fumigatus. Juichiro et al. (1963) reported that several strains of Rhizopus and Asphergillus niger as most potent Lipase formers. Successful isolation of the enzyme could be made from the bran-koji culture of Aspergillus niger. Ogundero (1981) stated that A. fumigatus and A. nidulans were able to degrade vegetable oils and triglycerides.
Among the UV irradiated bacterial strains in the present study, PSuv3 was found to be the predominant Lipase producing strain grown in both Sucrose and 10% Olive oil containing medium followed by PSuv4, PSuv2 and PSuv1. Among the UV irradiated fungal strains, ANuv50 was found the best Lipase producing strain grown in both Sucrose containing medium followed by AFuv50, ANuv40, ANuv60, ANuv30, AFuv60, PEuv60, PEuv50, PEuv40, AFuv40, PEuv30 and AFuv30.

Lawrence et al. (1967) studied the Lipase activity was considerably increased by nutritional and physical conditions from Pseudomonad. Suzuki et al. (1988) used the Olive oil as a carbon source for microbial growth and Lipase production by Pseudomonas sp. Ray et al. (1999) reported the Lipase activity of the isolated bacterial strain, Corynebacterium sp. was increased 2.3 fold by mutagenic technique. This was agreed with our present study, since all mutant strains tested were found to produce more Lipase activity, when compared to wild strains. The present study also indicated, the strain Pseudomonas sp. (PSeems120) treated with Ethyl Methane Sulphonate, produced 2-fold higher Lipase activity when compared to wild strain. PSeems120 which was the predominant Lipase producing strain, among all the strains used in the present study and the bacterial strains PSeems150, PSeems90, PSSa90 and PSSa120 were followed the strain PSeems120. ANems150 was the best strain for Lipase production among the chemical mutated fungal strains followed by ANems120, AFems120, Ansa90, AFems150, AFems90, ANsa60, PEsa120 and PEems150 grown in Sucrose containing medium. Ellaiah et al. (2002) isolated a fungal strain, which produced Lipase constitutively was used to produce mutants using physical and chemical agents and the mutant strain with Lipase productivity of 2-fold higher was obtained. Bapiraju et al. (2004) reported that the the UV and chemical treated fungal strains of Rhizopus sp. showed 133% to 232% higher
than the wild strains. Caob and Zhanga (2000) reported that the Lipase production of UV and chemical treated *Pseuomonas* mutant 3.25 fold higher than the wild strain.

The present study stated that the mutant strain PSems120, ANsa90, PSsa90, AFuv50 and PEsa120 showed 2 fold and PSems150, PEuv40, PEsa90, AFsa120 and PSsa90 showed 1.5 fold enhanced productivity of enzyme, over the wild strain. This was in agreement with findings of Mahadik *et al.* (2004), who reported that Mutant strains of *Aspergillus niger* showed seven to five fold enhanced productivity of Lipase, over the wild strain. Mala *et al.* (2001) isolated UV and nitrous acid derived mutants of *A. niger* selected on media containing bile salts. Nitrous acid mutants exhibited increased efficiency of Lipase production compared with wild strain. Mutation alters the genotype of microorganisms, when it expresses that leads to alter the character or death of microorganisms. The ultra-violet radiation forms thymine dimer in gene sequence. But the photolyase enzymes present in living system break the thymine dimer and correct it. The increasing exposure time to UV radiation may forms the thymine dimer in gene sequence that code photolyase enzyme. In this situation the thymine dimer can not break by the enzyme of living system (Freifelder, 1990; Radman, 1999). The chemical agents such as Sodium Azide, Ethyl Methane Sulphonate may alter the gene sequence by altering the base pairs. The azide ion alters the structure of cytosine such that it forms hydrogen bonds with adenine, rather than guanine. This produces a cytosine to thymine transition. Ethyl Methane Sulphonate is a strong mutagenic agent. It alkylates N7 of Guanine and severely alters the base pairing.

In the present study, all the bacterial strains were showed less Lipase activity, when compared to fungal strains grown in 10% Olive oil containing
medium and moreover all the strains, grown in 10% Olive oil containing medium were showed poorest Lipase activity. This may due to the inoculated strains failed to utilize more 10% Olive oil as a carbon source for their growth and subsequently reduction in Lipase activity. This was in agreement with Mahadik et al. (2004) who stated, higher concentration of oil in the medium did not help Lipase production in the case of mutant. On the other hand, Sucrose, Lactose, Mannitol and Glucose may support the growth initially and the inducer 1% Olive oil induced the inoculated strains for more Lipase production in latter stages. Many researchers have reported the positive effect of sugars on Lipase production by Aspergillus niger (Pal et al., 1978), by Rhizopus chinensis (Nakashima et al., 1988) and by Rhizopus oryzae (Salleh et al., 1993). On the contrary of present study, Muralidhar et al. (2001) stated that their experimental results indicate that Olive oil is a better carbon source for Lipase production by Candida cylindraceae compared to Glucose. This is supported by Dalmau et al., 2000; Gordillo et al., 1998 for Candida rugosa. Optimization of the quantity of Olive oil in the fermentation medium along with the other nutrients resulting in the rise in enzyme production also contradicts the fact that large quantities of Olive oil decrease the Lipase activity by for Candida rugosa (Gordillo et al., 1995).

Nahas (1988) reported that Carbohydrates were good sources of carbon for growth but low Lipase production was obtained by Rhizopus oligoporus. Lipase production was strongly repressed by higher concentration of Glucose. In present study revealed that fungal culture utilizing Olive oil as a carbon source exhibited lowest Lipase producing activity. This result was not in agreement with those of Paul and Carles (1992). Falony et al. (2006) reported that the A. niger strain showed more Lipase activity among the test strains used. The production of Lipase was more significant in culture medium added with lipids
as the carbon source than in the culture medium without lipids. Previous works on the physiology of Lipase production showed that the mechanisms regulating biosynthesis vary widely in different microorganisms. Results obtained with Calvatia (Christakopoulos et al., 1992), Rhizopus (Salleh et al., 1993), Aspergillus (Pokorny et al., 1994), and Rhodotorula (Papaparaskevas et al., 1992), showed that Lipase production seems to be constitutive and independent of the addition of lipid substrates to the culture medium, although their presence enhanced the level of Lipase activity produced. On the other hand, (Shimada et al., 1992; Rapp, 1995), lipid substrates are necessary for Lipase production by Geotrichium candidum and Fusarium oxysporum respectively and also, carbohydrates can act as repressors of its biosynthesis.

In the present study, Lipase production efficacy of mutant fungal strains (ANems120, AFems120, ANems150, PSem120 and PSems150) were demonstrated in the presence of various carbon and nitrogen sources. The effect of different carbon sources on Lipase production efficiency of bacterial and fungal strains was found to be in the ascending order Mannitol < Lactose < Glucose < Sucrose. In present study Aspergillus niger, and Penicillium sp., utilized Sucrose as a carbon source exhibited highest Lipase producing activities, these results are in agreement with those of Susumu and Yashio (1975). Elliah et al. (2002) reported that the Aspergillus niger utilized dextrose as carbon source for highest Lipase activity. The Lipase production was decreased in the order of Sucrose < Lactose < Mannitol < Dextrose by the mutant strain of Aspergillus niger. These results were in contrary to the present study results.

Petrovic et al. (1990) reported maximum Lipase production when Glucose and peptone were incorporated in the production medium using Penicillium roquefortii. In the present study, Glucose favoured the production
of Lipase. This is in contrary with Mahadik et al. (2004) who reported that there was no increase in enzyme levels was observed when mutant UV-10 was grown in medium supplemented with Glucose. However, the addition of Glucose in the medium resulted in increased levels of Lipase production by wild strain, *Aspergillus niger*. The maximum Lipase activity was found with the combination of Sucrose with Olive oil inducer and Glucose with Olive oil inducer in the present study. But the repressive effect of Glucose with fatty oil is reported by Dalmau et al. (2000) for *Candida rugosa*, Nahas (1988) for *Rhizopus oligosporus*, Baillargeon et al. (1989) for *Geotrichium candidum*, and Rapp (1995) for *Fusarium oxysporum*. but differ from those obtained for *C. rugosa* by Chang et al. (1994). Cordova (1998) reported that the carbon sources such as Glucose, Fructose, Glycerol, Xylose, Sucrose and Lactose are probably utilized before Lipase production, there were little or no differences with these substrates as carbon source.

Damaso et al. (2008) reported that the *Aspergillus niger* was found to be the best Lipase producer. Soap stock was the best substrate and inducer compare to Olive oil and they also explained the repressive effect Olive oil on Lipase production. Coca et al. (2001) stated that the *Aspergillus niger* and *Aspergillus fumigatus* were the best Lipase producer in the medium containing Olive oil as a carbon source. This is in contrary with our present results. The present study revealed that *Aspergillus fumigatus* utilizing Mannitol and Lactose as a carbon sources and exhibiting lowest Lipase producing activity. In case of Sucrose and Glucose exhibited highest Lipase producing activity.

Kakde and Chavan. (2011) found that carbon sources like Fructose and Sucrose induced Lipase activity while Lactose, Starch and Carboxyl Methyl Cellulose inhibited Lipase activity by *Penicillium chrysogenum*. Nitrogen
sources like Nitrate, Nitrite, Amide, Ammonium, and Protein showed its effect on Lipase enzyme of fungi. Casein and Peptone which are organic forms stimulated maximum Lipase enzyme production of storage fungi. Both the species of *Penicillium* viz. *Penicillium notatum* and *Penicillium chrysogenum* showed maximum extracellular Lipase activity in presence of Casein and Peptone. Urea which is an amide form and Ammonium Phosphate which is an ammonium form hampered the extracellular Lipase enzyme production of fungi. These reports were found to be similar to our present study results. Chavan and Kakde, (2009) studied that the Lipase enzyme activity of storage fungi under the influence of carbon and nitrogen sources. They found that carbon sources as like Fructose and Sucrose induces Lipase activity while Starch, Lactose and Carboxyl Methyl Cellulose inhibit Lipase activity. Nitrogen sources as like Nitrate, Nitrite, Amide, Ammonium, and Protein affect in different ways on Lipase enzyme of fungi. The results of the present study revealed that the Lactose and Urea was a poor carbon and nitrogen sources respectively in the tested bacterial strains like PSems120 and PSems150 and fungal strains like ANems120, AFems120 and ANems150.

The effect of different nitrogen sources on Lipase production efficiency of bacterial and fungal strains was found to be in the ascending order Urea < Ammonium Sulphate < Potassium Nitrate < Sodium nitrite in the present study. This was in accordance with Lima et al. (2003), who stated that the Lipase activity by *Penicillium* sp. was higher when the medium containing only the inorganic nitrogen sources and in the presence of Olive oil, Ammonium Sulphate produced less mycelial growth subsequently less Lipase activity. But the Ammonium Sulphate and Potassium Nitrate combination produced higher Lipase activity in short fermentation time. Other workers (Freire et al., 1997; Pereira-Meirelles et al., 1997; Samad et al., 1990) employed *Candida lipolytica,*
Penicillium restrictum and Rhizopus sp. reported maximum Lipase production, when organic nitrogen was used as a nitrogen source. Waller and Comeau (1990) reported that the incorporation of corn steep liquor into the production medium gave good Lipase activity by Candida sp. Rodriguez et al. (2006) reported that Ammonium Sulphate, Ammonium Nitrate and Sodium Nitrate except Urea reduced the production of Lipase activity compared to the initial medium containing yeast extract. With Urea, the activity was around six times higher. A similar observation has been reported using Penicillium sp. (Gombert et al., 1999). On the other hand, Lima et al. (2003) found that Lipase production in Penicillium sp. was stimulated using Ammonium Sulphate.

**Indole Acetic Acid**

The phosphate solubilizing wild and mutant strains of bacteria and fungi were analysed for their Indole Acetic Acid production efficacy qualitatively by Thin Layer Chromatography. Phosphate Solubilizing Bacteria are capable of producing physiologically active Auxins that may have pronounced effects on plant growth. (Ponmurugan and Gopi. 2006). Shahab et al. (2009) reported that the phosphate solubilizing microbes from Mung beans excreted the phytohormones like Auxin and ethylene.

The results of present study revealed that the wild strains of Pseudomonas sp., Aspergillus niger and Penicillium sp. have the ability to produce the plant hormone Indole Acetic Acid except Aspergillus fumigatus. Among the mutated bacterial and fungal strains, all the strains were able to produce Indole Acetic Acid except Ethyl Methane Sulphonate treated Aspergillus niger and all the strains of Aspergillus fumigatus. Since the wild strains failed to produce Indole Acetic Acid, the mutant Aspergillus fumigatus might not produced the Indole Acetic Acid.
Kulanthaivel et al. (2006) reported that *Azosprillum* sp. were treated with UV, Acridine orange and Ethidium bromide. The production of Indole Acetic Acid by most of mutant strains were higher when compared to wild strains. This is in agreement with the present study results, which stated most of the mutant produce more amount of Indole Acetic Acid when compared to wild strains. Garcia et al. (1980) stated that the mutant *Azosprillum brasilense* excreted Indole Acetic Acid more when compare to wild strains. Normanly et al. (1983) confirmed that the orangepericap mutant *Arabidopsis thaliana*, accumulate Auxin greater than wild type.

Tien et al. (1979) reported that *Azospirillum* and phosphobacteria isolated from the soil of pearl millet produced Indole Acetic Acid, gibberellic acid and cytokinin like substances. (Brown, 1972), and some of them are capable of dissolving phosphate (Barea et al., 1976). Production of Indole Acetic Acid varies greatly among different species and is also influenced by culture conditions, growth stage and availability of substrate (s) (Brown, 1972; Vijila, 2000).