Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 3(1) pp. 001-007, January, 2014.
Available online http://garj.org/garjas/index.htm

Review

Sustainable growth of rice in Ghana: The role of biofertilizers (Phosphate solubilizing microorganisms and Azolla anabaena) to rice improvement.

S. Asuming-Brempong

Department of Soil Science, College of Agriculture and Consumer Sciences, University of Ghana, Legon.
Email: sbrempong@yahoo.com

Accepted 16 December, 2013

Phosphorus deficiency in soil is a major constraint to food production in Ghana. This makes phosphate solubilizing microorganisms (PSM), organisms that make inorganic phosphates available for plant use important in the Ghanaian agriculture. Many PSMs have been isolated worldwide, some having been shown to be good solubilizers of inorganic phosphates and others not. Bioinformatic analysis showed that good phosphate solubilizers have the *pqq* and the *pho* genes. In a previous pot experiment, isolated PSM was tested on upland rice (Nerica 2). In field trials, the use of 45 kg P_2O_5/ha TSP + PSM out yielded the treatment with only 45 kg P_2O_5/ha TSP was applied to rice crop. The other important nutrient that constraints food production is inorganic N. Azolla is an aquatic fern that harbors an algal symbiont *Anabaena azollae*, capable of converting the tightly bound atmospheric nitrogen gas (N_2) which is unavailable to plants into inorganic N forms utilizable by plants. The successful cultivation and incorporation of Azolla into flooded soils where it commonly occurs results in the accumulation of large amounts of plant utilizable N for rice cultivation. Many azolla strains were introduced into the country and year round productivity trials were conducted. Promising azolla strains with high productivity and less variability in productivity from season to season were selected for further field trials. One azolla crop after incorporation before or after transplanting lowland rice increased the yield of rice by 17 % over the control treatment where no nitrogen fertilizer was used. Azolla supplemented the nitrogen requirement of rice and reduced the number of times one had to weed the rice farm, it was economical to use azolla.

Keywords: Rice improvement, Biofeterlizer, Phosphate solubilizing microorganisms and Azolla anabaena.

INTRODUCTION

The need to increase agricultural production from a steadily decreasing and degrading land resource base has placed strain on agro ecosystems. To maintain and improve agricultural productivity, the current strategy is to use chemical fertilizers. Yet, many synthetic fertilizers contain acids, such as sulphuric and hydrochloric acids, that tend to increase the acidity of the soil, reduce the soil's beneficial organism population and interfere with plant growth. Ammonium containing fertilizers such as ammonium sulphate upon application to the soil, the ammonium is nitrified and hydronium ions are produced in soil solution making the soil acidic. Bio fertilizers on the

other hand are inputs containing living microorganisms that are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes. One form of bio fertilizer is the use of phosphate solubilizing bacteria that are able to convert insoluble P forms into soluble phosphate forms and have been used to enhance the solubilization of re-precipitated soil P for crop improvement.

Some of the isolated phosphate solubilizing organisms are, *Pseudomonas putida, Rahnella aquatitis, Serratia marcescens, Klebsiella pneumonia, Burkholderia cepacia, Rhizobium sp., Aspergillus niger, Penicillium sp.*etc. These microorganisms have the genes for mineral phosphate solubilization and thus exhibiting the *Mps*$^{+}$ phenotype (mineral phosphate solubilizing phenotype). Some of the above microorganisms such as *Rhizobium, Pseudomonas* and *Bacillus* solubilize phosphate more efficiently than others (Rodriguez and Fraga, 1999).

The efficient mineral phosphate solubilization (*Mps*$^{+}$) phenotype in Gram negative bacteria results from extracellular oxidation of glucose via the quinoprotein glucose dehydrogenase to gluconic acid (Kpomblekou and Tabatabai, 1994). The resulting pH change and reduction potential are thought to be responsible for the dissolution of phosphate in culture medium. Gluconic acid biosynthesis is carried out by the glucose dehydrogenase (GDH) and the co-factor, pyrroloquinoline quinone (PQQ). Besides its relevant role in P solubilization, PQQ

is reported to be a potent growth-promoting factor for bacteria and plants, has antioxidant properties (Choi et al. 2008), and is directly related to the production of antimicrobial substances (de Werra et al. 2009, Guo et al. 2009, Schnider, 1995) as well as the induction of systemic plant defenses (Han et al. 2008). Hence, the cofactor PQQ has multiple plant beneficial effects. The genes involved in the synthesis of pyrroloquinoline quinone are, *pqqA, pqqB*, *pqqC*, *pqqD*, *pqqE*, *pqqF.* The *pqqA* gene encodes a small peptide of 23-29 glutamate and tyrosine residue at conserved position. The **expression of this gene** allowed the production of gluconic acid and mineral phosphate solubilization activity in *E. coli* HB101.

Other genes involved in organic phosphate solubilization are *phoA* that is an alkaline phosphatase required for the mineralization of organic substrates, *phoB* is a regulatory substance during organic phosphate mineralization and *phoC* is an acid phosphatase.

.

This paper examines

1) The differences in the phosphate solubilizing abilities of some phosphate solubilizing microorganisms using bioinformatics

2) The ability of PSM to influence the growth of upland rice.

3) The ability of *Azolla anabaena* association as a green manure for the sustainable growth of lowland rice.

Differences in solubilization of inorganic phosphates using bioinformation

To find out why some phosphate solubilizing microorganisms efficiently solubilize inorganic P more than others, bioinformation that has been deposited at both the NCBI Site (National Centre for Biotechnology Information) and the Kegg Data Base were used to obtain the amino acid sequences of the *pqq genes* as well as that for the other genes in the various phosphate solubilizing organisms and the results are presented in Table1. Table 1 shows that *Pseudomonas putida* for instance, has most of the pqq genes (*pqqA*, *pqqB*, *pqqC*, *pqqD*, *pqqE* and *pqqF* as well as *Pho B*). On the otherhand, *Rhizobium leguminosarium and Citrobacter* sp. have none of the *pqq genes* nor the *pho genes.* Also, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* have most of the *pqq genes* and can also produce the phosphatase enzyme (acid phosphatase, alkaline phosphate or produce both enzymes, Table 1). *Arthrobacter* sp. has only two of the *pqq* genes, probably it depends on other organisms with the full gene components to produce organic acids to solubilize inorganic P.

Pseudomonas aeruginosa, *Pseudomonas putida*, *Enterobacter aerogenes*, *Klebsiella pneumonia, Bradrhizobium japonicum* have most of the *pqq* genes and the *pho* genes and are likely to be good phosphate solubilizers. *Serratia marcenscens* has also the *pqq* genes and some of the *pho* genes but it is not a good phosphate solubilizer indicating that there are other factors that ought to be taken into consideration.

Phylogenetically, *Klebsiella pneumonia* is closely related to *Enterobacter* sp., *Burkholderia* is also closely related to the Pseudomonas sp. Using the 16S rRNA genes, three clusters are seen from the Figure. 1. Cluster 1 is made of *Klebsiella pneumomiae*, *Enterobacter* sp. and *Citrobacter rodentum*. Cluster 2 is the *Pseudomonas* sp and *Burkholderia* sp. Cluster 3 is also made of *Bradyrhizobium* sp., *Rhizobium* sp. and *Bacillus* sp. It is clear that the efficient phosphate solubilizers are scattered among the different clusters and that they are not phylogenetically grouped alone.

The *pqq C* gene encodes for the pyrroloquinoline quinone synthase *C* which is the best-characterized enzyme in the pathway and catalyzes the final step of the PQQ biosynthesis.

With the *pqqC* gene, amino acid sequence similarity also shows how closely related *Klebsiella pneumonia*, *Enterobacter aerogene* and *Serratia marcescens* are. *Pseudomonas* sp. and *Burkholderia* sp. also show another cluster, whilst *Bradyrhizobium japonicum* and *Arthrobacter* sp. also are further apart.

Phosphate solubilizing microorganisms were isolated and the promising isolates were used to grow upland rice in pot experiment using the Kokofu series (Paleudalf soil

Table 1. *pqq* genes in some bacterial isolates that show *Mps*$^+$ phenotype

Genes ⟶	*pqqA*	*pqqB*	*pqqC*	*pqqD*	*pqqE*	*pqqF*	Pqq-GDH	*pho A*	*pho B*	*pho C*
Pseudomonas putida	X	X	X	X	X	X	_	__	X	_
Bacillus polymxa	_	_	_	_	_	_	_	_	_	_
Rhizobium leguminosarium	_	_	_	_	_	_	_	_	_	_
Bradyrhizobium japonicum	X	X	X	X	X	_	_	_	_	X
Klebsiella pneumoniae	X	X	X	X	X	X	_	X	X	X
Serratia marcenscens	X	X	X	X	X	X	_	X	_	_
Citrobacter sp.	_	_	-	_	_	_	_	_	X	_
Burkhoderia cepacia	X	X	X	X	X	_	_	_	X	_
Pseudomonas aeruginosa	X	X	X	X	X	X	_	X	X	_
Enterobacter aerogenes	X	X	X	X	X	X	_	_	X	X
Arthrobacter sp.	_	_	X	X	_	_	_	_	_	_

- data not available either at the NCBI site nor at the KEGG DATA BASE
X – means that a particular gene under consideration is present.

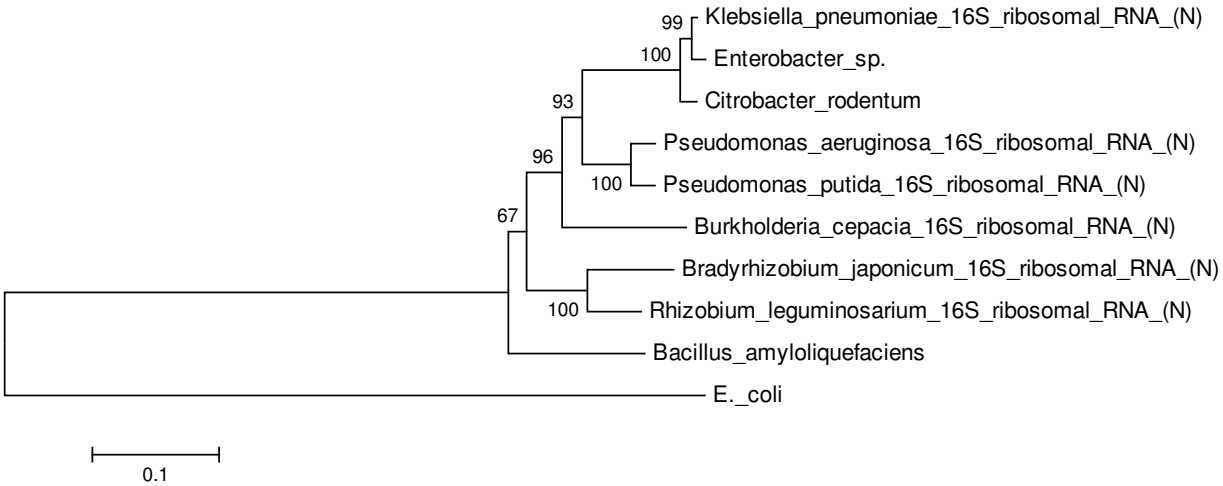

Figure 1. 16S rRNA genes of phosphate solubilizers.

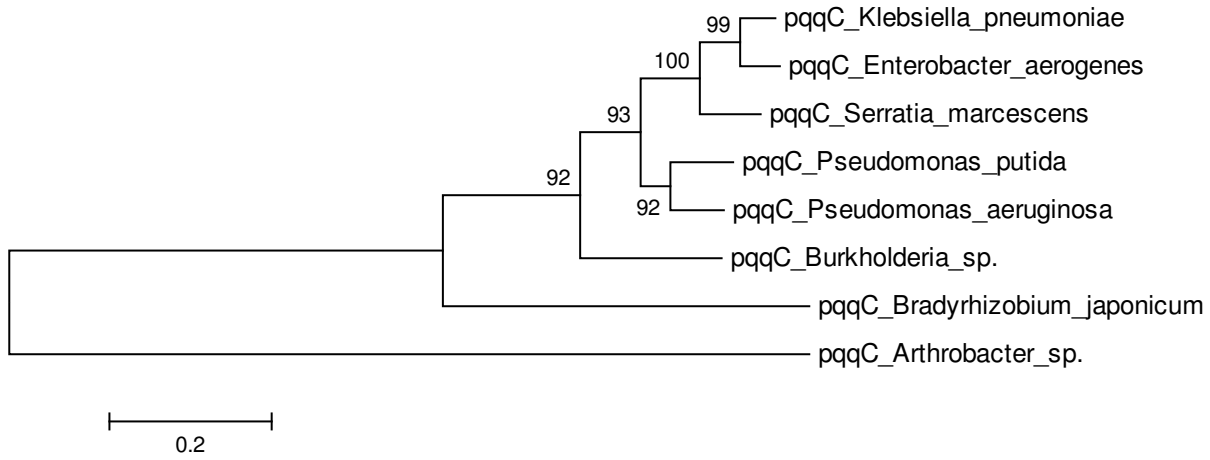

Figure 2. Cluster analysis of the *pqqC* gene of some phosphate solubilizing microorganisms

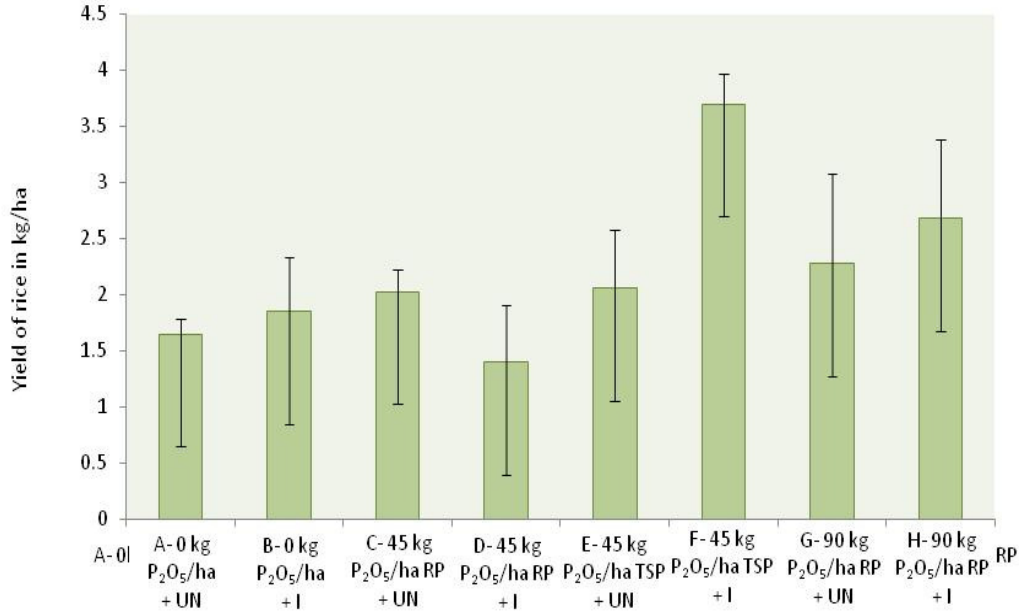

Figure 3. The use of rock phosphate on the growth of upland rice.

pH 5.2). The best treatment was when 45 kg P_2O_5/ha rock phosphate and PSM was used to grow the rice and the dry matter yield at day 40 after sowing the rice seed was comparable to the yield of 45 kg P_2O_5/ha TSP was used to grow rice.

Field trials were conducted using PSM (*Aspergillus niger* # ATCC 1274), the best treatment was treatment F where 45 kg P_2O_5/ha TSP + PSM was used to grow upland rice (Figure. 3). Thus, the yield of treatment F was higher treatment E where TSP alone was applied and the two treatments were significantly different from each other (Figure. 3). The treatments in the field trial involved the following;

A- 0 kg P_2O_5/ha + uninoculated with PSM, B -0 kg P_2O_5/ha + inoculated with PSM, C- 45 kg P_2O_5/ha rock phosphate (RP) + uninoculated, D – 45 kg P_2O_5/ha RP + inoculated with PSM, E – 45 kg P_2O_5/ha TSP (triple super phosphate) +uninoculated, F 45 kg P_2O_5/ha TSP +inoculated with PSM, G-90 kg P_2O_5/ha RP + uninoculated, H- 90 kg P_2O_5/ha RP + inoculated with PSM.

Table 2. List of Azolla strains.

Azolla strains	Country of origin	Source (Reporter/Collector)
# 175 Mi	Paraguay	Philippines, IRRI, 418
# 195 CA	Brazil	Brazil, A. Kagoni (Mali) per Montpellier
# 69 Mi	Ecuador, Galapagos	T. Lumpkin
# 137 Pi	Thailand, Bangkok	IRRI 5
# 62CA	Brazil, Negro river	M.F. Fiore
# 07 Pi	India, Cuttack	P.K. Pande
# 138 RP	AUSTRALIA	IRRI 701, C. Dixon
# 104 Mi	Mexico, Sinalou	R. Ferrera, Cerrata
# 44CA	Brazil, Solimoes river	C. Van Hove

Table 3. Productivities of Azolla strains during the rainy season

Azolla strains	Productivity g/fresh weight/day	Total N accumulated kg/ha/day
A. pinnata # 137 (Bangkok)	140	3.2
A. pinnata # 7 (India)	97	2.8
A. caroliniana # 195	123	3.1
A. caroliniana # 301	103.9	1.78
A. pinnata var. pinnata	67.3	0.8

The use of azolla as green manure in lowland rice cultivation

Azolla is an aquatic fern that is normally found growing in rivers, ditches, ponds and other stagnant waters. The Azolla plant is triangular or polygonal in shape and floats on water surface individually or in mats. Each leaf has two lobes, the ventral lobe which is achlorophyllous and the dorsal lobe that is chlorophyllous. Azolla has been described by the Chinese and the Vietnamese as miniature nitrogen fertilizer factories. Indeed the Vietnamese call them indestructible fertilizer factories since azolla continued to produce nitrogen fertilizer for the Vietnamese rice paddies at the height of the Vietnamese war.

With an average nitrogen fixing rate ranging from 1.0 to 3.6 kg N/ha/day, in 30 days about 40-60 kg N/ha is fixed under favourble condition. Azolla can therefore be a complete or partial substitute for chemical nitrogen fertilizer in lowland rice production. The yields of rice obtained when one crop of azolla (20-30 t/ha) is incorporated before or after transplanting the rice and intercropping is equivalent to 30 kg N/ha urea or ammonium sulphate. The nitrogen fixed by azolla mostly becomes available to the rice after the “azolla mat” is incorporated into the soil and its nitrogen begins to be released through decomposition. After 6 weeks of incorporation in the soil, 62-75 % of the total nitrogen in Azolla would have been released as ammonia and it becomes available to rice plant. The use of Azolla is new in Ghana and therefore there was the need to investigate the growth and green manuring performances of azolla in Ghana.

The Agricultural Research Station at Kpong was supplied with a number of exotic Azolla strains and the local azolla strain was included (Table 2). These strains were supplied by the International Rice Research Institute (IRRI) and the West African Rice Development Association (WARDA). Productivity trials were conducted in collaboration with the Catholic Louvian University of Belgium. Azolla strains were grown in productivity nets of 1 m^2 diameter and the fresh weight of azolla was taken every week and thinned down to the initial weight to continue azolla growth . This was continued until the end of one year. Results showed that *A. pinnata* # 137 had the highest productivity of 140 g/fresh weight/m^2/day especially during the rainy season and had a % N of 3-5 whilst *A. pinnata* # 7 did well under very unfavourable conditions from January to April or on the onset of rain (Table 3).

After a year round productivity trial, promising azolla strains were selected (Table 3). Generally, the productivity of Azolla in the dry season was about half that of the rainy season partly because the high environmental temperature did not favour azolla growth. Also, water seeped into the deep cracks of the vertisol making it difficult to retain water in the basins that Azolla strains were growing.

Table 4. The use of azolla as a supplement of chemical N fertilizer in a major rainy season trial

Treatment	Mean Grain yield (t/ha)
A- no N fertilizer	2.16 [d]
B- Ammonium sulphate, 60 kg N/ha	2.94 [b]
C- Ammonium sulphate, 90 kg N/ha	3.07 [a]
D- Azolla incorporated 2 weeks before transplanting	2.54 [bc]
E- Azolla incorporated 2 weeks before transplanting and a second incorporation 1 month after transplanting	2.63 [bc]
F- Azolla incorporated once as in Treatment D + 60 kg N/ha	3.05 [ab]

Means followed by the same letters are not significantly different from each other at the 5 % level of significance according to the Duncan Multiple Range Test (DMRT).

The local Azolla strain, *A. pinnata var. pinnata* or *A. pinnata var. africana* had a low productivity and a lower %N.

Further studies were conducted to know why the local Azolla strain had a lower productivity and total N yield per day as compared to the exotic azolla strains.

Test conducted involved squeezing the dorsal lobe on a microscope slide and gently putting on a cover slip and squeezing the frond in a drop of water. The whole set up was then examined under the microscope. Three types of cells were observed, the akinete, the heterocyst and the vegetative cells. The heterocyst is where nitrogen fixation takes place and the fixation of carbon dioxide take place in the vegetative cell. The function of the akinete is yet unknown. Results showed that the heterocyst frequency in the cells of the exotic strain was 15 -25 %. For almost every 5 vegetative cells a heterocyst was found. For the local Azolla strain it was difficult observing any heterocyst. One observed a number of vegetative cells and in most cases no heterocyst was found. Heterocysts are the sites where active fixation of atmospheric nitrogen take place. On very rare occasion one observed heterocyst, thus the heterocyst frequency was very low suggesting a very low nitrogen fixing rate since such fixation sites were either few or nonexistent for the local azolla strain.

To further test the ability of azolla to supplement the nitrogen fertilizer needs of lowland rice, field experiments were conducted. Exotic azolla strain *A. microphylla* # 175 was used as green manure. Results showed that one azolla incorporation after transplanting rice and adding 60 kg N/ha ammonium sulphate fertilizer, the yield of rice was as equal as the treatment where 90 kgN/ha of ammonium sulphate fertilizer had been used. Thus one azolla incorporation supplemented 30 kg N/ha of N fertilizer (Table 4).

In a previous study by Nyalemegbe and Oteng (1988), single incorporation of azolla increased the rice yield over the control by 17 %. Double azolla incorporation resulted in 54.5 % yield increase over the control rice yield.

Azolla is economical to use when it partly substitutes for the N fertilizer requirement of rice crop. This is because instead of a farmer weeding twice, weeding is done once because azolla suppresses weeds when it is grown in dual cropping with lowland rice. The use of azolla as a green manure reduces the cost of nitrogen fertilizer use for the rice crop by 40 % as compared to when only chemical fertilizer is used. This is because fertilizer N cannot suppress weeds so extra expenses are incurred in weeding and also meeting all the fertilizer requirement of rice.

CONCLUSION

The use of biofertilizers in the Ghanaian is very promising. Every effort should be made to explore the use of other forms of biofertilizers such as blue green algae, Azotobacter, Azospirillium etc. in rice and other crop production.

ACKNOWLEDGEMENT

I am grateful to some scientists at Dow AgroSciences, Indianapolis, USA for the aiding me to carry out the Bioinformatic studies and the opportunity given me to do my post doctoral studies.

REFERENCES

Choi O, Kim J, Kim JG, Jeong Y, Moon JS, Hwang I (2008). Pyrroloquinoline quinone is a plant growth promotionfactor produced by Pseudomonas fluorescens B16. Plant Physiol. 146:657–668.

de Werra P, Pechy-Tarr M, Keel C, Maurhofer M (2009). Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas fluorescens* CHA0. Appl. Environ. Microbiol. 75:4162–4174.

Guo YB, Li J, Li L, Chen F, Wu W, Wang J, Wang H (2009). Mutations that disrupt either the *pqq* or the *gdh* gene of *Rahnella aquatilis* abolish the production of an antibacterial substance and result in reduced biological control of grapevine crown gall. Appl. Environ. Microbiol. 75:6792–6803.

Han SH, Kim CH, Lee JH, Park JY, Cho SM, Park SK, Kim KY, Krishnan HB, Kim YC (2008). Inactivation of *pqq* genes of *Enterobacter intermedium* 60-2G reduces antifungal activity and induction of systemic resistance. FEMS Microbiol. Lett. 282**:**140–146.

Kpomblekou A, Tabatabai MA (1994). Effect of organic acids on release of phosphorus from phosphate rocks. Soil Sci. 158:442–453.

Nyalemegbe KN, Oteng JW (1988). The use of azolla as a green manure in the vertisol. University of Ghana Annual Report, 1989, 104 pp.

Rodríguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Advances. 17: 319–339.

Schnider U, Keel CC, Voisard G, De´fago Haas D (1995). Tn5- directed cloning of pqq genes from Pseudomonas fluorescens CHA0: mutational inactivation of the genes results in overproduction of the antibiotic pyoluteorin. Appl. Environ. Microbiol. 61:3856–3864.