PREFACE

Cereals, legumes, pulses and oilseed crops are rich and cheap source of nutrients for the animal kingdom, including humans and hence these crops are grown over 90% of the world's harvested area. But one of the major problems related to the consumption of plant-based products is the presence of antinutritional compounds in vegetarian diets. Phytic acid and its cationic salt phytate are examples such antinutritional factors which are actually the major source of phosphorus and energy in plants for seed germination. Monogastric animals do not contain the mechanism to hydrolyze phytate and hence are needed inorganic phosphate supplements in diet to meet their nutritional requirements or processed food with lower levels of phytate to avoid its antinutritional effects. Moreover, the unutilized phytate from vegetable feed when gets excreted out by animals is becoming an environmental pollutant in areas of intensive livestock production. All these problems could be solved by hydrolysis of phytate using supplemental phytases which represent a specific group of enzymes that can catalyse the sequential hydrolysis of phytate to less phosphorylated myo-inositol derivatives with concomitant release of inorganic phosphate. Therefore, requirements for better animal feed, lower food and feed cost and also concerns about environmental protection and human health have prompted a good array of research on phytases and their applications.

The work presented in this thesis is focused on two novel bacterial phytase enzymes isolated from environmental samples. Here, the chapter 1 deals with a general discussion and literature review about phytates and phytases. The chemistry and importance of phytates, diversity of phytases, structural information on various phytases along with their biophysical and biochemical properties, cloning and expression studies and applications are summarized in this chapter. The chapter 2 features isolation, characterization, gene cloning and expression of phytase from Shigella sp. CD2. The phytase enzyme from Shigella sp. CD2 was purified and characterized. Then the gene encoding the cell-bound phytase was cloned and expressed in two heterologous hosts and the properties of the recombinant proteins were compared after purification. The chapter 3 focuses on isolation, characterization and gene cloning of phytase from Bacillus sp. RS1 and application in plant growth. The extracellular enzyme was purified to near homogeneity and characterized followed by gene cloning and sequencing studies. Further, the use of appropriate cultivation strategies resulted in a high-level production of the enzyme. Finally, both the bacterium and purified enzyme were found effective in improving germination and growth of chickpea seedlings.

FOREWORDS

Echoing the words of Leonardo De Vinci- "The sun has never seen its shadow", we can say science can never see its end; being an ever expanding knowledge, it is a good servant unless we make a bad master of it. To make it serve us, it is in November, 2008 that I stepped into the lab of Department of Biotechnology at the University of North Bengal under the inspiring guidance and care of Dr. Shilpi Ghosh. With her showing me the way, I got into an unchartered venture to trace some truth underlying the system of nature. Pursuing this I had to carry out in phases a lot of experiments, trying at the same time to follow some of the intrinsic problems of the microbial world. Hidden bacteria are omnipotent and omnipresent organisms, reproductive and proliferating in quick succession, as if endless and deathless like God who is also invisible but palpable. A peep into the microbial kingdom will enable us to know that bacterium is an all-pervasive entity predominating over all. It is a paradoxical truth that even though they are universally believed to be bad and harmful, they are mostly beneficial to mankind. It cannot be gainsaid that their positive role played in human life makes itself so evidently prominent that they are now prodigiously used to contribute in every aspect of life, ensuring further betterment.

The success of doctoral pursuits depends inter alia on an excellent ability of communication, presentation and solid grounding in the subject dealt with. One bursting with talent, I know, cannot make much headway if he does not find any suitable exposure for showcasing his dream-child. For me, my ambitious journey was not always a smooth sail; it was rather beset with many a stumbling block. Sometimes my breath was caught in the throat whenever I got stuck and felt like whimpering as a lost pup. Again, at other times my heart brimmed over with profuse joy at every stage of success. Poring over books or test tubes for hours with no fruitful results sometimes seemed to be so disheartening that I could not help thinking it to be a wild goose chase, a matter not fit for holding a candle to. However, without losing heart I stuck to my ground till success smiled on me. The fruit of success, as we know, is always sweet. On the whole, I enjoyed the work despite some hurdles getting in the way to my peril and now I feel the true import underlying the Shakespearean line: "All's well that ends well". Above all it's my pleasure to seize this occasion to express my heartfelt gratitude to all the people coming up with their help and active support to see me through the journey.

In acknowledgement, let me first mention the name of my mentor-supervisor Dr. Shilpi Ghosh, Assistant Professor, Department of Biotechnology, University of North Bengal to whom I owe a great deal for a fruitful end. Words fail me to place on record

how deeply she got involved in the job I was pursuing, sometimes with faltering steps which she had corrected. To be frank without any simulation, she was always ready with her valuable suggestions followed by unflagging guidance during the whole period of research to help me make it. Without her efficient leadership my success would have gone farther away like the Ramayanic golden deer. In short, my indebtedness to her is too great to reduce it to words.

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I cannot help calling up the names with a thankful heart who gave me good advice and/or the yeoman's service whenever I found things getting messed up and confusive, like- Kamal Krishna Singh, Subhabrata Dutta, Deepika Mazumdar and Shyama Prasad Saha. Further, I express my gratefulness to Madhumita Poddar, Amrita Acharya, Payel Sarkar, Khusboo Lepcha, Vaskar Das, Priyam Chettri, Pranita Pradhan and labmates, senior and junior, who were always kind enough to share their experience with me, as and when required, besides discussing things during critical hours.

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Side by side, I feel called upon to recapitulate the memories of Late Nirmala Pal Roy, my paternal grandmother (a widow under thirty hailing from Bangladesh with three minor sons to educate by serving as a Nurse), Late Bibhuti Bhusan Nag, my maternal grandfather (ex-Manager, Ramjhora T.E.), Late Indu Prabha Nag, my maternal grandmother (who was as if the second mother for me), Late Ajit Baran Pal Roy (ex-professor, Malda Polytechnic) who wanted me to pursue a higher berth of academic career. Here I gratefully remember the invaluable patronage always extended to me by Mr. Partha Mitra, Mrs. Rima Mitra, Mr. Subhojit Banerjee and

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As regards my parents, Mr. Dinesh Pal Roy and Mrs. Utpala Pal Roy, who have ever been a perennial source of my inspiration and steadfastness, were always confident of my innovative and original abilities. I fumble for suitable words to express my feelings towards them. It is better left unsaid than said because all parents are the best well-wishers of their offspring. So, I am afraid, I may not be immune from exaggeration while speaking about my parents.

At last, I have to conclude my prefatory notes, dry and prolix, lest it should provoke disfavour and distaste among many. To speak the truth, success bagged after a long sweating struggle continued against thick and thin, tastes sweet. It appears to be exceedingly exhilarating when victory in the cause comes in the face of adverse and obtrusive realities. Be it as it may, now that I have made it translating my cherished dreams into reality, I feel too much happy.

Moushree Pal Roy