INTRODUCTION
1.0 INTRODUCTION

Salinity has detrimental effect in microbes, plants and animals. Salinity stress negatively impacts agricultural yield throughout the world and affect production whether it is for subsistence or economic gain. The United Nations Environment Program (UNEP) estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Flowers and Yeo, 1995). Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability.

Many technical measures, physical, chemical and mechanical, have been developed to reclaim the soil, however, due to economic viability and ecological concerns those technologies became less attractive to the farmers. Under these circumstances, the only viable alternative is to identify or develop suitable salt tolerant crops to cultivate in these saline soils. However, developing salt tolerant crop is not an easy task because salt tolerance is a polygenic trait. Therefore, integration of knowledge on physiological, biochemical and genetic aspects of salt tolerance is essential to make any progress in this regard (Ashraf and Foolad, 2007). To combat over this problem, recently many bacterial genes involved in biosynthesis of compatible solutes were successfully transferred into plants, for example, the carbohydrate trehalose (Holmstrom et al., 1996; Pilon-Smits et al., 1998), the polyols mannitol (Tarczynski et al., 1993; Thomas et al., 1995), amino acid derivatives glycine betaine (Lilius et al., 1996; Hayashi et al., 1997; Nuccio et al., 1998; Waditee et al., 2005) and ectoine (Nakayama et al., 2000). The transgenic approach offers one of the attractive techniques for the genetic improvement of cultivars. Although, it required exploration of novel bacterial proteins/genes, to understand the molecular basis of
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Salt tolerance mechanisms, which, helps in to develop, salinity tolerance in plant (Feng-Yun et al., 2007; Wright, 2007).

It is widely accepted that the microbial diversity present in the environment has been severely underestimated. Torsvik and co-workers (1990) employed DNA re-association kinetic analysis on microbial community DNA and estimated the bacterial diversity in the order of $10^3$ taxa per sample. Amman and co-workers (1995) elsewhere demonstrated that direct microscopic visualization of a natural sample yielded a population count of one to two orders of magnitude higher than estimates from culturing the same sample. It is now accepted in the wider microbial ecology scientific community that as much as 99% of the microorganisms present in nature are not cultivated by the standard techniques (Amman et al., 1995; Schloss and Handelsman, 2003; Daniel, 2005). The exploitation of this genetic reservoir has now been made possible by advances in our ability to recover significantly more genetic information from environmental samples in a culture independent manner. The realization that these standard microbiological methods provide limited access to the true microbial diversity and therefore the available microbial genetic diversity (collectively termed the metagenome) has resulted in the development of environmental nucleic acid extraction technologies designed to access this wealth of genetic information. The approach to metagenomic DNA extraction broadly remains similar to that used in the extraction of DNA from pure cultures. Basic steps include cell lysis, separation of the DNA and cell debris, and DNA recovery and purification (Rochelle et al., 1992; More et al., 1994; Frostegard et al., 1999). It is followed by library construction which includes: the generation of suitably sized DNA fragments, cloning of these fragments into an appropriate vector and screening for the desired gene. The ease with which the target gene is recovered from the library depends on the strategy followed to screen the library. Screening strategy either based on function or on sequence similarities can be used to
access the vast genetic and functional information from metagenomic libraries to isolate novel genes. There have been a number of reports describing both the construction of metagenomic DNA libraries from diverse environments, and the subsequent recovery of novel genes from these libraries. Such as chitinase, 4-hydroxybutyrate dehydrogenase, lipase, esterase, and genes encoding Na$^+/H^+$ antiporter activity (Cottrell et al., 1999; Henne et al., 1999; Henne et al., 2000; Majernik et al., 2001). The isolated genes from metagenomic library can be ascertained by the bioinformatic analysis based on the homology search in the databases.

In this work, functional metagenomic approach was employed in an attempt to recover novel bacterial salt stress tolerance genes from uncultured bacteria. Efforts have been made to construct metagenomic libraries from environmental samples and function based screening was done to isolate novel salt stress tolerance genes. The present study was designed to achieve the following objectives:

(i) Preparation of metagenomic libraries from environmental samples.

(ii) Isolation of salt tolerance clones from environmental metagenomic library/ ies.

(iii) Identification and characterization of novel salt stress tolerance genes.