

Abstract

Rhizobia, the diazotrophic bacteria forming mutualistic association with legume hosts are commonly used as N-fixing biofertilizer, however with an often inadequate outcome. The biggest constraint in effective application of elite N-fixing rhizobial strains is their failure to quantitatively nodulate the host in the presence of autochthonous native strains present in the soil which are usually more adept at nodulation. This dominance of the native rhizobia is known as nodulation competitiveness. One of the most crucial steps of nodulation is the initial colonization of the root by nodulating rhizobia and while the process of nodule formation in mutualistic relationship between legumes and rhizobia is understood in significant details, the principles driving the early colonization of root by rhizobia and how its modulation affects the colonization outcomes remain to be studied in more details. The process of root-bacterial interaction is an outcome of several individual processes most which are governed by quorum sensing (QS). QS— the cell density dependent mechanism of regulation of gene expression, in rhizobia contributes to modulation of these ‘symbiotically important’ phenotypes and is thus reported to alter nodulation competitiveness of rhizobia. QS, in different species of nodule-forming diazotrophs is diverse in all of its aspects including the genetic elements that code for the regulatory elements, the signaling molecules and the downstream targets. Present thesis was aimed at understanding the role of QS in early steps of colonization by rhizobia in different host-symbiont settings.

This work is divided in three parts. The first part deals isolation of rhizobia nodulating pigeon pea- one of the most important legume crop of India and global south. Investigation into the QS of the screened strain(s) led to identification of QS gene homologues and canonical n-acyl homoserine lactone (AHL) signaling molecules as well as conservation of the scheme of regulation of symbiotically important phenotypes. On the other hand the strains differed significantly in terms of sequence of the detected QS homologues with interesting pattern of distribution of amino acid variation in them and identification of AHLs novel to the

Rhizobiaceae family. This part of the work is possibly the first to describe QS in the rhizobia nodulating pigeon pea.

The second part of the work deals with homologous overexpression of the master QS circuit in two of the well-studied rhizobia— *R. leguminosarum* strain 3841 and *Ensifer meliloti* 8530. Results indicated that overexpression of the QS in each of the strains removed the cell-density mediated regulation of QS, and made it constitutively active at a greater magnitude. This was found to lead to amplification of phenotypes promoting the colonization and spread of the rhizobia such as increased biofilm formation and exopolysaccharide production on host root and confirmed with transcriptional regulation of relevant genes. These effects however did not reflect into significant amelioration of nodulation competitiveness, an observation attributed to the complex social nature of the AHL mediated QS and the interference faced by QS and the downstream phenotypes by secondary regulators—collectively equating the observation between the compared experimental groups.

The final part of the work dealt with understanding the colonization dynamics of rhizobia – when they are used as inoculants or when they are present in the soil at different abundances or in combinations. The rhizobia were found to be restricted in the spread over the roots of pigeon pea when coated on the root in different magnitudes even without a competing strain. These differences were attributed to the abilities of the tested strain to secretion of polymers and biofilm formation. While an earlier presence of a competing strain was found to restrict the colonization of the other strain, the strain with greater biofilm and EPS matrices strain seemed to be aiding in the spread of the other strain. A time lapse imaging in the microfluidic setup revealed a rapid colonization of root hairs of alfalfa roots by its nodulant Em8530 and successive colonization pattern similar to observed effects in the soil. Thick and strong root cap biofilms were observed in this experiment to form within 20h.