12 A *hsfA* gene, a gene for host-specific nitrogen fixation is expressed only at specific stage

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The *hsfA* gene of *Bradyrhizobium japonicum* was shown to be responsible for the host-specificity of the nitrogen-fixing bacteria and to be bacteroid-specific. The gene was cloned and found to encode a 11kDa-sized polypeptide. This gene was shown to be required for many legume species. To understand its regulation, serial deletions of the *hsf*A promotor were constructed and fused with GUS (β-glucuronidase) gene. These constructs were transferred into *B. japonicum* by triparental mating. The 60bp-fragment of *hsfA* promoter showed almost the same level of GUS expression as its longer fragment (465bp). It appears that the minimal fragment is fully enough for *hsfA* gene expression and so will contain *cis*-activating site. In addition, this fragment of *hsfA* promoter showed the temporal expression. In 10-day old nodules, GUS expression was not found, although rhizobia were present. GUS expression was found in 13- to 17-day-old nodules, but not in 28-day old nodules. To clarify the stage-specific expression of *hsfA* gene between 17- and 28-day-old nodule , *in situ* hybridization was performed using a *hsfA* gene as probe It was concluded that the 60bp-fragment of *hsfA* promoter is responsible for the stage-specific expression of *hsfA* gene.