An *Azotobacter* with partially deleted *nifL* and constitutive *nifA* can save a good amount of urea fertilizer without affecting wheat yield

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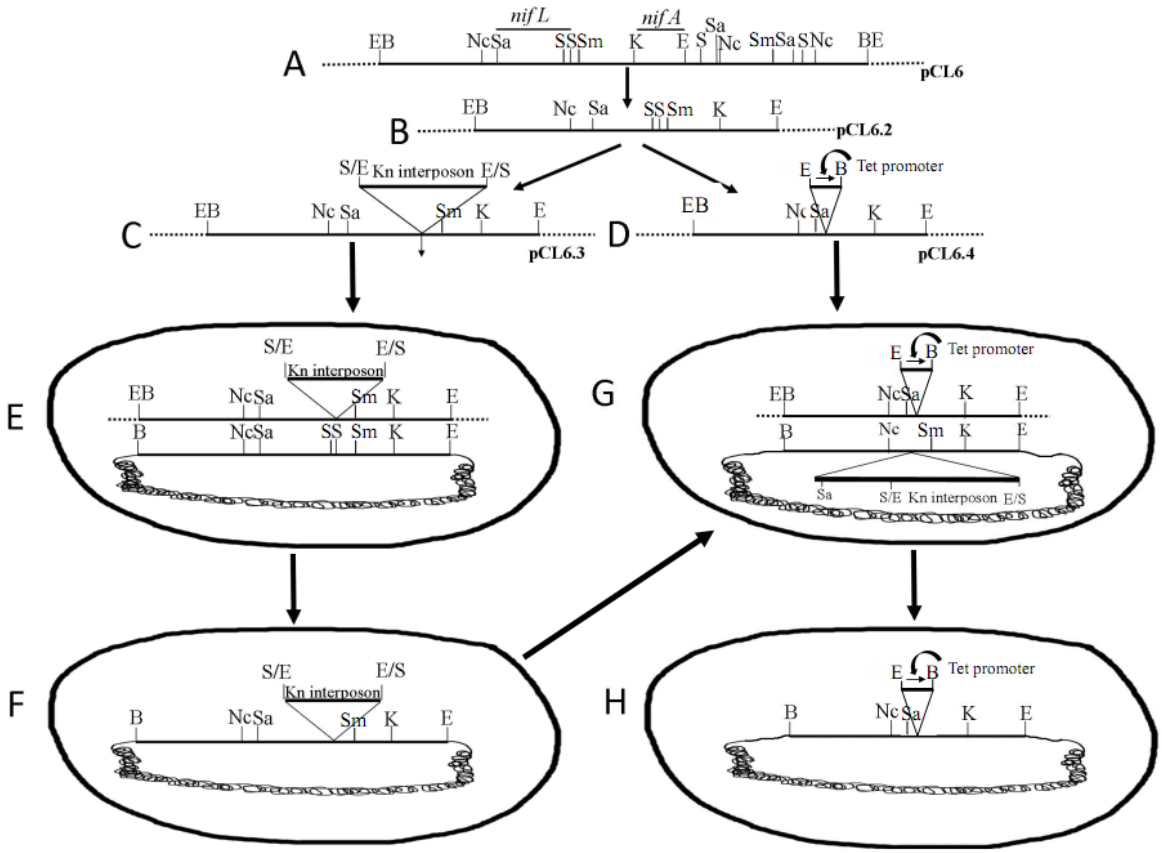
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Abstract

Soil bacteria belonging to the genus *Azotobacter* can reduce atmospheric nitrogen into ammonia, which can be assimilated by plants. About 20 genes are involved in the process of nitrogen reduction. Expression of all these genes is dependent on a positive regulator NifA. In the presence of ammonia, the negative regulator NifL, gets activated and interacts with NifA to neutralize it. As a result no nitrogen is reduced by wild type *Azotobacter* when ammonia is formed or produced by nitrogenous fertilizers. In two species of *Azotobacter* studied by us, the *nifL* and *nifA* genes were present in the same operon, *nifL* being proximal and *nifA* distal to the promoter. The *nifLA* promoter was also regulated by ammonia. We partially deleted the *nifL* gene and inserted a constitutive promoter there, thus bringing the *nifA* gene under its control. Ammonium only marginally affected acetylene reduction (a measure of nitrogen reduction) by the engineered *Azotobacter* strain. When wheat seeds were inoculated with the engineered strain and sown, the crop yield was enhanced by 60%, without any application of urea or other chemical nitrogenous fertilizer. The crop yield, however, was enhanced only by 10% when the wheat seeds were inoculated with the wild type strain. When we

applied urea, the wheat plants arising from the seeds inoculated by the engineered strain, could yield the same amount of crop using ~85kg less urea (~40kg less nitrogen) than the usual ~257 kg urea (~120 kg nitrogen) per hectare. Wheat plants from the seeds inoculated with the engineered strain had much higher dry weight and nitrogen content and assimilated molecular ^{15}N much better than plants from seeds inoculated with the wild type strain. Inoculation of the wheat seeds with the engineered strain did not adversely affect the microbial population in the rhizosphere soil.

Image



Legend of image. Cloning steps involved for construction of engineered *Azotobacter*.
(A) Partial restriction map of the ~7.5 kb *Bam*HI genomic fragment from *A. chroococcum* CBD15, which contains regions homologous to *nifL* and *nifA* of *A. vinelandii* UW, cloned in the *Bam*HI site of pUC7. The construct has been designated pCL6. The restriction sub-fragments that hybridize with *nifL* and *nifA* of *A. vinelandii* UW are overlined. *(B).* The ~4.8 kb *Eco*RI fragment from pCL6 containing the regions homologous to *nifL* and *nifA* of *A. vinelandii* UW was cloned in pUC7 and this construct

has been designated pCL6.2. *(C)* The ~2.0 kb *Eco*RI fragment from pHP45ΩKm containing the interposon ΩKm was inserted into the *Sal*I sites of pCL6.2 and this construct has been designated pCL6.3. *(D)* The construct pCL6.2 was digested with *Sal*I and deletion of 1112 bp was achieved by subsequent exonuclease treatment; the 374 bp *Eco*RI-*Bam*HI fragment from pBR322 containing the Tet promoter was cloned there in the correct orientation. This construct has been designated pCL6.4. *(E)* The construct pCL6.3 was introduced into *A. chroococcum* CBD15 by electroporation. *(F)* Insertion of the kanamycin interposon into the *nifL* gene in the genome of *A. chroococcum* CBD15, as a result of homologous recombination between pCL6.3 and the *A. chroococcum* CBD15 genome. *(G)* The construct pCL6.4 was introduced by electroporation into *A. chroococcum* CBD15, which already had the kanamycin interposon inserted into the *nifL* gene in its genome. *(H)* Replacement of the region of the *nifL* gene comprising 1112 bp around the two *Sal*I sites and the kanamycin interposon in the genome of *A. chroococcum* CBD15, as a result of homologous recombination with pCL6.4. Abbreviations: E, *Eco*RI; B, *Bam*HI; Nc, *Nco*I; S, *Sal*I; Sa, *Sac*II; Sm, *Sma*I; K, *Kpn*I; Kn, kanamycin; Tet, tetracycline resistance gene.

Recent Publications

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