# THE ISOLATION AND CHARACTERIZATION OF *RHIZOBIUM LOTI* EXOPOLYSACCHARIDE MUTANTS

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## ABSTRACT

PN184, a streptomycin resistant derivative of the broad host range *Rhizobium loti* strain NZP2037, was shown to be Calcofluor-bright due to the production of a Calcofluor-binding exopolysaccharide (EPS) with both high and low molecular weight forms, the low molecular weight form being predominant.

Eight Calcofluor-dark EPS mutants (three smooth and five rough) were generated by Tn5 mutagenesis of PN184. Each mutant was shown to carry a single, independent Tn5 insertion. Cosmids that complemented the mutation carried by each of the rough, PN184-derived EPS mutants were isolated from a pLAFR1 gene library to NZP2037 by complementation of the Calcofluor-dark phenotype. The genetic regions identified were shown to be located on the chromosome, and were not closely linked. The mutants were divided into three (complementation) groups.

While the rough, PN184-derived EPS mutants failed to synthesize EPS, the smooth, PN184-derived EPS mutants were found to synthesize an EPS which failed to bind Calcofluor, and which was shown, by $^{1}$H-NMR spectroscopy, to be significantly less acetylated than the EPS produced by PN184. Furthermore, PN1177, one of the smooth, PN184-derived EPS mutants, was shown to produce only a small amount of high molecular weight EPS compared to PN184.

All the PN184-derived EPS mutants induced the formation of fully effective ($Nod^{+}Fix^{+}$) nodules on *Lotus pedunculatus*, a determinate nodulating host legume, but, in contrast, induced the formation of ineffective ($Nod^{+}Fix^{-}$) nodules on *Leucaena leucocephala*, an indeterminate nodulating host legume. Each rough, PN184-derived EPS mutant, carrying its complementing cosmid, was fully effective on *L. leucocephala*.

PN4115, a streptomycin resistant derivative of the restricted, effective host range *R. loti* strain NZP2213, was shown to be Calcofluor-dark. PN4115 was shown to produce an EPS, which fails to bind Calcofluor, that is acetylated to approximately the same extent as the EPS produced by PN184. Like PN1177, PN4115 was shown to produce only a small amount of high molecular weight EPS. Examination of $^{1}$H-NMR spectra of EPS from PN4115 and the smooth, PN184-derived EPS mutants suggests that these strains produce an EPS of similar structure, with the exception of the degree of *O*-acetylation.

Three non-mucoid, Calcofluor-bright, EPS mutants were generated by Tn5 mutagenesis of PN4115. Each mutant was shown to carry a single, independent Tn5 insertion. Cosmids could not be isolated which stably complemented the mutation carried by each mutant. None of the mutants produced EPS, but all three mutants produce a Calcofluor-binding EPS, possibly cellulose.

All three PN4115-derived EPS mutants induced the formation of fully effective nodules on *Lotus corniculatus*, a determinate nodulating host legume. On *L. leucocephala*, PN4115 induced the formation of both small, ineffective, nodular swellings and large, ineffective, tumour-like structures. Occasionally, a low level of nitrogen fixation was observed. In contrast, the PN4115-derived EPS mutants all induced the formation of only small, ineffective, nodular swellings.

These results, obtained in isogenic *Rhizobium* backgrounds, support suggestions that EPS is required for effective nodulation of indeterminate nodulating legumes, but is not required for effective nodulation of determinate nodulating legumes.

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