500. The typA gene is required for stress adaptation as well as for symbiosis of Sinorhizobium meliloti 1021 with certain Medicago truncatula lines

By Kiss Erno; Huguet Thierry; Poinsot Verena; Batut Jacques
From Molecular plant-microbe interactions : MPMI (2004), 17(3), 235-44, Language: English, Database: MEDLINE

In this article, we describe the typA gene of Sinorhizobium meliloti, the orthologue of typA/bipA genes found in a wide range of bacteria. We found that typA was required for survival of S. meliloti under certain stress conditions, such as growth at low temperature or low pH and in the presence of sodium dodecyl sulfate (SDS). The cold-sensitive phenotype of both Escherichia coli bipA and S. meliloti typA mutants were cross-complemented, indicating that the two genes are functionally equivalent. typA was indispensable for symbiosis on Medicago truncatula Jemalong and F83005.5 and contributes to the full efficiency of symbiosis on other host plant lines such as DZA315.16 or several cultivars of M. sativa. Hence, the symbiotic requirement for typA is host dependent. Interestingly, the symbiotic defect was different on Jemalong and F83005.5 plants, thus indicating that typA is required at a different stage of the symbiotic interaction.

~14 Citings

501. A Medicago sativa haem oxygenase gene is preferentially expressed in root nodules

By Baudouin Emmanuel; Frendo Pierre; Le Gleuher Marie; Puppo Alain
From Journal of experimental botany (2004), 55(394), 43-7, Language: English, Database: MEDLINE

Haem oxygenases (HO) are ubiquitous enzymes catalysing the oxidative degradation of haem into biliverdin, iron and carbon monoxide. Whereas animal HOs participate in multiple cellular functions including haemoglobin catabolism, antioxidant defence and iron homeostasis, to date, plant HOs have so far only been involved in phytochrome metabolism. The expression of the HO1 gene was studied in Medicago sativa, especially during the interaction with its symbiotic partner, Sinorhizobium meliloti. Transcript accumulation was higher in mature root nodules than in roots and leaves and was correlated to HO1 protein immunodetection. The analysis of HO1 expression following alfalfa root inoculation with S. meliloti indicates that transcripts do not accumulate during the early steps of symbiosis, but rather in the mature nodules. These results correlate with the expression of the leghaemoglobin gene, which encodes the major haem-containing protein present in the nodule. Contrary to its animal counterpart, alfalfa HO1 was not induced by pro-oxidant compounds including H(2)O(2), paraquat and sodium nitroprusside, suggesting that it is not involved in the antioxidant defence. The results suggest that HO1 could play a role in the alfalfa mature nodule and its involvement in leghaemoglobin metabolism is hypothesized.

~4 Citings

502. Cell surface characteristics of two halotolerant strains of Sinorhizobium meliloti

By Bhattacharya Indranil; Das Hasi R
From Microbiological research (2003), 158(2), 187-94, Language: English, Database: MEDLINE

The halotolerant Sinorhizobium meliloti strain Rmd201 and its variant Rmd201 a were examined for their cell surface properties. The variant strain formed rough colonies and was found to be more hydrophobic. Growth of the variant strain was not affected appreciably when NaCl concentration of the medium was increased from 2 mM to 700 mM. Exopolysaccharide (EPS) and the lipopolysaccharide (LPS) content of the variant strain was found to be 7 and 14 times less, respectively, than the parent strain. However, enhanced synthesis of high molecular weight LPS bands were observed in SDS-PAGE analysis in the variant strain when the NaCl concentration was raised from 2 mM to 700 mM. Ribose and glucosamine were present in the variant LPS only. Mannose appeared as a major LPS constituent of the variant when grown in high salt containing medium. All these cell surface characteristics indicated that there were significant differences between the halotolerant strains of S. meliloti. The changes in the cell surface of the variant strain indicated the possible mutation in the gene(s) of cell surface polysaccharide biosynthesis.

~2 Citings

503. Purification and characterization of homodimeric methylmalonyl-CoA mutase from Sinorhizobium meliloti

By Miyamoto Emi; Watanabe Fumio; Charles Trevor C; Yamaji Ryoichi; Inui Hiroshi; Nakano Yoshihisa
From Archives of microbiology (2003), 180(2), 151-4, Language: English, Database: MEDLINE

High activity (>60 munit/mg protein) of 5'-deoxyadenosylcobalamin-dependent methylmalonyl-CoA mutase (EC 5.4.99.2) was constantly found during growth of a strain of the root-nodule-forming bacterium Sinorhizobium meliloti harboring an extra plasmid-encoded copy of the methylmalonyl-CoA-mutase-encoding bhbA gene. The enzyme was purified to homogeneity and characterized. The purified enzyme was found to be a colorless apo-form. The apparent molecular weight of the enzyme was calculated to be 165,000+/-5,000 by Superdex 200 HR gel filtration. SDS-PAGE of the purified enzyme resolved one protein band with an apparent molecular mass of 80.0+/-2.0 kDa, indicating that the S. meliloti enzyme is composed of two identical subunits. The NH(2)-terminal sequence was identical to that predicted from the bhbA nucleotide sequence. Monovalent cations were required for enzyme activity.

~2 Citings

504. The response of some common Egyptian plants to ozone and their use as biomonitors

By El-Khatib A A
From Environmental pollution (Barking, Essex : 1987) (2003), 124(3), 419-28, Language: English, Database: MEDLINE

Relative sensitivity of five common Egyptian plant species namely, Senecio vulgaris, Malva parviflora, Sonchus oleraceus, Medicago sativa and Melilotus indicus to elevated levels of ozone has been studied. The plants were exposed to charcoal filtered air (CFA) and different levels of O3 (50 and 100 ppb) for 5 h per day. The studied parameters were recorded for five consecutive days after fumigation. The foliar injury varied significantly among species in a dose-dependent manner. Severe injury symptoms were recorded on the leaves of M. sativa. With the exception of M. parviflora, all species exhibited significant increases in the percentage reduction of the above-ground dry weight as a result of reductions in both leaf and stem dry weights. M. sativa showed a marked reduction in its relative growth rate at elevated levels of O3. The extent of chlorophyll a destruction was higher in both M. sativa and S. oleraceus than in the other species tested. No differences in the sensitivity of chlorophylls a+b and carotenoids to ozone levels were recorded in this work. Percentage reduction of ascorbic acid was higher in M. sativa and S. oleraceus, compared with the other species studied. With respect to relative percentages of proline, there was a significant difference in the responses of plants to ozone. According to the ozone resistance (R%), measured as relative growth rate, the test species were arranged in the descending order: M. parviflora>M. Indicus>S. Vulgaris>S. Oleraceus>M. sativa. In M. sativa, both determinant and correlation coefficients are well reflected in the relationship between its physiological response, its performance and ozone levels, supporting its recommendation as a candidate for biomonitoring in Egypt.

~0 Citings

505. Symbiotic characterization of isoleucine+valine and leucine auxotrophs of Sinorhizobium meliloti

By Hassani Raad; Prasad C Krishna; Vineetha K E; Vij Neeraj; Singh Prachi; Sud Reeteka; Yadav Sangeeta; Randhawa Gursharn S
From Indian journal of experimental biology (2002), 40(10), 1110-20, Language: English, Database: MEDLINE

Ten isoleucine+valine and three leucine auxotrophs of Sinorhizobium meliloti Rmd201 were obtained by random mutagenesis with transposon Tn5 followed by screening of Tn5 derivatives on minimal medium supplemented with modified Holliday pools. Based on intermediate feeding, intermediate accumulation and cross-feeding studies, isoleucine+valine and leucine auxotrophs were designated as ilvB/ilvG, ilvC and ilvD, and leuC/leuD and leuB mutants, respectively. Symbiotic properties of all ilvD mutants with alfalfa plants were similar to those of the parental strain. The ilvB/ilvG and ilvC mutants were Nod-. Inoculation of alfalfa plants with ilvB/ilvG mutant did not result in root hair curling and infection thread formation. The ilvC mutants were capable of curling root hairs but did not induce infection thread formation. All leucine auxotrophs were Nod+ Fix-. Supplementation of leucine to the plant nutrient medium did not restore symbiotic effectiveness to the auxotrophs. Histological studies revealed that the nodules induced by the leucine auxotrophs did not develop fully like those induced by the parental strain. The nodules induced by leuB mutants were structurally more advanced than the leuC/leuD mutant induced nodules. These results indicate that ilvB/ilvG, ilvC and one or two leu genes of S. meliloti may have a role in symbiosis. The position of ilv genes on the chromosomal map of S. meliloti was found to be near ade-15 marker.

~1 Citing

506. Biotin limitation in Sinorhizobium meliloti strain 1021 alters transcription and translation

By Heinz Elke B; Streit Wolfgang R
From Applied and environmental microbiology (2003), 69(2), 1206-13, Language: English, Database: MEDLINE

Most Sinorhizobium meliloti strains lack several key genes involved in microbial biotin biosynthesis, and it is assumed that this may be a special adaptation which allows the microbe to down-regulate metabolic activities in the absence of a host plant. To further explore this hypothesis, we employed two different strategies. (i) Searches of the S. meliloti genome database in combination with the construction of nine different gusA reporter fusions identified three genes involved in a biotin starvation response in this microbe. A gene coding for a protein-methyl carboxyl transferase (pcm) exhibited 13.6-fold-higher transcription under biotin-limiting conditions than cells grown in the presence of 40 nM biotin. Consistent with this observation, biotin-limiting conditions resulted in a significantly decreased survival of pcm mutant cells compared to parental cells or cells grown in the presence of 40 nM biotin. Further studies indicated that the autoinducer synthase gene, sinI, was transcribed at a 4.5-fold-higher level in early stationary phase in biotin-starved cells than in biotin-supplemented cells. Lastly, we observed that open reading frame smc02283, which codes for a putative copper resistance protein (CopC), was 21-fold down-regulated in response to biotin starvation. (ii) In a second approach, proteome analysis identified 10 proteins which were significantly down-regulated under the biotin-limiting conditions. Among the proteins identified by using matrix-assisted laser desorption ionization-time of flight mass spectrometry were the pi subunit of the RNA polymerase and the 50S ribosomal protein L7/L12 (L8) subunit, indicating that biotin-limiting conditions generally affect transcription and translation in S. meliloti.

~1 Citing

507. Functional analysis of Sinorhizobium meliloti genes involved in biotin synthesis and transport

By Entcheva Plamena; Phillips Donald A; Streit Wolfgang R
From Applied and environmental microbiology (2002), 68(6), 2843-8, Language: English, Database: MEDLINE

External biotin greatly stimulates bacterial growth and alfalfa root colonization by Sinorhizobium meliloti strain 1021. Several genes involved in responses to plant-derived biotin have been identified in this bacterium, but no genes required for biotin transport are known, and not all loci required for biotin synthesis have been assigned. Searches of the S. meliloti genome database in combination with complementation tests of Escherichia coli biotin auxotrophs indicate that biotin synthesis probably is limited in S. meliloti 1021 by the poor functioning or complete absence of several key genes. Although several open reading frames with significant similarities to genes required for synthesis of biotin in gram-positive and gram-negative bacteria were found, only bioB, bioF, and bioH were demonstrably functional in complementation tests with known E. coli mutants. No sequence or complementation evidence was found for bioA, bioC, bioD, or bioZ. In contrast to other microorganisms, the S. meliloti bioB and bioF genes are not localized in a biotin synthesis operon, but bioB is cotranscribed with two genes coding for ABC transporter-like proteins, designated here bioM and bioN. Mutations in bioM and bioN eliminated growth on alfalfa roots and reduced bacterial capacity to maintain normal intracellular levels of biotin. Taken together, these data suggest that S. meliloti normally grows on exogenous biotin using bioM and bioN to conserve biotin assimilated from external sources.

~11 Citings

508. The Sinorhizobium meliloti stringent response affects multiple aspects of symbiosis

By Wells Derek H; Long Sharon R
From Molecular microbiology (2002), 43(5), 1115-27, Language: English, Database: MEDLINE

Sinorhizobium meliloti and host legumes enter into a nitrogen-fixing, symbiotic relationship triggered by an exchange of signals between bacteria and plant. S. meliloti produces Nod factor, which elicits the formation of nodules on plant roots, and succinoglycan, an exopolysaccharide that allows for bacterial invasion and colonization of the host. The biosynthesis of these molecules is well defined, but the specific regulation of these compounds is not completely understood. Bacteria control complex regulatory networks by the production of ppGpp, the effector molecule of the stringent response, which induces physiological change in response to adverse growth conditions and can also control bacterial development and virulence. Through detailed analysis of an S. meliloti mutant incapable of producing ppGpp, we show that the stringent response is required for nodule formation and regulates the production of succinoglycan. Although it remains unknown whether these phenotypes are connected, we have isolated suppressor strains that restore both defects and potentially identify key downstream regulatory genes. These results indicate that the S. meliloti stringent response has roles in both succinoglycan production and nodule formation and, more importantly, that control of bacterial physiology in response to the plant and surrounding environment is critical to the establishment of a successful symbiosis.

~40 Citings

509. Temporal effects on the composition of a population of Sinorhizobium meliloti associated with Medicago sativa and Melilotus alba

By Bromfield E S; Butler G; Barran L R
From Canadian journal of microbiology (2001), 47(6), 567-73, Language: English, Database: MEDLINE

An assessment was made of the impact of temporal separation on the composition of a population of Sinorhizobium meliloti associated with Medicago sativa (alfalfa) and Melilotus alba (sweet clover) grown at a single site that had no known history of alfalfa cultivation. Root nodules were sampled on six occasions over two seasons, and a total of 1620 isolates of S. meliloti were characterized on the basis of phage sensitivity using 16 typing phages. Plant infection tests indicated that symbiotic S. meliloti were deficient in the soil at the time of planting and that these bacteria were present at low density during the first season (<10(2)/g of soil); in the second season numbers increased markedly to about 10(5)/g of soil. Overall, 37 and 51 phage types, respectively, were encountered among the nodule isolates from M. sativa and M. alba. The data indicate significant temporal shifts in the frequency and diversity of types associated with the two legume species. Apparent temporal variation with respect to the frequency of types appeared largely unpredictable and was not attributable to any one sampling time. The results indicate an apparent reduction in phenotypic diversity over the course of the experiment. Differential host plant selection of specific types with respect to nodule occupancy was indicated by significant interactions between legume species and either the frequency or diversity of phage types. Isolates from M. sativa that were resistant to lysis by all typing phages (type 14) were unusual in that they were predominant on this host at all sampling times (between 53% and 82% nodule occupancy) and were relatively homogeneous on the basis of DNA hybridization with 98% of the isolates analysed sharing the same nod EFG hybridization profile. In contrast, those isolates from M. alba comprising type 14 were encountered at low total frequency (2%) and were genetically heterogeneous on the basis of Southern hybridization. The implications of the observed temporal and host plant variation for ecological studies are discussed.

~2 Citings

510. Cloning and characterization of the pyruvate carboxylase from Sinorhizobium meliloti Rm1021

By Dunn M F; Araiza G; Finan T M
From Archives of microbiology (2001), 176(5), 355-63, Language: English, Database: MEDLINE

The gene encoding pyruvate carboxylase (pyc) was isolated from a Sinorhizobium meliloti Rm1021 cosmid bank by complementation of a Rhizobium tropici pyc mutant. PYC-negative mutants of S. meliloti Rm1021 were isolated by transposon mutagenesis and were unable to grow with glucose or pyruvate as sole carbon sources, but were symbiotically competent in combination with alfalfa plants. PYC activity assays, pyc::lacZ gene fusion studies and an in vivo biotinylation assay showed that PYC activity in S. meliloti was dependent mainly on biotin availability and not on changes in gene transcription. The subunit and holo-enzyme molecular masses of the S. meliloti PYC indicated that the enzyme was an alpha4 homotetramer. The S. meliloti PYC had a high apparent Ka (0.23 mM) for the allosteric activator acetyl-CoA and was product-inhibited by sub-millimolar concentrations of oxaloacetate. In contrast to other bacterial alpha4-PYCs which have been characterized, the S. meliloti enzyme was not strongly inhibited by L-aspartate.

~6 Citings

511. Ultrastructural studies on nodules induced by pyrimidine auxotrophs of Sinorhizobium meliloti

By Vineetha K E; Vij N; Prasad C K; Hassani R; Randhawa G S
From Indian journal of experimental biology (2001), 39(4), 371-7, Language: English, Database: MEDLINE

Twenty three pyrimidine auxotrophs of Sinorhizobium meliloti Rmd201 were generated by random mutagenesis with transposon Tn5. On the basis of biochemical characters these auxotrophic mutants were classified into car, pyrC and pyrE/pyrF categories. All auxotrophs induced white nodules which were ineffective in nitrogen fixation. Light and electron microscopic studies revealed that the nodules induced by pyrC mutants were more developed than the nodules of car mutants. Similarly the nodules induced by pyrE/pyrF mutants had more advanced structural features than the nodules of pyrC mutants. The nodule development in case of pyrE/pyrF mutants was not to the extent observed in the parental strain. These results indicated that some of the intermediates and/or enzymes of pyrimidine biosynthetic pathway of S. meliloti play a key role in bacteroidal transformation and nodule development.

~2 Citings

512. Identification and structure of the Rhizobium galegae common nodulation genes: evidence for horizontal gene transfer

By Suominen L; Roos C; Lortet G; Paulin L; Lindstrom K
From Molecular biology and evolution (2001), 18(6), 907-16, Language: English, Database: MEDLINE

Rhizobia are soil bacteria able to fix atmospheric nitrogen in symbiosis with leguminous plants. In response to a signal cascade coded by genes of both symbiotic partners, a specific plant organ, the nodule, is formed. Rhizobial nodulation (nod) genes trigger nodule formation through the synthesis of Nod factors, a family of chitolipooligosaccharides that are specifically recognized by the host plant at the first stages of the nodulation process. Here, we present the organization and sequence of the common nod genes from Rhizobium galegae, a symbiotic member of the RHIZOBIACEAE: This species has an intriguing phylogenetic position, being symbiotic among pathogenic agrobacteria, which induce tumors instead of nodules in plant shoots or roots. This apparent incongruence raises special interest in the origin of the symbiotic apparatus of R. galegae. Our analysis of DNA sequence data indicated that the organization of the common nod gene region of R. galegae was similar to that of Sinorhizobium meliloti and Rhizobium leguminosarum, with nodIJ downstream of nodABC and the regulatory nodD gene closely linked to the common nod operon. Moreover, phylogenetic analyses of the nod gene sequences showed a close relationship especially between the common nodA sequences of R. galegae, S. meliloti, and R. leguminosarum biovars viciae and trifolii. This relationship in structure and sequence contrasts with the phylogeny based on 16S rRNA, which groups R. galegae close to agrobacteria and separate from most other rhizobia. The topology of the nodA tree was similar to that of the corresponding host plant tree. Taken together, these observations indicate that lateral nod gene transfer occurred from fast-growing rhizobia toward agrobacteria, after which the symbiotic apparatus evolved under host plant constraint.

~6 Citings

513. Sugar-binding activity of pea lectin enhances heterologous infection of transgenic alfalfa plants by Rhizobium leguminosarum biovar viciae

By van Rhijn P; Fujishige N A; Lim P O; Hirsch A M
From Plant physiology (2001), 126(1), 133-44, Language: English, Database: MEDLINE

Transgenic alfalfa (Medicago sativa L. cv Regen) roots carrying genes encoding soybean lectin or pea (Pisum sativum) seed lectin (PSL) were inoculated with Bradyrhizobium japonicum or Rhizobium leguminosarum bv viciae, respectively, and their responses were compared with those of comparably inoculated control plants. We found that nodule-like structures formed on alfalfa roots only when the rhizobial strains produced Nod factor from the alfalfa-nodulating strain, Sinorhizobium meliloti. Uninfected nodule-like structures developed on the soybean lectin-transgenic plant roots at very low inoculum concentrations, but bona fide infection threads were not detected even when B. japonicum produced the appropriate S. meliloti Nod factor. In contrast, the PSL-transgenic plants were not only well nodulated but also exhibited infection thread formation in response to R. leguminosarum bv viciae, but only when the bacteria expressed the complete set of S. meliloti nod genes. A few nodules from the PSL-transgenic plant roots were even found to be colonized by R. leguminosarum bv viciae expressing S. meliloti nod genes, but the plants were yellow and senescent, indicating that nitrogen fixation did not take place. Exopolysaccharide appears to be absolutely required for both nodule development and infection thread formation because neither occurred in PSL-transgenic plant roots following inoculation with an Exo(-) R. leguminosarum bv viciae strain that produced S. meliloti Nod factor.

~11 Citings

514. Two RpoH homologs responsible for the expression of heat shock protein genes in Sinorhizobium meliloti

By Ono Y; Mitsui H; Sato T; Minamisawa K
From Molecular & general genetics : MGG (2001), 264(6), 902-12, Language: English, Database: MEDLINE

We identified two rpoH-related genes encoding sigma32-like proteins from Sinorhizobium meliloti, a nitrogen-fixing root-nodule symbiont of alfalfa. The genes, rpoH1 and rpoH2, are functionally similar to rpoH of Escherichia coli because they partially complemented an E. coli rpoH null mutant. We obtained evidence indicating that these genes are involved in the heat shock response in S. meliloti. Following an increase in temperature, synthesis of several putative heat shock proteins (Hsps) was induced in cultures of wild-type cells: the most prominent were 66- and 60-kDa proteins, both of which are suggested to represent GroEL species. The other Hsps could divided into two groups based on differences in synthesis kinetics: synthesis of the first group peaked 5-10 min, and expression of the other group 30 min, after temperature upshift. In the rpoH1 mutant, inducible synthesis of the former group was markedly reduced, whereas that of the latter group was not affected. Synthesis of both the 66- and 60-kDa proteins was partially reduced. While no appreciable effect was observed in the rpoH2 single mutant, the rpoH2 mutation had a synergistic effect on the 60-kDa protein in the rpoH1- background. The results indicate that two distinct mechanisms are involved in the heat shock response of S. meliloti: one requires the rpoH1 function, while rpoH2 can substitute in part for the rpoH1 function. Moreover, the rpoH1 mutant and rpoH1 rpoH2 double mutant exhibited Nod+ Fix- and Nod- phenotypes, respectively, on alfalfa.

~12 Citings

515. glnD and mviN are genes of an essential operon in Sinorhizobium meliloti

By Rudnick P A; Arcondeguy T; Kennedy C K; Kahn D
From Journal of bacteriology (2001), 183(8), 2682-5, Language: English, Database: MEDLINE

To evaluate the role of uridylyl-transferase, the Sinorhizobium meliloti glnD gene was isolated by heterologous complementation in Azotobacter vinelandii. The glnD gene is cotranscribed with a gene homologous to Salmonella mviN. glnD1::Omega or mviN1::Omega mutants could not be isolated by a powerful sucrose counterselection procedure unless a complementing cosmid was provided, indicating that glnD and mviN are members of an indispensable operon in S. meliloti.

~12 Citings

516. Effect of field inoculation with Sinorhizobium meliloti L33 on the composition of bacterial communities in rhizospheres of a target plant (Medicago sativa) and a non-target plant (Chenopodium album)-linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria

By Schwieger F; Tebbe C C
From Applied and environmental microbiology (2000), 66(8), 3556-65, Language: English, Database: MEDLINE

Fourteen weeks after field release of luciferase gene-tagged Sinorhizobium meliloti L33 in field plots seeded with Medicago sativa, we found that the inoculant also occurred in bulk soil from noninoculated control plots. In rhizospheres of M. sativa plants, S. meliloti L33 could be detected in noninoculated plots 12 weeks after inoculation, indicating that growth in the rhizosphere preceded spread into bulk soil. To determine whether inoculation affected bacterial diversity, 1,119 bacteria were isolated from the rhizospheres of M. sativa and Chenopodium album, which was the dominant weed in the field plots. Amplified ribosomal DNA restriction analysis (ARDRA) revealed plant-specific fragment size frequencies. Dominant ARDRA groups were identified by 16S rRNA gene nucleotide sequencing. Database comparisons indicated that the rhizospheres contained members of the Proteobacteria (alpha, beta, and gamma subgroups), members of the Cytophaga-Flavobacterium group, and gram-positive bacteria with high G+C DNA contents. The levels of many groups were affected by the plant species and, in the case of M. sativa, by inoculation. The most abundant isolates were related to Variovorax sp., Arthrobacter ramosus, and Acinetobacter calcoaceticus. In the rhizosphere of M. sativa, inoculation reduced the numbers of cells of A. calcoaceticus and members of the genus Pseudomonas and increased the number of rhizobia. Cultivation-independent PCR-single-strand conformation polymorphism (SSCP) profiles of a 16S rRNA gene region confirmed the existence of plant-specific rhizosphere communities and the effect of the inoculant. All dominant ARDRA groups except Variovorax species could be detected. On the other hand, the SSCP profiles revealed products which could not be assigned to the dominant cultured isolates, indicating that the bacterial diversity was greater than the diversity suggested by cultivation.

~11 Citings

517. Sinorhizobium meliloti putA gene regulation: a new model within the family Rhizobiaceae

By Soto M J; Jimenez-Zurdo J I; van Dillewijn P; Toro N
From Journal of bacteriology (2000), 182(7), 1935-41, Language: English, Database: MEDLINE

Proline dehydrogenase (PutA) is a bifunctional enzyme that catalyzes the oxidation of proline to glutamate. In Sinorhizobium meliloti, as in other microorganisms, the putA gene is transcriptionally activated in response to proline. In Rhodobacter capsulatus, Agrobacterium, and most probably in Bradyrhizobium, this activation is dependent on an Lrp-like protein encoded by the putR gene, located immediately upstream of putA. Interestingly, sequence and genetic analysis of the region upstream of the S. meliloti putA gene did not reveal such a putR locus or any other encoded transcriptional activator of putA. Furthermore, results obtained with an S. meliloti putA null mutation indicate the absence of any proline-responsive transcriptional activator and that PutA serves as an autogenous repressor. Therefore, the model of S. meliloti putA regulation completely diverges from that of its Rhizobiaceae relatives and resembles more that of enteric bacteria. However, some differences have been found with the latter model: (i) S. meliloti putA gene is not catabolite repressed, and (ii) the gene encoding for the major proline permease (putP) does not form part of an operon with the putA gene.

~7 Citings

518. Characterization of an atypical superoxide dismutase from Sinorhizobium meliloti

By Santos R; Bocquet S; Puppo A; Touati D
From Journal of bacteriology (1999), 181(15), 4509-16, Language: English, Database: MEDLINE

Sinorhizobium meliloti Rm5000 is an aerobic bacterium that can live free in the soil or in symbiosis with the roots of leguminous plants. A single detectable superoxide dismutase (SOD) was found in free-living growth conditions. The corresponding gene was isolated from a genomic library by using a sod fragment amplified by PCR from degenerate primers as a probe. The sodA gene was located in the chromosome. It is transcribed monocistronically and encodes a 200-amino-acid protein with a theoretical M(r) of 22,430 and pI of 5. 8. S. meliloti SOD complemented a deficient E. coli mutant, restoring aerobic growth of a sodA sodB recA strain, when the gene was expressed from the synthetic tac promoter but not from its own promoter. Amino acid sequence alignment showed great similarity with Fe-containing SODs (FeSODs), but the enzyme was not inactivated by H(2)O(2). The native enzyme was purified and found to be a dimeric protein, with a specific activity of 4,000 U/mg. Despite its Fe-type sequence, atomic absorption spectroscopy showed manganese to be the cofactor (0.75 mol of manganese and 0.24 mol of iron per mol of monomer). The apoenzyme was prepared from crude extracts of S. meliloti. Activity was restored by dialysis against either MnCl(2) or Fe(NH(4))(2)(SO(4))(2), demonstrating the cambialistic nature of the S. meliloti SOD. The recovered activity with manganese was sevenfold higher than with iron. Both reconstituted enzymes were resistant to H(2)O(2). Sequence comparison with 70 FeSODs and MnSODs indicates that S. meliloti SOD contains several atypical residues at specific sites that might account for the activation by manganese and resistance to H(2)O(2) of this unusual Fe-type SOD.

~14 Citings

519. Mutational analysis of the Rhizobium etli recA operator

By Tapias A; Barbe J
From Journal of bacteriology (1998), 180(23), 6325-31, Language: English, Database: MEDLINE

Based upon our earlier studies (A. Tapias, A. R. Fernandez de Henestrosa, and J. Barbe, J. Bacteriol. 179:1573-1579, 1997) we hypothesized that the regulatory sequence of the Rhizobium etli recA gene was TTGN11CAA. However, further detailed analysis of the R. etli recA operator described in the present work suggests that it may in fact be GAACN7GTAC. This new conclusion is based upon PCR mutagenesis analysis carried out in the R. etli recA operator, which indicates that the GAAC and GTAC submotifs found in the sequence GAACN7GTAC are required for the maximal stimulation of in vivo transcription and in vitro DNA-protein complex formation. This DNA-protein complex is also detected when the GAACN7GTAC wild-type sequence is modified to obtain GAACN7GAAC, GTACN7GTAC, or GAACN7GTTC. The wild-type promoters of the Rhizobium meliloti and Agrobacterium tumefaciens recA genes, which also contain the GAACN7GTAC sequence, compete with the R. etli recA promoter for the DNA-protein complex formation but not with mutant derivatives in any of these motifs, indicating that the R. etli, R. meliloti, and A. tumefaciens recA genes present the same regulatory sequence.

~4 Citings

520. Biosynthetic control of molecular weight in the polymerization of the octasaccharide subunits of succinoglycan, a symbiotically important exopolysaccharide of Rhizobium meliloti

By Gonzalez J E; Semino C E; Wang L X; Castellano-Torres L E; Walker G C
From Proceedings of the National Academy of Sciences of the United States of America (1998), 95(23), 13477-82, Language: English, Database: MEDLINE

Succinoglycan, a symbiotically important exopolysaccharide of Rhizobium meliloti, is composed of polymerized octasaccharide subunits, each of which consists of one galactose and seven glucoses with succinyl, acetyl, and pyruvyl modifications. Production of specific low molecular weight forms of R. meliloti exported and surface polysaccharides, including succinoglycan, appears to be important for nodule invasion. In a previous study of the roles of the various exo gene products in succinoglycan biosynthesis, exoP, exoQ, and exoT mutants were found to synthesize undecaprenol-linked fully modified succinoglycan octasaccharide subunits, suggesting possible roles for their gene products in polymerization or transport. Using improved techniques for analyzing succinoglycan biosynthesis by these mutants, we have obtained evidence indicating that R. meliloti has genetically separable systems for the synthesis of high molecular weight succinoglycan and the synthesis of a specific class of low molecular weight oligosaccharides consisting of dimers and trimers of the octasaccharide subunit. Models to account for our unexpected findings are discussed. Possible roles for the ExoP, ExoQ, and ExoT proteins are compared and contrasted with roles that have been suggested on the basis of homologies to key proteins involved in the biosynthesis of O-antigens and of certain exported or capsular cell surface polysaccharides.

~23 Citings

521. The nos (nitrous oxide reductase) gene cluster from the soil bacterium Achromobacter cycloclastes: cloning, sequence analysis, and expression

By McGuirl M A; Nelson L K; Bollinger J A; Chan Y K; Dooley D M
From Journal of inorganic biochemistry (1998), 70(3-4), 155-69, Language: English, Database: MEDLINE

The nitrous oxide (N2O) reductase (nos) gene cluster from Achromobacter cycloclastes has been cloned and sequenced. Seven protein coding regions corresponding to nosR, nosZ (structural N2O reductase gene), nosD, nosF, nosY, nosL, and nosX are detected, indicating a genetic organization similar to that of Rhizobium meliloti. To aid homology studies, nosR from R. meliloti has also been sequenced. Comparison of the deduced amino acid sequences with corresponding sequences from other organisms has also allowed structural and functional inferences to be made. The heterologous expression of NosD, NosZ (N2O reductase), and NosL is also reported. A model of the CuA site in N2O reductase, based on the crystal structure of this site in bovine heart cytochrome c oxidase, is presented. The model suggests that a His residue of the CuA domain may be a ligand to the catalytic CuZ site. In addition, the origin of the spectroscopically-observed Cys coordination to CuZ is discussed in terms of the sequence alignment of seven N2O reductases.

~4 Citings

522. Characterization and splicing in vivo of a Sinorhizobium meliloti group II intron associated with particular insertion sequences of the IS630-Tc1/IS3 retroposon superfamily

By Martinez-Abarca F; Zekri S; Toro N
From Molecular microbiology (1998), 28(6), 1295-306, Language: English, Database: MEDLINE

By sequence analysis of Sinorhizobium meliloti strain GR4 plasmid pRmeGR4b, we have identified a group II intron named RmInt1 inserted within the insertion sequence ISRm2011-2 of the IS630-Tc1/IS3 retroposon superfamily. Like some other group II introns, RmInt1 possesses, in addition to the structurally conserved ribozyme core, an open reading frame (ORF) with homology to reverse transcriptases. Using a T7 expression system in Escherichia coli, we show that the intron is active in splicing in vivo and that splicing efficiency requires the intron-encoded ORF, which suggests that the putative intron encoded protein has a maturase function. DNA hybridization studies indicate that intron RmInt1 is widespread within S. meliloti native populations and appears to be mostly located within this IS element. Nevertheless, some S. meliloti strains harbour one copy of RmInt1 at a different location. DNA sequence analysis of the 5' exon of one of these heterologous intron insertion sites revealed the presence of a putative IS element closely related to insertion sequence ISRm2011-2. The intron-binding sites (IBS1 and IBS2 motifs) are conserved, although a transition of a G-->A in the IBS1 has occurred. Our results demonstrate an association of intron RmInt1 with particular insertion sequences of the IS630-Tc1/IS3 retroposon superfamily that may have ensured the spread and maintenance of this group II intron in S. meliloti.

~19 Citings

523. Distribution of free seleno-amino acids in plant tissue of Melilotus indica L. grown in selenium-laden soils

By Guo X; Wu L
From Ecotoxicology and environmental safety (1998), 39(3), 207-14, Language: English, Database: MEDLINE

Accumulation of specific groups of seleno-amino acids in plant tissue reflects not only the Se tolerance of a plant species, but also Se toxicity to animals. The distribution of seleno-amino acids in a Se-tolerant grassland legume species (Melilotus indica L.) grown in Se-laden soils was studied using high-resolution gas chromatography- and gas chromatography-mass spectrometry. Five seleno-amino acids including selenocystine, selenomethionine, selenocysteine, Se-methylselenocysteine, and gamma-glutamyl-Se-methylselenocysteine were identified and measured for their plant tissue concentrations. Se-methylselenocysteine, a nonprotein seleno-amino acid, was found in the plant tissue. Its concentration ranged from 15.3 mumol kg-1 for the plants growing in soil of low Se concentration to 109.8 mumol kg-1 for the plants grown in soil of high Se concentration. Accumulation of the nonprotein seleno-amino acid in this species resembles that in Se accumulator plants. gamma-Glutamyl-Se-methylselenocysteine was detected in the plant. However, its concentration was very low. It might not become a toxic element in the food chain. Results of plant tissue Se accumulation analysis indicated that there was a five-fold increase in tissue selenocysteine concentration when the total tissue Se increased from 5.07 to 22.02 mg kg-1, but there was no further increase in tissue selenocysteine concentration when the tissue total Se concentration increased from 22.0 to 117.4 mg kg-1. Selenomethinone constituted more than 50% of the total seleno-amino acid in the plant. More research is needed to reveal whether the mechanisms limiting the accumulation of selenocysteine and preferential accumulation of selenomethionine found in this study play any role in Se tolerance in this species.

~0 Citings

524. The Rhizobium etli FixL protein differs in structure from other known FixL proteins

By D'hooghe I; Michiels J; Vanderleyden J
From Molecular & general genetics : MGG (1998), 257(5), 576-80, Language: English, Database: MEDLINE

The central heme-binding domain in the FixL proteins of Sinorhizobium meliloti, Bradyrhizobium japonicum, Rhizobium leguminosarum biovar viciae and Azorhizobium caulinodans, is highly conserved. The similarity with the corresponding domain in the Rhizobium etli FixL protein is considerably less. This observation prompted us to analyze the heme-binding capacities of the R. etli FixL protein. The R. etli fixL gene was overexpressed in Escherichia coli. In the presence of S. meliloti FixJ, the overexpressed R. etli FixL protein was able to enhance FixJ-mediated activation of an S. meliloti pnifA-lacZ fusion, indicating that the R. etli FixL protein possesses an active conformation in E. coli. Subsequently, using a non-denaturing gel assay for heme, we analyzed the heme-binding capacity of the R. etli FixL protein expressed in E. coli, taking the S. meliloti FixL protein as a positive control. The R. etli FixL protein expressed in E. coli does not contain a heme group, in contrast to the S. meliloti FixL protein. Therefore we conclude that the R. etli FixL is a non-heme protein in the nif regulatory cascade.

~4 Citings

525. Exopolysaccharide II production is regulated by salt in the halotolerant strain Rhizobium meliloti EFB1

By Lloret J; Wulff B B; Rubio J M; Downie J A; Bonilla I; Rivilla R
From Applied and environmental microbiology (1998), 64(3), 1024-8, Language: English, Database: MEDLINE

The halotolerant strain Rhizobium meliloti EFB1 modifies the production of extracellular polysaccharides in response to salt. EFB1 colonies grown in the presence of 0.3 M NaCl show a decrease in mucoidy, and in salt-supplemented liquid medium this organism produces 40% less exopolysaccharides. We isolated transposon-induced mutant that, when grown in the absence of salt, had a colony morphology (nonmucoid) similar to the colony morphology of the wild type grown in the presence of salt. Calcofluor fluorescence, proton nuclear magnetic resonance spectroscopy, and genetic analysis of the mutant indicated that galactoglucan, which is not produced under normal conditions by other R. meliloti strains, is produced by strain EFB1 and that production of this compound decreases when the organism is grown in the presence of salt. The mutant was found to be affected in a genetic region highly homologous to genes for galactoglucan production in R. meliloti Rm2011 (expE genes). However, sequence divergence occurs in a putative expE promoter region. A transcriptional fusion of the promoter with lacZ demonstrated that, unlike R. meliloti Rm2011, galactoglucan is produced constitutively by EFB1 and that its expression is reduced 10-fold during exponential growth in the presence of salt.

~11 Citings

526. Heme compounds as iron sources for nonpathogenic Rhizobium bacteria

By Noya F; Arias A; Fabiano E
From Journal of bacteriology (1997), 179(9), 3076-8, Language: English, Database: MEDLINE

Many animal-pathogenic bacteria can use heme compounds as iron sources. Like these microorganisms, rhizobium strains interact with host organisms where heme compounds are available. Results presented in this paper indicate that the use of hemoglobin as an iron source is not restricted to animal-pathogenic microorganisms. We also demonstrate that heme, hemoglobin, and leghemoglobin can act as iron sources under iron-depleted conditions for Rhizobium meliloti 242. Analysis of iron acquisition mutant strains indicates that siderophore-, heme-, hemoglobin-, and leghemoglobin-mediated iron transport systems expressed by R. meliloti 242 share at least one component.

~18 Citings

527. Rhizobium nodulation protein NodC is an important determinant of chitin oligosaccharide chain length in Nod factor biosynthesis

By Kamst E; Pilling J; Raamsdonk L M; Lugtenberg B J; Spaink H P
From Journal of bacteriology (1997), 179(7), 2103-8, Language: English, Database: MEDLINE

Synthesis of chitin oligosaccharides by NodC is the first committed step in the biosynthesis of rhizobial lipochitin oligosaccharides (LCOs). The distribution of oligosaccharide chain lengths in LCOs differs between various Rhizobium species. We expressed the cloned nodC genes of Rhizobium meliloti, R. leguminosarum bv. viciae, and R. loti in Escherichia coli. The in vivo activities of the various NodC proteins differed with respect to the length of the major chitin oligosaccharide produced. The clearest difference was observed between strains with R. meliloti and R. loti NodC, producing chitintetraose and chitinpentaose, respectively. In vitro experiments, using UDP-[14C]GlcNAc as a precursor, show that this difference reflects intrinsic properties of these NodC proteins and that it is not influenced by the UDP-GlcNAc concentration. Analysis of oligosaccharide chain lengths in LCOs produced by a R. leguminosarum bv. viciae nodC mutant, expressing the three cloned nodC genes mentioned above, shows that the difference in oligosaccharide chain length in LCOs of R. meliloti and R. leguminosarum bv. viciae is due only to nodC. The exclusive production of LCOs which contain a chitinpentaose backbone by R. loti strains is not due to NodC but to end product selection by Nod proteins involved in further modification of the chitin oligosaccharide. These results indicate that nodC contributes to the host specificity of R. meliloti, a conclusion consistent with the results of several studies which have shown that the lengths of the oligosaccharide backbones of LCOs can strongly influence their activities on host plants.

~3 Citings

528. The common nodABC genes of Rhizobium meliloti are host-range determinants

By Roche P; Maillet F; Plazanet C; Debelle F; Ferro M; Truchet G; Prome J C; Denarie J
From Proceedings of the National Academy of Sciences of the United States of America (1996), 93(26), 15305-10, Language: English, Database: MEDLINE

Symbiotic bacteria of the genus Rhizobium synthesize lipo-chitooligosaccharides, called Nod factors (NFs), which act as morphogenic signal molecules on legume hosts. The common nodABC genes, present in all Rhizobium species, are required for the synthesis of the core structure of NFs. NodC is an N-acetylglucosaminyltransferase, and NodB is a chitooligosaccharide deacetylase; NodA is involved in N-acylation of the aminosugar backbone. Specific nod genes are involved in diverse NF substitutions that confer plant specificity. We transferred to R. tropici, a broad host-range tropical symbiont, the ability to nodulate alfalfa, by introducing nod genes of R. meliloti. In addition to the specific nodL and nodFE genes, the common nodABC genes of R. meliloti were required for infection and nodulation of alfalfa. Purified NFs of the R. tropici hybrid strain, which contained chitin tetramers and were partly N-acylated with unsaturated C16 fatty acids, were able to elicit nodule formation on alfalfa. Inactivation of the R. meliloti nodABC genes suppressed the ability of the NFs to nodulate alfalfa. Studies of NFs from nodA, nodB, nodC, and nodI mutants indicate that (i) NodA of R. meliloti, in contrast to NodA of R. tropici, is able to transfer unsaturated C16 fatty acids onto the chitin backbone and (ii) NodC of R. meliloti specifies the synthesis of chitin tetramers. These results show that allelic variation of the common nodABC genes is a genetic mechanism that plays an important role in signaling variation and in the control of host range.

~12 Citings

529. Sinorhizobium medicae sp. nov., isolated from annual Medicago spp

By Rome S; Fernandez M P; Brunel B; Normand P; Cleyet-Marel J C
From International journal of systematic bacteriology (1996), 46(4), 972-80, Language: English, Database: MEDLINE

The taxonomic position of isolates of a new genomic species (designated genomic species 2) obtained from several annual Medicago species and originating from different geographical locations was established through the results of phenotypic tests (including the results of auxanographic and biochemical tests and symbiotic properties) and 16S rRNA phylogenetic inferences. A comparison of the complete 16S rRNA sequence of a representative of genomic species 2 (strain A 321T [T = type strain]) with the 16S rRNA sequences of other members of the Rhizobiaceae and closely related taxa showed that genomic species 2 was phylogenetically related to Sinorhizobium meliloti, Sinorhizobium fredii, Sinorhizobium saheli, and Sinorhizobium teranga. The levels of sequence similarity and observed numbers of nucleotide substitutions in Sinorhizobium strains indicated that A 321T and S. meliloti exhibited the highest level of sequence similarity (99.7%), with four nucleotide substitutions and one deletion. The results of a numerical analysis based on data from 63 auxanographic and biochemical tests clearly separated genomic species 2 isolates from S. meliloti. Genomic species 2 isolates nodulated and fixed nitrogen with Medicago polymorpha, whereas S. meliloti isolates were ineffective and formed rudimentary nodules on this host plant. On the basis of phenotypic and 16S sequence analysis data, genomic species 2 isolates cannot be assigned to a previously described species. We propose that these isolates belong to a new species, Sinorhizobium medicae.

~22 Citings

530. Bioextraction of selenium by forage and selected field legume species in selenium-laden soils under minimal field management conditions

By Van Mantgem P J; Wu L; Banuelos G S
From Ecotoxicology and environmental safety (1996), 34(3), 228-38, Language: English, Database: MEDLINE

A forage plant, tall fescue (Festuca arundinacea), and a selected field legume species, sour clover (Melilotus indica), were examined for their selenium (Se) bioextraction abilities in Se-laden soils under minimal management conditions. Natural vegetations in a 2-acre plot adjacent to the forage plots were also studied for Se accumulation comparisons. During the dry season, in the fall of 1994, the field plots were either irrigated weekly or without irrigation. No fertilization and weed control were applied. The plants were harvested in May 1995. There were considerable differences in the ability of Se uptake between the forage and the legume species and among the naturally established plant species; the amount of Se accumulated per land area was largely dependent on their respective biomass production. Comparing Se concentration between preplant and postharvest, there was a detectable reduction in the soil selenate, selenite, and water-extractable organic Se in the tall fescue and melilotus plots. The field irrigation provided more favorable conditions for bioextractions and dissipation of Se by the plants. However, the available soil Se only accounts for less than 10% of the total soil Se and no detectable reduction of total soil Se was found. This may be due to the large inventory and variation of Se concentrations in the field soils and therefore obscured the detectable differences. For practical considerations, the forage plants can be repeatedly harvested and used for rangelands of Se deficiency currently seen in some northern California counties.

~0 Citings

531. Thermoregulation of kpsF, the first region 1 gene in the kps locus for polysialic acid biosynthesis in Escherichia coli K1

By Cieslewicz M; Vimr E
From Journal of bacteriology (1996), 178(11), 3212-20, Language: English, Database: MEDLINE

The kps locus for biosynthesis of the capsular polysialic acid virulence factor in Escherichia coli K1 contains at least two convergently transcribed operons, designated region 1 and regions 2 plus 3. On the basis of DNA sequence analysis, kpsF appeared to be a good candidate for the first gene of region 1 (M. J. Cieslewicz, S. M. Steenbergen, and E. R. Vimr, J. Bacteriol. 175:8018-8023, 1993). A preliminary indication that kpsF is required for capsule production is the capsule-negative phenotype of an aph T insertion in the chromosomal copy of kpsF. The present communication describes the isolation and phenotypic characterization of this mutant. Although transcription through kpsF was required for capsule production, complementation analysis failed to indicate a clear requirement for the KpsF polypeptide. However, since E. coli contains at least two other open reading frames that could code for homologs of KpsF, the apparent dispensability of KpsF remains provisional. DNA sequence analysis of 1,100 bp upstream from the kpsF translational start site did not reveal any open reading frames longer than 174 nucleotides, consistent with kpsF being the first gene of region 1. Since kpsF appeared to be the first gene of a region whose gene products are required for polysialic acid transport and because capsule production is known to be thermoregulated, primer extension analyses were carried out with total RNA isolated from cells grown at permissive (37 degrees C) and nonpermissive (20 degrees C) temperatures. The results revealed a potentially complex kpsF promoter-like region that was transcriptionally silent at the nonpermissive temperature, suggesting that thermoregulation of region 1 may be exerted through variations in kpsF expression. Additional evidence supporting this conclusion was obtained by demonstrating the effects of temperature on expression of the gene kpsE, immediately downstream of kpsF. Chloramphenicol acetyltransferase assays were carried out with constructs containing the kpsF 5' untranslated region fused to a promoterless cat cassette, providing further evidence that kpsF is thermoregulated. Although the function of KpsF is unclear, primary structure analysis indicated two motifs commonly observed in regulatory proteins and homology with glucosamine synthase from Rhizobium meliloti.

~16 Citings

532. Extension of the Rhizobium meliloti succinoglycan biosynthesis gene cluster: identification of the exsA gene encoding an ABC transporter protein, and the exsB gene which probably codes for a regulator of succinoglycan biosynthesis

By Becker A; Kuster H; Niehaus K; Puhler A
From Molecular & general genetics : MGG (1995), 249(5), 487-97, Language: English, Database: MEDLINE

Two new genes, designated exsA and exsB, were identified adjacent to the 24 kb exo gene cluster of Rhizobium meliloti, which is involved in succinoglycan (EPS I) biosynthesis. The derived amino acid sequence of ExsA displayed significant homologies to ATP binding cassette (ABC) transporter proteins. R. meliloti strains mutated in exsA were characterized by a decreased ratio of HMW to LMW EPS I, indicating a function for ExsA in EPS I biosynthesis. The R. meliloti NdvA protein, which is involved in the transport of cyclic beta-(1,2)-glucans, was identified as the closest homologue of ExsA. R. meliloti exsB mutants produced a three-fold increased amount of EPS I in comparison to the wild-type strain. In contrast, high copy number of exsB resulted in a decrease in the EPS I level to 20% of wild type, indicating that the exsB gene product can negatively influence EPS I biosynthesis. It was demonstrated that this influence is not due to transcriptional regulation of the exo genes by the exsB gene product. By plasmid integration it was shown that exsA and exsB represent monocistronic transcription units.

~28 Citings

533. Expression vectors for the use of eukaryotic luciferases as bacterial markers with different colors of luminescence

By Cebolla A; Vazquez M E; Palomares A J
From Applied and environmental microbiology (1995), 61(2), 660-8, Language: English, Database: MEDLINE

An easy way to identify microorganisms is to provide them with gene markers that confer a unique phenotype. Several genetic constructions were developed to use eukaryotic luciferase genes for bacterial tagging. The firefly and click bettle luciferase genes, luc and lucOR, respectively, were cloned under constitutive control and regulated control from different transcriptional units driven by P1, lambda PR, and Ptrc promoters. Comparison of the expression of each gene in Escherichia coli cells from identical promoters showed that bioluminescence produced by luc could be detected luminometrically in a more sensitive manner. In contrast, luminescence from intact lucOR-expressing cells was much more stable and resistant to high temperatures than that from luc-expressing cells. To analyze the behavior of these constructions in other gram-negative bacteria, gene fusions with luc genes were cloned on broad-host-range vectors. Measurements of light emission from Rhizobium meliloti, Agrobacterium tumefaciens, and Pseudomonas putida cells indicated that both luciferases were poorly expressed from P1 in most bacterial hosts. In contrast, the lambda promoter PR yielded constitutively high levels of luciferase expression in all bacterial species tested. PR activity was not regulated by temperature when the thermosensitive repressor cI857 was present in the bacterial species tested, except for E. coli. In contrast, the regulated lacIq-Ptrc::lucOR fusion expression system behaved in a manner similar to that observed in E. coli cells. After IPTG (isopropyl-beta-D-thiogalactopyranoside) induction, this system produced the highest levels of lucOR expression in all bacterial species tested.(ABSTRACT TRUNCATED AT 250 WORDS)

~4 Citings

534. A homolog of the Rhizobium meliloti nitrogen fixation gene fixN is involved in the production of a microaerobically induced oxidase activity in the phytopathogenic bacterium Agrobacterium tumefaciens

By Schluter A; Ruberg S; Kramer M; Weidner S; Priefer U B
From Molecular & general genetics : MGG (1995), 247(2), 206-15, Language: English, Database: MEDLINE

Hybridization analysis using the Rhizobium meliloti nitrogen fixation gene fixN as a probe revealed the presence of a homologous DNA region in the phytopathogenic bacterium Agrobacterium tumefaciens. Hybridization signals were also detected with total DNAs of Rhizobium leguminosarum bv. phaseoli, Rhodobacter capsulatus and Escherichia coli, but not those of Xanthomonas campestris pv. campestris and Pseudomonas putida. The hybridizing fragment from A. tumefaciens was cloned and sequenced. The predicted gene product of one of the two open reading frames identified on the sequenced fragment shows homology to FixN of different Rhizobiaceae as well as a low but significant similarity to subunit I of heme copper oxidases from various bacteria. The presence of five strictly conserved histidine residues previously implicated in forming ligands to heme and CuB in oxidases and the predicted membrane topology provide evidence that the A. tumefaciens fixN-like gene product is a component of the heme copper oxidase superfamily. The incomplete open reading frame starting only 8 nucleotides downstream of the fixN-like gene exhibits homology to Rhizobium fixO. Using an uidA (GUS) gene fusion it could be shown that the A. tumefaciens fixN-like gene is preferentially expressed under microaerobic conditions. Expression of the uidA fusion is abolished in R. meliloti fixJ and fixK mutants, indicating that an Fnr-like protein is involved in transcriptional regulation of the fixN-like gene in A. tumefaciens. The presence of an upstream DNA sequence motif identical to the Fnr-consensus binding site (anaerobox) further supports this hypothesis. A. tumefaciens mutated in the fixN-like gene shows decreased TMPD-specific oxidase activity under microaerobic conditions, indicating that the fixN-like gene or operon codes for proteins involved in respiration under reduced oxygen availability.

~6 Citings

535. Cloning of the second adenylate cyclase gene (cya2) from Rhizobium meliloti F34: sequence similarity to eukaryotic cyclases

By Archdeacon J; Talty J; Boesten B; Danchin A; O'Gara F
From FEMS microbiology letters (1995), 128(2), 177-84, Language: English, Database: MEDLINE

A second adenylate cyclase (cya2) gene was isolated from a Rhizobium meliloti F34 gene bank. Complemented E. coli delta cya mutants were capable of utilizing a number of, but not all, carbon sources known to be regulated by cAMP. DNA hybridization studies showed cya2 to be unique to R. meliloti strains. The cya2 nucleotide sequence was determined and found to encode a protein of 363 amino acids. Residues were identified within the C-terminal domain which are conserved in both eukaryotic adenylate and guanylate cyclases, including a putative ATP binding site. Similar residues were also found in the prokaryotic R. meliloti Cya1 protein. A R. meliloti cya1/cya2 double mutant was constructed and characterized; however, cAMP production was still observed in this strain indicating the presence of a third cya gene.

~5 Citings

536. The cycHJKL genes of Rhizobium meliloti involved in cytochrome c biogenesis are required for "respiratory" nitrate reduction ex planta and for nitrogen fixation during symbiosis

By Kereszt A; Slaska-Kiss K; Putnoky P; Banfalvi Z; Kondorosi A
From Molecular & general genetics : MGG (1995), 247(1), 39-47, Language: English, Database: MEDLINE

We report the genetic and biochemical analysis of Rhizobium meliloti mutants defective in symbiotic nitrogen fixation (Fix-) and "respiratory" nitrate reduction (Rnr-). The mutations were mapped close to the ade-1 and cys-46 chromosomal markers and the mutated locus proved to be identical to the previously described fix-14 locus. By directed Tn5 mutagenesis, a 4.5 kb segment of the chromosome was delimited in which all mutations resulted in Rnr- and Fix- phenotypes. Nucleotide sequence analysis of this region revealed the presence of four open reading frames coding for integral membrane and membrane-anchored proteins. Biochemical analysis of the mutants showed that the four proteins were necessary for the biogenesis of all cellular c-type cytochromes. In agreement with the nomenclature proposed for rhizobial genes involved in the formation of c-type cytochromes, the four genes were designated cycH, cycJ, cycK, and cycL, respectively. The predicted protein product of cycH exhibited a high degree of similarity to the Bradyrhizobium japonicum counterpart, while CycK and CycL shared more than 50% amino acid sequence identity with the Rhodobacter capsulatus Cc11 and Cc12 proteins, respectively. cycJ encodes a novel membrane anchored protein of 150 amino acids. We suggest that this gene cluster codes for (parts of) a multisubunit cytochrome c haem lyase. Moreover, our results indicate that in R. meliloti c-type cytochromes are required for respiratory nitrate reduction ex planta, as well as for symbiotic nitrogen fixation in root nodules.

~16 Citings

537. Interspecies regulation of the recA gene of gram-negative bacteria lacking an E. coli-like SOS operator

By Riera J; Fernandez de Henestrosa A R; Garriga X; Tapias A; Barbe J
From Molecular & general genetics : MGG (1994), 245(4), 523-7, Language: English, Database: MEDLINE

The recA genes of Agrobacterium tumefaciens, Rhizobium meliloti, Rhizobium phaseoli and Rhodobacter sphaeroides, species belonging to the alpha-group bacteria of the Proteobacteria class, have been fused in vitro to the lacZ gene of Escherichia coli. By using a mini-Tn5 transposon derivative, each of these recA-lacZ fusions was introduced into the chromosome of each of the four species, and into that of E. coli. The recA genes of three of the alpha bacteria are induced by DNA damage when inserted in A. tumefaciens, R. phaseoli or R. meliloti chromosomes. The expression of the recA gene of R. sphaeroides is DNA damage-mediated only when present in its own chromosome; none of the genes is induced in E. coli. Likewise, the recA gene of E. coli is not induced in any of the four alpha species. These data indicate that A. tumefaciens, R. meliloti and R. phaseoli possess a LexA-like repressor, which is able to block the expression of their recA genes, as well as that of R. sphaeroides, but not the recA gene of E. coli. The LexA repressor of R. sphaeroides does not repress the recA gene of A. tumefaciens, R. meliloti, R. phaseoli or E. coli.

~4 Citings

538. Effects of elevated selenium concentration on selenium accumulation and nitrogen fixation symbiotic activity of Melilotus indica L

By Wu L; Emberg A; Biggar J A
From Ecotoxicology and environmental safety (1994), 27(1), 50-63, Language: English, Database: MEDLINE

Biological and soil factors which contribute to the successful colonization of an annual legume species. Melilotus indica L., in soils with elevated selenium (Se) were studied. This species was introduced into the Kesterson Reservoir in the fresh top soil that was brought in under the Kesterson Cleanup Action to fill lowering pond sites and prevent the formation of ephemeral pools containing hazardous levels of Se. In 4 years since its introduction, it has expanded its range of colonization from the fresh soil fill sites to the native soil sites and contributed 10 to 50% of biomass to the grassland communities. The plant and nodule tissue Se concentrations of the field grown plants were found to be negatively correlated with the soil sulfate concentration. Nutrient solution culture studies discovered that M. indica was able to accumulate 500 micrograms Se g-1 dry weight without a reduction of growth rate. Plants without nodulation were found to accumulate a greater amount of Se and more vulnerable to Se toxicity. Acetylene reduction rate measurements indicate that the nitrogen fixation symbiotic activity appears to be more susceptible to an elevated Se concentration than its host plant. M. indica is a winter weed, and it occurs naturally in the Se-rich soils. It grows actively over the winter and spring and complete its life cycle in May. If the root nodules and root tissues are incorporated into the soil, the rate of soil Se volatilization may be accelerated over the warm summer months. For disposal of the Se-rich plant materials the plant shoot tissues may be harvested for Se-deficient forage supplementation. Therefore, this species may be useful for field management and reclamation of Se-contaminated soils.

~1 Citing

539. Rhizobium meliloti homologs of Escherichia coli mur genes

By Leach F; Wacks D B; Signer E R
From Gene (1994), 148(1), 87-90, Language: English, Database: MEDLINE

The pectate-lyase-encoding gene pelB of Erwinia chrysanthemi Ec16 was used as a probe for hybridization to Rhizobium meliloti Rm1021 chromosomal DNA under low-stringency conditions. An Rm1021 DNA fragment that hybridized to this probe was cloned and sequenced. Results of RNA hybridization indicate that a portion of the cloned fragment is transcribed in R. meliloti. Although the Rm1021 fragment shares no significant nucleotide sequence identity with Ec16 pelB, it includes an ORF (open reading frame) that shares a high degree of nt sequence identity with the Escherichia coli murD gene. This gene codes for UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase, which catalyzes a step in the synthesis of the E. coli cell wall. The R. meliloti putative murD sequence is preceded by a partial ORF that shares sequence identity with mraY. The orientation of the two ORFs in R. meliloti is similar to that of the E. coli murD and mraY genes.

~0 Citings

540. A 4.6 kb DNA region of Rhizobium meliloti involved in determining urease and hydrogenase activities carries the structural genes for urease (ureA, ureB, ureC) interrupted by other open reading frames

By Miksch G; Arnold W; Lentzsch P; Priefer U B; Puhler A
From Molecular & general genetics : MGG (1994), 242(5), 539-50, Language: English, Database: MEDLINE

A 4.6 kb DNA region of the Rhizobium meliloti strain AK631 was found to contain seven open reading frames (ORFs), all oriented in the same direction. The putative gene products of four of these ORFs were highly homologous to UreA, UreB and UreC of Klebsiella aerogenes, Proteus mirabilis, Proteus vulgaris and Canavalia ensiformis. The overall organisation of the DNA region analysed was ORF1, ureA (ORF2), ORF3, ureB (ORF4), ORF5, ORF6 and ureC (ORF7), indicating that the organisation of the urease structural genes in R. meliloti differs from that of other urease genes so far characterized. ORF1 was incomplete; only the 3' end of the coding region was present. The six complete ORFs coded for polypeptides of 11.1 (UreA), 8.9 (ORF3), 10.8 (UreB), 15.0 (ORF5), 13.8 (ORF6) and 60.7 kDa (UreC). No sequence homology to known polypeptides could be detected for the gene products of ORF1, ORF3, ORF5 and ORF6. Using a lacZ fusion and insertional mutagenesis it was shown that the seven ORFs identified were all located in the same transcription unit. For mutational analysis a resistance gene cassette was introduced into each of the complete ORFs resulting in apolar mutations. Mutations in ureA, ureB and ureC, but not in ORF3, ORF5 and ORF6, abolished urease activity in R. meliloti. The determination of hydrogen uptake in these R. meliloti mutants revealed that only ORF6 and ureB are necessary for hydrogen uptake.

~5 Citings

541. Cloning and characterization of multiple groEL chaperonin-encoding genes in Rhizobium meliloti

By Rusanganwa E; Gupta R S
From Gene (1993), 126(1), 67-75, Language: English, Database: MEDLINE

Heat-shock treatment of Rhizobium meliloti cells causes major enhancement in the synthesis of several proteins with apparent molecular weights in the range of 58-60 kDa. Using the polymerase chain reaction and degenerate oligodeoxyribonucleotide primers for conserved regions of the 60-kDa heat-shock protein (HSP60) or GroEL protein family, a 0.6-kb probe for the R. meliloti hsp60 gene was prepared. Southern blot analysis of R. meliloti DNA digested with different restriction enzymes and hybridized to R. meliloti hsp60 probes indicated the presence of between four and five hsp60 or groEL in this species. From the cloning and sequencing of several of these fragments, we have been able to deduce the complete nucleotide sequences of three groEL in R. meliloti. The deduced amino acid (aa) sequences of these proteins show extensive similarity to each other (78-85% aa identity) and to other GroEL homologues. In the upstream regions of two of the groEL, but not the third, open reading frames corresponding to GroES proteins were also identified. Analysis of various prokaryotic GroEL sequences suggests that the multiple groEL of R. meliloti have evolved by means of gene duplication events within this or a related group of organisms. Results presented in this paper also show that some of the groEL in R. meliloti are located on the two megaplasmids present in these cells. The presence of multiple GroEL homologues in R. meliloti suggests a possible role of the GroEL or HSP60 chaperonins in the nodulation (symbiosis) and nitrogen fixation processes.

~15 Citings

542. Natural establishment and selenium accumulation of herbaceous plant species in soils with elevated concentrations of selenium and salinity under irrigation and tillage practices

By Wu L; Enberg A; Tanji K K
From Ecotoxicology and environmental safety (1993), 25(2), 127-40, Language: English, Database: MEDLINE

The effects of irrigation and tillage practices were studied on species richness, biomass, and selenium accumulation of naturally established herbaceous plants in soils with elevated levels of selenium (Se) and salinity at Kesterson Reservoir, Merced County, California. The four different irrigation-tillage practice combinations were (1) no irrigation, no tillage; (2) irrigation, no tillage; (3) no irrigation, tillage; and (4) irrigation, tillage. The fields were allowed to become colonized naturally by herbaceous plant species. For the Mediterranean climate in the study site, irrigation was conducted biweekly through the summer months, and tillage was done in 3-month intervals. Biomass and Se accumulation of Atriplex patula L, Bassia hyssopifolia Kuntze, Rev. Gen. Pl., Melilotus indica (L.) All., and Salsola kali L. were substantially affected by irrigation. The degree and direction of the effects were found to be species dependent. The field plots which were tilled at 3-month intervals remained bare throughout the experiment. The total soil Se concentrations in the top 15 cm soil horizon were found to be in the range of 40 to 70 mg kg-1 dry wt. Soil Se concentrations below 25 cm soil depth were much lower and within a range of 2 to 4 mg kg-1. Less than 1/10th of the total soil Se inventory in the top soil horizon was water extractable, and the distribution of the Se inventory did not change significantly over the period of 1990 and 1991 despite the irrigation and tillage practices suggesting that a large portion of the Se inventory was not remobilized. The water-extractable soil Se concentration was found to be significantly lower in soils with the greatest biomass production suggesting an effective bioextraction of soil selenium by the native herbaceous plants.

~0 Citings

543. The Rhizobium leguminosarum FnrN protein is functionally similar to Escherichia coli Fnr and promotes heterologous oxygen-dependent activation of transcription

By Schluter A; Patschkowski T; Unden G; Priefer U B
From Molecular microbiology (1992), 6(22), 3395-404, Language: English, Database: MEDLINE

An open reading frame from Rhizobium leguminosarum bv. viciae strain VF39, previously identified and found to be similar to Escherichia coli fnr and Rhizobium meliloti fixK (orf240, thereafter called fnrN), was further analysed. Analysis of the expression of an fnrN-lacZ transcriptional fusion revealed that fnrN is preferentially expressed under oxygen limitation. Using R. meliloti fixN-lacZ fusions it was shown that the fnrN gene product only mediates transcriptional activation under microaerobiosis, indicating that the FnrN protein responds, directly or indirectly, to oxygen. Plasmids which expressed fnrN under the control of an E. coli promoter were able to complement an E. coli fnr mutant with respect to anaerobic growth on nitrate but not fumarate, and to promote anaerobic but not aerobic activation of the Fnr-dependent E. coli genes narGHJI, nirB and fdnGHI coding for nitrate reductase, NADH-dependent nitrite reductase and formate dehydrogenase-N, respectively. Fumarate and DMSO reductase activities were not induced by FnrN. The E. coli fnr gene substituted for fnrN in oxygen-regulated transcription of nirB- and fixN-lacZ fusions in R. leguminosarum. The results indicate that Fnr and FnrN are functionally very similar and share a common mode of oxygen-dependent transcriptional activation. From hybridization studies, it appeared that fnrN-like genes are present in a number of different R. leguminosarum strains.

~5 Citings

544. Synthesis of the ferredoxin-like protein FdxN from Rhizobium meliloti bacteroids as a fusion protein in Escherichia coli

By Riedel K U; Masepohl B; Klipp W; Puhler A
From Canadian journal of microbiology (1992), 38(6), 534-40, Language: English, Database: MEDLINE

To analyze the overexpression of the Rhizobium meliloti fdxN gene in Escherichia coli, different translational and transcriptional fusions were constructed. The translational signals of R. meliloti fdxN were recognized in E. coli as demonstrated by the use of in-frame lac fusions. Translational fusions consisting of the lacZ or the lpp gene fused in frame to the 3' end of the entire fdxN gene were expressed at high levels in E. coli. In contrast, the wild-type R. meliloti FdxN protein without a C-terminal fusion could only be detected using the very sensitive T7 promoter-polymerase system and not in immunoblots with antibodies against an FdxN-LacZ hybrid protein. Evidently, translational fusions to the 3' end of fdxN had a stabilizing effect on the expression of the fdxN gene. A constitutively expressed transcriptional fdxN fusion, which did not mediate detectable amounts of FdxN protein either in E. coli or in free-living R. meliloti cells, complemented the Fix- phenotype of an R. meliloti fdxN::[Tc] mutant strain to wild-type levels. Therefore, either low amounts of the wild-type FdxN protein are sufficient for symbiotic nitrogen fixation or there are stabilizing factors, which are present only in R. meliloti bacteroids but not in free-living R. meliloti cells. Fusion proteins consisting of FdxN and LacZ or a partial Lpp protein restored the Fix- phenotype of an R. meliloti fdxN mutant to 3 and 11%, respectively, indicating that a C-terminal fusion did not completely abolish the function of FdxN.

~0 Citings

545. Transcription of the Azospirillum brasilense nifH gene is positively regulated by NifA and NtrA and is negatively controlled by the cellular nitrogen status

By Vande Broek A; Michiels J; de Faria S M; Milcamps A; Vanderleyden J
From Molecular & general genetics : MGG (1992), 232(2), 279-83, Language: English, Database: MEDLINE

The expression of a translational Azospirillum brasilense nifH-uidA fusion was studied in A. brasilense and in Rhizobium meliloti strains with mutations in nifA, ntrA and ntrC. Induction of the fusion was observed in the R. meliloti wild-type and NtrC- strains on incubation under microaerobic conditions but not in the NifA- and NtrA- strains, showing the absolute requirement of both sigma 54 and NifA for activation of the nifH promoter. Histochemical analysis of the root nodules elicited by R. meliloti wild-type showed expression of the fusion in the late symbiotic zone but not in the meristematic and the early symbiotic zones. No induction of the nifH-uidA fusion was observed in the R. meliloti wild-type or NifA- strains incubated aerobically in nitrogen-free medium, indicating that, in contrast to R. meliloti nifH, A. brasilense nifH cannot be activated directly by NtrC. Expression of the nifH gene in A. brasilense only occurs under nitrogen-limiting, microaerobic conditions, suggesting the presence of a nitrogen-dependent control system for nif gene expression.

~3 Citings

546. Rhizobium meliloti exopolysaccharides: genetic analyses and symbiotic importance

By Reuber T L; Reed J; Glazebrook J; Glucksmann M A; Ahmann D; Marra A; Walker G C
From Biochemical Society transactions (1991), 19(3), 636-41, Language: English, Database: MEDLINE

Genetic experiments have indicated that succinoglycan (EPS I), the acidic Calcofluor-binding exopolysaccharide, of the nitrogen-fixing bacterium Rhizobium meliloti strain Rm1021 is required for nodule invasion and possibly for later events in nodule development on alfalfa and other hosts. Fourteen exo loci on the second megaplasmid have been identified that are required for, or affect, the synthesis of EPS I. Mutations in certain of these loci completely abolish the production of EPS I and result in mutants that form empty Fix- nodules. We have identified two loci, exoR and exoS, that are involved in the regulation of EPS I synthesis in the free-living state. Certain exo mutations which completely abolish EPS I production are lethal in an exoR95 or exoS96 background. Histochemical analyses of the expression of exo genes during nodulation using exo::TnphoA fusions have indicated that the exo genes are expressed most strongly in the invasion zone. In addition, we have discovered that R. meliloti has a latent capacity to synthesize a second exopolysaccharide (EPS II) that can substitute for the role(s) of EPS I in nodulation of alfalfa but not of other hosts. Possible roles for exopolysaccharides in symbiosis are discussed.

~6 Citings

547. Species richness and selenium accumulation of plants in soils with elevated concentration of selenium and salinity

By Huang Z Z; Wu L
From Ecotoxicology and environmental safety (1991), 22(3), 251-66, Language: English, Database: MEDLINE

Field studies were conducted in soils with elevated concentrations of Se and salinity at Kesterson, California. Biomass distribution, species richness, and selenium accumulation of plants were examined for two sites where 15 cm of surface soil was removed and replaced with fill dirt in the fall of 1989, and two sites were native soil cover. The Se concentrations in the top 15 cm of fill dirt ranged from undetectable to 36 ng g-1. For the native soil sites, Se levels ranged from 75 to 550 ng g-1. Soil Se concentrations below 15 cm ranged from 300 to 700 ng g-1 and were comparable between the fill dirt and the native soil sites. At least 20 different plant species were brought into the two fill dirt sites with the top soil. Avena fatua L., Bassia hyssopifolia Kuntze Rev. Gen. Pl., Centaurea solstitialis L., Erysimum officianale L., Franseria acanthicarpa Cav. Icon., and Melilotus indica (L.) All. contributed over 60% of the total biomass. Only 5 species were found in the native soil sites, and salt grass (Distichlis spicata L.) was the predominant species and accounted for over 80% of the total biomass. Between 1989 and 1990, two years after the surface soil replacement, the two fill dirt sites had a 70% reduction in species richness. Plant tissue selenium concentrations were found to be quite variable between plant species and between sites of sampling. At the fill dirt sites, the plant species with deep root systems accumulated greater amounts of selenium than the shallow-rooted species. The soil selenium concentration of the field soil had no negative effect on pollen fertility, seed set, and seed germination for the plant species examined. However, seedling growth was impaired by the soil selenium concentrations. This suggests that a selection pressure of soil Se concentration may have been imposed on plant species such as M. indica in an early stage of its life cycle.

~0 Citings

548. Mutations that affect activity of the Rhizobium meliloti trpE(G) promoter in Rhizobium meliloti and Escherichia coli

By Bae Y M; Stauffer G V
From Journal of bacteriology (1991), 173(18), 5831-6, Language: English, Database: MEDLINE

The cloned Rhizobium meliloti trpE(G) gene is not expressed in Escherichia coli. Oligonucleotide-directed mutagenesis was used to introduce base substitution mutations in the promoter region of this gene. Three separate mutations that increased homology of the putative -10 region of this promoter with the E. coli -10 promoter consensus sequence by 1 bp converted this promoter to an active promoter in E. coli. A deletion extending to position -43 from the 5' side had a minor effect on transcription in R. meliloti. However, transcription was nearly eliminated when a deletion extended to position -33, indicating that the crucial domain of the R. meliloti trpE(G) promoter begins in the region downstream of position -43. The R. meliloti trpE(G) promoter has two regions that show homology with the E. coli -35 and -10 promoter consensus sequences. Mutations in these putative -35 and -10 regions, but not in the spacer region, affected promoter strength in R. meliloti. By comparing four known R. meliloti promoter sequences, we identified a highly conserved trimer near position -35 (5'-TTG-3') but no noticeably conserved sequence near position -10.

~2 Citings

549. Cloning and characterization of a Rhizobium meliloti homolog of the Escherichia coli cell division gene ftsZ

By Margolin W; Corbo J C; Long S R
From Journal of bacteriology (1991), 173(18), 5822-30, Language: English, Database: MEDLINE

The ftsZ gene is essential for initiation of cell division in Escherichia coli and Bacillus subtilis. To begin our studies of division arrest during differentiation of Rhizobium meliloti bacteroids, we isolated a R. meliloti ftsZ homolog, ftsZRm. Degenerate primers directed towards a conserved region of ftsZ were used to amplify a segment of R. meliloti DNA by polymerase chain reaction, and the product of this reaction was then used to isolate positive clones from a bacteriophage library. The DNA sequence of an open reading frame containing the region of homology indicated that the R. meliloti FtsZ protein (FtsZRm) is 50% homologous to the known E. coli and B. subtilis FtsZ proteins, but at 590 amino acids (63 kDa), it is predicted to be nearly 50% larger. Strong expression of an approximately 70-kDa labeled protein in a coupled in vitro transcription-translation system supports this prediction. The additional 200 amino acids appear to fall in a single internal domain highly enriched for proline and glutamine residues. When we regulated R. meliloti ftsZ (ftsZRm) expression on a high-copy-number plasmid in E. coli with Plac and laclq, cells were smaller than normal in the presence of low FtsZRm levels (with no isopropyl-beta-D-thiogalactopyranoside [IPTG]) and filamentous when FtsZRm was overproduced (with IPTG). These results suggest that low levels of FtsZRm stimulate E. coli cell division, while high levels may be inhibitory.

~33 Citings

550. ndvF, a novel locus located on megaplasmid pRmeSU47b (pEXO) of Rhizobium meliloti, is required for normal nodule development

By Charles T C; Newcomb W; Finan T M
From Journal of bacteriology (1991), 173(13), 3981-92, Language: English, Database: MEDLINE

Rhizobium meliloti strains carrying either of two overlapping deletions (delta 5408 and delta F114) of the megaplasmid pRmeSU47b form nodules on alfalfa which fail to fix N2 (Fix-). Strains carrying these deletions also fail to fluoresce on media containing calcofluor, indicating a defect in synthesis of the acidic exopolysaccharide (Exo-) of R. meliloti. We have isolated cosmid clones (pTH21 and pTH22) which complement the Fix- but not the Exo- phenotype of the strains carrying the delta 5408 and delta F114 deletions. In addition, cosmid clones which complement the Exo- phenotype fail to complement the Fix- phenotype of these deletions; thus, the Exo- phenotype is not related to the Fix- phenotype. A 5-kb region within a 7.3-kb BamHI restriction fragment was found to be required for complementation of the Fix- phenotype of the delta 5408 and delta F114 deletion strains. Tn5 insertions in the 5-kb region generated a Fix- phenotype when recombined into the wild-type genome. We have designated this locus ndvF, for nodule development. TnphoA mutagenesis of this region generated active alkaline-phosphatase gene fusions, indicating that ndvF encodes extracytoplasmic protein(s). Induction of nodules by the ndvF mutants was delayed by 2 to 3 days compared with induction by the wild-type strain. Light microscopy of nodules elicited by strains carrying the large 150-kb delta F114 deletion, a 12-kb deletion removing ndvF, or an individual ndvF::Tn5 insertion mutation demonstrated that many nodules contained few infected cortical cells, indicating that nodule development was blocked early in the infection process, before the release of bacteria from the infection threads.

~15 Citings

551. Genetic analysis of the attenuator of the Rhizobium meliloti trpE(G) gene

By Bae Y M; Stauffer G V
From Journal of bacteriology (1991), 173(11), 3382-8, Language: English, Database: MEDLINE

It was previously reported that transcription of the Rhizobium meliloti trpE(G) gene starts at the adenine residue of the AUG codon of the leader peptide coding sequence (trpL), suggesting that translation of the trpL sequence starts without the Shine-Dalgarno sequence. We constructed mutations replacing the AUG codon of the trpL sequence with AAG or ACG. These mutations reduced the expression of a trpL'-'lacZ fusion gene to 0.1 and 0.2% of the wild-type level, respectively, indicating that the AUG codon is the translation initiation codon for the trpL coding sequence. In addition, these mutations, as well as a mutation converting the eighth codon (UCG) of the trpL sequence to UGA, abolished regulation by attenuation when introduced upstream of the tandem tryptophan codons in a trpE'-'lacZ fusion. Mutations affecting the stability of the probable antiterminator and terminator secondary structures in trpL mRNA were also constructed. Studies using these mutations indicate that the attenuator of R. meliloti functions in a way analogous to that of the Escherichia coli trp attenuator.

~2 Citings

552. Functional and evolutionary relatedness of genes for exopolysaccharide synthesis in Rhizobium meliloti and Rhizobium sp. strain NGR234

By Zhan H J; Gray J X; Levery S B; Rolfe B G; Leigh J A
From Journal of bacteriology (1990), 172(9), 5245-53, Language: English, Database: MEDLINE

Rhizobium meliloti SU47 and Rhizobium sp. strain NGR234 produce distinct exopolysaccharides that have some similarities in structure. R. meliloti has a narrow host range, whereas Rhizobium strain NGR234 has a very broad host range. In cross-species complementation and hybridization experiments, we found that several of the genes required for the production of the two polysaccharides were functionally interchangeable and similar in evolutionary origin. NGR234 exoC and exoY corresponded to R. meliloti exoB and exoF, respectively. NGR234 exoD was found to be an operon that included genes equivalent to exoM, exoA, and exoL in R. meliloti. Complementation of R. meliloti exoP, -N, and -G by NGR234 R'3222 indicated that additional equivalent genes remain to be found on the R-prime. We were not able to complement NGR234 exoB with R. meliloti DNA. In addition to functional and evolutionary equivalence of individual genes, the general organization of the exo regions was similar between the two species. It is likely that the same ancestral genes were used in the evolution of both exopolysaccharide biosynthetic pathways and probably of pathways in other species as well.

~7 Citings

553. The Rhizobium meliloti trpE(G) gene is regulated by attenuation, and its product, anthranilate synthase, is regulated by feedback inhibition

By Bae Y M; Crawford I P
From Journal of bacteriology (1990), 172(6), 3318-27, Language: English, Database: MEDLINE

In Rhizobium meliloti, the genes involved in biosynthesis of the amino acid tryptophan are found at three separate chromosomal locations. Of the three gene clusters, trpE(G), trpDC, and trpFBA, only the trpE(G) gene is regulated by the end product of the pathway, tryptophan. We found that trpE(G) mRNA contains a leader transcript that terminates at a stem-loop structure in a putative transcription attenuator. The level of this leader transcript was constant regardless of the amount of tryptophan in the growth medium. However, the level of full-length trpE(G) mRNA decreased as the amount of tryptophan increased. The beta-galactosidase activity of an R. meliloti strain carrying a trpL'-'lacZ fusion remained constant at different tryptophan concentrations, but the beta-galactosidase activity of the same strain carrying a trpE(G)'-'lacZ fusion decreased as the tryptophan concentration increased. These data indicate that transcription of the R. meliloti trpE(G) gene is regulated only by attenuation. We also found that the product of the trpE(G) gene, anthranilate synthase, is feedback inhibited by tryptophan.

~8 Citings

554. Rhizobium meliloti glutamate synthase: cloning and initial characterization of the glt locus

By Lewis T A; Gonzalez R; Botsford J L
From Journal of bacteriology (1990), 172(5), 2413-20, Language: English, Database: MEDLINE

The genetic locus glt, encoding glutamate synthase from Rhizobium meliloti 1021, was selected from a pLAFR1 clone bank by complementation of the R. meliloti 41 Glt- mutant AK330. A fragment of cloned DNA complementing this mutant also served to complement the Escherichia coli glt null mutant PA340. Complementation studies using these mutants suggested that glutamate synthase expression requires two complementation groups present at this locus. Genomic Southern analysis using a probe of the R. meliloti 1021 glt region showed a close resemblance between R. meliloti 1021, 41, and 102f34 at glt, whereas R. meliloti 104A14 showed many differences in restriction fragment length polymorphism patterns at this locus. R. meliloti 102f34, but not the other strains, showed an additional region with sequence similarity to glt. Insertion alleles containing transposable kanamycin resistance elements were constructed and used to derive Glt- mutants of R. meliloti 1021 and 102f34. These mutants were unable to assimilate ammonia and were Nod+ Fix+ on alfalfa seedlings. The mutants also showed poor or no growth on nitrogen sources such as glutamate, aspartate, arginine, and histidine, which are utilized by the wild-type parental strains. Strains that remained auxotrophic but grew nearly as well as the wild type on these nitrogen sources were readily isolated from populations of glt insertion mutants, indicating that degradation of these amino acids is negatively regulated in R. meliloti as a result of disruptions of glt.

~11 Citings

555. Symbiotic pseudorevertants of Rhizobium meliloti ndv mutants

By Dylan T; Nagpal P; Helinski D R; Ditta G S
From Journal of bacteriology (1990), 172(3), 1409-17, Language: English, Database: MEDLINE

Nodule development (ndv) mutants of Rhizobium meliloti cannot invade alfalfa to establish a nitrogen-fixing symbiosis and instead induce the formation of small, white, unoccupied nodules on alfalfa roots. Such mutants also fail to produce the unusual cyclic oligosaccharide beta-(1----2)-glucan and show defects in several aspects of vegetative growth and function. Here we show that ndv mutants are severely reduced, although not totally deficient, in the ability to attach to and initiate infection threads on alfalfa seedlings, and we demonstrate that the symbiotic deficiency can be separated from the rest of the mutant phenotype by isolating second-site pseudorevertants. Pseudorevertants selected for restoration of motility, a vegetative property, regained a substantial amount of attachment capability but only slight infection thread initiation and symbiotic ability. Such strains also regained partial tolerance to growth at low osmolarity, even though they did not recover the ability to synthesize periplasmic beta-(1----2)-glucan. Pseudorevertants selected on alfalfa for restoration of symbiosis were unrestored for beta-(1----2)-glucan production or any other vegetative property and regained little or no attachment or infection thread initiation capability. We take these data to indicate that wild-type R. meliloti normally has considerable excess capability for both attachment and infection thread initiation and that the symbiotic block in ndv mutants lies further along the developmental pathway than either of these processes, probably at the level of infection thread extension. Further, the fact that neither type of pseudorevertant recovered the ability to produce periplasmic beta-(1----2)-glucan raises the possibility that this oligosaccharide is not directly required for nodule development.

~18 Citings

556. Expression of the adenyl cyclase-encoding gene (cya) of Rhizobium meliloti F34: existence of two cya genes?

By O'Regan M; Kiely B; O'Gara F
From Gene (1989), 83(2), 243-9, Language: English, Database: MEDLINE

To gain insight into the role of cyclic AMP (cAMP) in Gram-negative soil bacteria, we have studied the expression of an adenyl cyclase-encoding gene 'cya' of Rhizobium meliloti F34. In both Escherichia coli and Bradyrhizobium japonicum, the gene is expressed from a promoter(s) contained on a 2.6-kb fragment of the cloned insert, which may indicate the presence of a functional 'cya' promoter or the coincidental presence of sequences which can function as promoters in these two species. The study of 'cya'-lac fusion activity in R. meliloti indicated that the 'cya' gene is not expressed at detectable levels and, thus, may not contribute to the modulation of cAMP levels under the growth conditions employed. R. meliloti strains carrying defined genomic mutations at the 'cya' locus were still capable of the synthesis of near wild-type levels of cAMP. These results suggest that the cloned 'cya' gene is not the key determinant responsible for cAMP synthesis under the culture conditions employed.

~2 Citings

557. A novel exopolysaccharide can function in place of the calcofluor-binding exopolysaccharide in nodulation of alfalfa by Rhizobium meliloti

By Glazebrook J; Walker G C
From Cell (1989), 56(4), 661-72, Language: English, Database: MEDLINE

We have found that R. meliloti strain Rm1021, which is known to synthesize a Calcofluor-binding exopolysaccharide (EPS I), also has a cryptic capacity to synthesize a second exopolysaccharide (EPS II). Structural analysis of EPS II has shown that it differs in many respects from EPS I. Genetic analysis indicates that EPS II synthesis requires the products of at least seven loci on the second symbiotic megaplasmid of R. meliloti, and is induced by a mutation, expR101, which causes increased transcription of these genes. Synthesis of EPS II suppresses the symbiotic defects of EPS I-deficient strains on Medicago sativa (alfalfa), but not on four other plants that are normally hosts for Rm1021. These observations suggest that structural features of bacterial exopolysaccharides are involved in the determination of host range. The implications of these results for models of exopolysaccharide function, such as serving as signals to the plant or shielding the bacteria from plant defense responses, are discussed.

~91 Citings

558. The DNA-binding domain of the transcriptional activator protein NifA resides in its carboxy terminus, recognises the upstream activator sequences of nif promoters and can be separated from the positive control function of NifA

By Morett E; Cannon W; Buck M
From Nucleic acids research (1988), 16(24), 11469-88, Language: English, Database: MEDLINE

The positive control protein NifA activates transcription of nitrogen fixation promoters in Klebsiella pneumoniae. NifA is believed to bind to specific sites, the upstream activator sequences (UAS's), of the nif promoters which it activates. We have now shown by mutation of the carboxy terminus of NifA that this is the DNA-binding domain and that the DNA-binding and positive activator functions of NifA can be separated. Mutational analysis of the nifH UAS and in vivo methylation protection analysis of the interaction of NifA with the nifH promoter demonstrates that the UAS is recognised by the carboxy terminus of NifA. The UAS's of K. pneumoniae nif promoters are also required for activation by the Rhizobium meliloti NifA indicating that this activator also possesses DNA-binding activity.

~39 Citings

559. Rhizobium meliloti host range nodH gene determines production of an alfalfa-specific extracellular signal

By Faucher C; Maillet F; Vasse J; Rosenberg C; van Brussel A A; Truchet G; Denarie J
From Journal of bacteriology (1988), 170(12), 5489-99, Language: English, Database: MEDLINE

The Rhizobium meliloti nodH gene is involved in determining host range specificity. By comparison with the wild-type strain, NodH mutants exhibit a change in host specificity. That is, although NodH mutants lose the ability to elicit root hair curling (Hac-), infection threads (Inf-), and nodule meristem formation (Nod-) on the homologous host alfalfa, they gain the ability to be Hac+ Inf+ Nod+ on a nonhomologous host such as common vetch. Using root hair deformation (Had) bioassays on alfalfa and vetch, we have demonstrated that sterile supernatant solutions of R. meliloti cultures, in which the nod genes had been induced by the plant flavone luteolin, contained symbiotic extracellular signals. The wild-type strain produced at least one Had signal active on alfalfa (HadA). The NodH- mutants did not produce this signal but produced at least one factor active on vetch (HadV). Mutants altered in the common nodABC genes produced neither of the Had factors. This result suggests that the nodABC operon determines the production of a common symbiotic factor which is modified by the NodH product into an alfalfa-specific signal. An absolute correlation was observed between the specificity of the symbiotic behavior of rhizobial cells and the Had specificity of their sterile filtrates. This indicates that the R. meliloti nodH gene determines host range by helping to mediate the production of a specific extracellular signal.

~19 Citings

560. Genetic analysis of carbamoylphosphate synthesis in Rhizobium meliloti 104A14

By Kerppola T K; Kahn M L
From Journal of general microbiology (1988), 134(4), 921-9, Language: English, Database: MEDLINE

We have previously isolated ineffective (Fix-) mutants of Rhizobium meliloti 104A14 requiring both arginine and uracil, and thus probably defective in carbamoylphosphate synthetase. We describe here the molecular and genetic analysis of the R. meliloti genes coding for carbamoylphosphate synthetase. Plasmids that complement the mutations were isolated from a R. meliloti gene bank. Restriction analysis of these plasmids indicated that complementation involved two unlinked regions of the R. meliloti chromosome, carA and carB. Genetic complementation between the plasmids and mutants demonstrated a single complementation group for carA, but two overlapping complementation groups for carB. The cloned R. meliloti genes hybridize to the corresponding E. coli carA and carB genes which encode the two subunits of carbamoylphosphate synthetase. Transposon Tn5 mutagenesis was used to localize the carA and carB genes on the cloned R. meliloti DNA. The cloned R. meliloti carA and carB genes were unable to complement E. coli carA or carB mutants alone or in combination. We speculate on the mechanism of the unusual pattern of genetic complementation at the R. meliloti carB locus.

~4 Citings

561. Cascade regulation of nif gene expression in Rhizobium meliloti

By David M; Daveran M L; Batut J; Dedieu A; Domergue O; Ghai J; Hertig C; Boistard P; Kahn D
From Cell (1988), 54(5), 671-83, Language: English, Database: MEDLINE

We report the discovery of two genes from Rhizobium meliloti, fixL and fixJ, which are positive regulators of symbiotic expression of diverse nitrogen fixation (nif and fix) genes. nif gene regulation is shown to consist of a cascade: the fixLJ genes activate nifA, which in turn activates nifHDK and fixABCX. Like nifA, fixN can be induced in free-living microaerobic cultures of R. meliloti, indicating a major physiological role for oxygen in nif and fix gene regulation. Microaerobic expression of fixN and nifA depends on fixL and fixJ. The FixL and FixJ proteins belong to a family of two-component regulatory systems widely spread among prokaryotes and responsive to the cell environment. We propose that FixL, which has features of a transmembrane protein, senses an environmental signal and transduces it to FixJ, a transcriptional activator of nif and fix genes.

~111 Citings

562. Rhizobium fix genes mediate at least two communication steps in symbiotic nodule development

By Putnoky P; Grosskopf E; Ha D T; Kiss G B; Kondorosi A
From The Journal of cell biology (1988), 106(3), 597-607, Language: English, Database: MEDLINE

To identify bacterial genes involved in symbiotic nodule development, ineffective nodules of alfalfa (Medicago sativa) induced by 64 different Fix-mutants of Rhizobium meliloti were characterized by assaying for symbiotic gene expression and by morphological studies. The expression of leghemoglobin and nodulin-25 genes from alfalfa and of the nifHD genes from R. meliloti were monitored by hybridizing the appropriate DNA probes to RNA samples prepared from nodules. The mutants were accordingly divided into three groups. In group I none of the genes were expressed, in group II only the plant genes were expressed and in group III all three genes were transcribed. Light and electron microscopical analysis of nodules revealed that nodule development was halted at different stages in nodules induced by different group I mutants. In most cases nodules were empty lacking infection threads and bacteroids or nodules contained infection threads and a few released bacteroids. In nodules induced by a third mutant class bacteria were released into the host cells, however the formation of the peribacteroid membrane was not normal. On this basis we suggest that peribacteroid membrane formation precedes leghemoglobin and nodulin-25 induction, moreover, after induction of nodulation by the nod genes at least two communication steps between the bacteria and the host plants are necessary for the development of the mature nodule. By complementing each mutant of group I with a genomic R. meliloti library made in pLAFRI, four new fix loci were identified, indicating that several bacterial genes are involved in late nodule development.

~18 Citings

563. Rhizobium meliloti has three functional copies of the nodD symbiotic regulatory gene

By Honma M A; Ausubel F M
From Proceedings of the National Academy of Sciences of the United States of America (1987), 84(23), 8558-62, Language: English, Database: MEDLINE

We have identified two Rhizobium meliloti genes (nodD2 and nodD3) that are highly homologous and closely linked to the regulatory gene nodD (nodD1). R. meliloti strains containing mutations in the three nodD genes in all possible combinations were constructed and their nodulation phenotypes were assayed on Medicago sativa (alfalfa) and Melilotus alba (sweet clover). A triple nodD1-nodD2-nodD3 mutant exhibited a Nod- phenotype on alfalfa and sweet clover, indicating that nodD is an essential nodulation gene in R. meliloti. A nodD2 mutant exhibited no discernable defect in nodulation and nodD3 mutants exhibited a delayed nodulation phenotype of 2-3 days when inoculated onto either host. Alfalfa nodules elicited by a nodD1 mutant appeared 5-6 days after wild-type nodules, and sweet clover nodules elicited by a nodD1 mutant appeared 2-3 days after wild-type nodules. nodD1-nodD2 double mutants formed nodules with the same delay as single nodD1 mutants on both hosts. nodD2-nodD3 double mutants elicited sweet clover nodules at the same rate as single nodD3 mutants, but this same double mutant was slightly more delayed in alfalfa nodule formation than the nodD3 mutant. The nodD1-nodD3 mutant exhibited an extremely delayed nodulation phenotype on alfalfa and elicited no nodules on sweet clover. These experiments indicate that nodD1 and nodD3 have equivalent roles in nodulating sweet clover but that nodD1 plays a more important role than nodD3 in eliciting nodules on alfalfa. The nodD2 gene appears to have some effect on alfalfa nodulation and none on sweet clover. Our results indicate that R. meliloti has three functional nodD genes that modulate the nodulation process in a host-specific manner.

~40 Citings

564. Identification and characterization of the Rhizobium meliloti ntrC gene: R. meliloti has separate regulatory pathways for activation of nitrogen fixation genes in free-living and symbiotic cells

By Szeto W W; Nixon B T; Ronson C W; Ausubel F M
From Journal of bacteriology (1987), 169(4), 1423-32, Language: English, Database: MEDLINE

We show here that Rhizobium meliloti, the nitrogen-fixing endosymbiont of alfalfa (Medicago sativa), has a regulatory gene that is structurally homologous to previously characterized ntrC genes in enteric bacteria. DNA sequence analysis showed that R. meliloti ntrC is homologous to previously sequenced ntrC genes from Klebsiella pneumoniae and Bradyrhizobium sp. (Parasponia) and that an ntrB-like gene is situated directly upstream from R. meliloti ntrC. Similar to its counterparts in K. pneumoniae and Escherichia coli, R. meliloti ntrC is expressed when the cells are grown in nitrogen-limiting media. In addition, R. meliloti ntrC is required for growth on media containing nitrate as the sole nitrogen source and for the ex planta transcription of several R. meliloti nif genes. On the other hand, root nodules elicited by R. meliloti ntrC mutants fix nitrogen as well as nodules elicited by wild-type R. meliloti. These latter results indicate that R. meliloti has separate regulatory pathways for activating nif gene expression ex planta and during symbiotic nitrogen fixation.

~49 Citings

565. Rhizobium meliloti nodulation genes: identification of nodDABC gene products, purification of nodA protein, and expression of nodA in Rhizobium meliloti

By Egelhoff T T; Long S R
From Journal of bacteriology (1985), 164(2), 591-9, Language: English, Database: MEDLINE

A set of conserved, or common, bacterial nodulation (nod) loci is required for host plant infection by Rhizobium meliloti and other Rhizobium species. Four such genes, nodDABC, have been indicated in R. meliloti 1021 by genetic analysis and DNA sequencing. An essential step toward understanding the function of these genes is to characterize their protein products. We used in vitro and maxicell Escherichia coli expression systems, together with gel electrophoresis and autoradiography, to detect proteins encoded by nodDABC. We facilitated expression of genes on these DNA fragments by inserting them downstream of the Salmonella typhimurium trp promoter, both in colE1 and incP plasmid-based vectors. Use of the incP trp promoter plasmid allowed overexpression of a nodABC gene fragment in R. meliloti. We found that nodA encodes a protein of 21 kilodaltons (kDa), and nodB encodes one of 28 kDa; the nodC product appears as two polypeptide bands at 44 and 45 kDa. Expression of the divergently read nodD yields a single polypeptide of 33 kDa. Whether these represent true Rhizobium gene products must be demonstrated by correlating these proteins with genetically defined Rhizobium loci. We purified the 21-kDa putative nodA protein product by gel electrophoresis, selective precipitation, and ion-exchange chromatography and generated antiserum to the purified gene product. This permitted the immunological demonstration that the 21-kDa protein is present in wild-type cells and in nodB- or nodC-defective strains, but is absent from nodA::Tn5 mutants, which confirms that the product expressed in E. coli is identical to that produced by R. meliloti nodA. Using antisera detection, we found that the level of nodA protein is increased by exposure of R. meliloti cells to plant exudate, indicating regulation of the bacterial nod genes by the plant host.

~41 Citings

566. Conservation of symbiotic nitrogen fixation gene sequences in Rhizobium japonicum and Bradyrhizobium japonicum

By Masterson R V; Prakash R K; Atherly A G
From Journal of bacteriology (1985), 163(1), 21-6, Language: English, Database: MEDLINE

Southern hybridization with nif (nitrogen fixation) and nod (nodulation) DNA probes from Rhizobium meliloti against intact plasmid DNA of Rhizobium japonicum and Bradyrhizobium japonicum strains indicated that both nif and nod sequences are on plasmid DNA in most R. japonicum strains. An exception is found with R. japonicum strain USDA194 and all B. japonicum strains where nif and nod sequences are on the chromosome. In R. japonicum strains, with the exception of strain USDA205, both nif and nod sequences are on the same plasmid. In strain USDA205, the nif genes are on a 112-megadalton plasmid, and nod genes are on a 195-megadalton plasmid. Hybridization to EcoRI digests of total DNA to nif and nod probes from R. meliloti show that the nif and nod sequences are conserved in both R. japonicum and B. japonicum strains regardless of the plasmid or chromosomal location of these genes. In addition, nif DNA hybridization patterns were identical among all R. japonicum strains and with most of the B. japonicum strains examined. Similarly, many of the bands that hybridize to the nodulation probe isolated from R. meliloti were found to be common among R. japonicum strains. Under reduced hybridization stringency conditions, strong conservation of nodulation sequences was observed in strains of B. japonicum. We have also found that the plasmid pRjaUSDA193, which possess nif and nod sequences, does not possess sequence homology with any plasmid of USDA194, but is homologous to parts of the chromosome of USDA194. Strain USDA194 is unique, since nif and nod sequences are present on the chromosome instead of on a plasmid as observed with all other strains examined.

~12 Citings

567. Application of immunodiffusion to the identification of Rhizobium meliloti strains competing for nodulation on Medicago sativa

By Sinha R C; Bromfield E S; Peterson E A
From Antonie van Leeuwenhoek (1984), 50(2), 155-60, Language: English, Database: MEDLINE

The immunodiffusion technique was successfully used to unambiguously recognize four strains of Rhizobium meliloti in a study of competition for nodulation with Medicago sativa cv. Apollo inoculated with two-, three- and four-strain mixtures. The serological reactions of all R. meliloti strains revealed no significant changes following plant passage indicating that the antigens involved in immunodiffusion were stable. R. meliloti 102F70 formed 50% or more of the nodules on M. sativa inoculated with two-, three- and four-strain mixtures. The remaining three strains were less competitive and produced similar proportions of nodules (14-20%) on plants inoculated with three- and four-strain mixtures. Cases of mixed-strain occupancy of nodules involving either two of three strains were detected in a sub-sample of nodules. The data also indicated considerable variation in the proportions of strains in the nodules of individual plants.

~0 Citings

568. Transfer of an indigenous plasmid of Rhizobium loti to other rhizobia and Agrobacterium tumefaciens

By Pankhurst C E; Broughton W J; Wieneke U
From Journal of general microbiology (1983), 129(8), 2535-43, Language: English, Database: MEDLINE

Rhizobium loti strains NZP2037 and NZP2213 were each found to contain a single large plasmid: pRlo2037a (240 MDal) and pRlo2213a (120 MDal), respectively. Plasmid DNA present in crude cell lysates of each strain and purified pRlo2037a DNA did not hybridize with pID1, a recombinant plasmid containing part of the nitrogen fixation (nif) region of R. meliloti, indicating that nif genes were not present on these plasmids. The transposon Tn5 was inserted into pRlo2037a and this plasmid was then transferred into R. leguminosarum, R. meliloti and Agrobacterium tumefaciens. All transconjugants failed to nodulate Lotus pedunculatus, suggesting that the ability to nodulate this legume was also not carried on pRlo2037a. Transfer of pRlo2037a to R. loti strain NZP2213 did not alter the Nod+ Fix- phenotype of this strain for L. pedunculatus. Determinants for flavolan resistance, believed to be necessary for effective nodulation of L. pedunculatus, were not carried on pRlo2037a. These data suggest that nodulation, nitrogen fixation and flavolan resistance genes are not present on the large plasmid in R. loti strain NZP2037.

~7 Citings

569. Identification of a Rhizobium trifolii plasmid coding for nitrogen fixation and nodulation genes and its interaction with pJB5JI, a Rhizobium leguminosarum plasmid

By Christensen A H; Schubert K R
From Journal of bacteriology (1983), 156(2), 592-9, Language: English, Database: MEDLINE

Rhizobium trifolii T37 contains at least three plasmids with sizes of greater than 250 megadaltons. Southern blots of agarose gels of these plasmids probed with Rhizobium meliloti nif DNA indicated that the smallest plasmid, pRtT37a, contains the nif genes. Transfer of the Rhizobium leguminosarum plasmid pJB5JI, which codes for pea nodulation and the nif genes and is genetically marked with Tn5, into R. trifolii T37 generated transconjugants containing a variety of plasmid profiles. The plasmid profiles and symbiotic properties of all of the transconjugants were stably maintained even after reisolation from nodules. The transconjugant strains were placed into three groups based on their plasmid profiles and symbiotic properties. The first group harbored a plasmid similar in size to pJB5JI (130 megadaltons) and lacked a plasmid corresponding to pRtT37a. These strains formed effective nodules on peas but were unable to nodulate clover and lacked the R. trifolii nif genes. This suggests that genes essential for clover nodulation as well as the R. trifolii nif genes are located on pRtT37a and have been deleted. The second group harbored hybrid plasmids formed from pRtT37a and pJB5JI which ranged in size from 140 to ca. 250 megadaltons. These transconjugants had lost the R. leguminosarum nif genes but retained the R. trifolii nif genes. Strains in this group nodulated both peas and clover but formed effective nodules only on clover. The third group of transconjugants contained a hybrid plasmid similar in size to pRtT37b. These strains contained the R. trifolii and R. leguminosarum nif genes and formed N2-fixing nodules on both peas and clover.

~10 Citings

570. Active site probes of flavoproteins. Determination of the solvent accessibility of the flavin position 8 for a series of flavoproteins

By Schopfer L M; Massey V; Claiborne A
From The Journal of biological chemistry (1981), 256(14), 7329-37, Language: English, Database: MEDLINE

The chemical reactivity of 8-chloroflavins and 8-mercaptoflavins has been exploited in order to examine the orientation of protein-bound flavins relative to solvent. The apoprotein form of a series of flavoproteins was prepared and the native flavin was replaced by either 8-Cl-flavin or 8-mercaptoflavin (FAD, FMN, or riboflavin form as was appropriate). The reconstituted proteins were exposed to reagents capable of reacting with the group at position 8. The 8-Cl-proteins were challenged with sodium sulfide and thiophenol, while the 8-mercaptoproteins were faced with iodoacetamide and iodoacetic acid. The kinetics of the ensuing reactions served as a measure of the solvent availability of position 8 for the protein-bound flavin. These studies indicated that position 8 of flavin bound to melilotate hydroxylase, D-amino acid oxidase, old yellow enzyme, p-OH-benzoate hydroxylase, and flavodoxin is accessible to solvent, while position 8 on L-lactate oxidase, glucose oxidase, putrescine oxidase, and riboflavin-binding protein appears to be inaccessible. For luciferase, D-lactate dehydrogenase, and xanthine oxidase, the data suggest that position 8 is exposed but the results are inconclusive. The effect of ligand binding on the accessibility of position 8 was also studied. NADPH binding to 8-mercapto old yellow enzyme and benzoate binding to 8-Cl-D-amino acid oxidase results in complete blockage of previously available position 8. On the other hand, p-OH-benzoate hydroxylase and melilotate hydroxylase bind their respective substrates (p-OH-benzoate and melilotate) without significantly altering the reactivity of position 8.

~4 Citings

571. Large plasmids of fast-growing rhizobia: homology studies and location of structural nitrogen fixation (nif) genes

By Prakash R K; Schilperoort R A; Nuti M P
From Journal of bacteriology (1981), 145(3), 1129-36, Language: English, Database: MEDLINE

A single large plasmid was isolated from multiplasmid-harboring strains Rhizobium leguminosarum 1001 and R. trifolii 5. These single plasmids, as well as the largest plasmid detectable in R. phaseoli 3622, hybridized with part of the nif structural genes of Klebsiella pneumoniae. In contrast, the plasmids of R. meliloti strains V7 and L5-30 did not show hybridization with the nif genes of K. pneumoniae, indicating that these genes might be located either on the chromosome or on a much larger plasmid which as yet has not been isolated. Studies of the homology between plasmids of fast-growing Rhizobium species showed that a specific deoxyribonucleic acid sequence, which carries the structural genes for nitrogenase, is highly conserved on a plasmid in R. leguminosarum, R. trifolii, and R. phaseoli. Furthermore, it was found that this type of plasmid in the different species shares extensive deoxyribonucleic acid homology, suggesting that strains in the R. leguminosarum cluster have preserved a nif plasmid.

~26 Citings

572. Heterozygosis of phage 16-3 of Rhizobium meliloti: moderate level of mismatch repair or gene conversion

By Orosz L; Pay A; Dallmann G
From Molecular & general genetics : MGG (1980), 179(1), 163-7, Language: English, Database: MEDLINE

Analysis of clear/turbid mottled (heterozygotic plaques) of Rhizobium meliloti temperate phage 16-3 indicates that the efficiency of repair at three sites (ti3, ti4, and ti5) in the C cistron is 2 to 20-fold less than that observed in E. coli phage lambda. In agreement with this conclusion, heterozygotic plaques were observed at similar frequency in crosses where point and small deletion mutants were combined, suggesting that in Rhizobium, DNA molecules with short single-stranded loops can escape from repair as efficiently as the simple mismatches.

~7 Citings

573. Restriction mapping of DNA of temperate Rhizobium meliloti phage 16-3: comparison of genetic and physical maps indicates a long, genetically silent chromosomal arm

By Dallmann G; Orosz L; Sain B
From Molecular & general genetics : MGG (1979), 176(3), 439-48, Language: English, Database: MEDLINE

The complete restriction map of DNA (61.57 Kb) of temperate Rhizobium meliloti phage 16-3 has been constructed for enzymes BglII, HindIII, HpaI, KpnI, and a partial map for EcoRI. The strategy employed for mapping included the analysis of double, triple and partial digests; comparison of wild type and deletion mutants; and detailed analysis of subfragments, exploiting the presence of cohesive ends of the phage. Comparison of the genetic and physical maps indicates that one arm of the chromosome is genetically silent and/or contains nonessential genes.

~7 Citings

574. Relatedness among Rhizobium and Agrobacterium species determined by three methods of nucleic acid hybridization

By Gibbins A M; Gregory K F
From Journal of bacteriology (1972), 111(1), 129-41, Language: English, Database: MEDLINE

Deoxyribonucleic acid (DNA) was isolated from 20 strains of Rhizobium and Agrobacterium and from one strain of Serratia marcescens; the guanine plus cytosine content of each DNA sample was determined by thermal denaturation. Radioactive DNA was isolated from three reference strains following the uptake of [2-(14)C]thymidine in the presence of deoxyadenosine. Ribonucleic acid (RNA) polymerase was used to synthesize radioactive RNA on DNA templates from the three reference strains. Radioactive DNA and RNA from the three reference strains were each hybridized with filter-bound DNA from all of the 21 test strains in 6 x SSC (standard saline citrate) and 50% formamide at 43 C for 40 hr. DNA/DNA relatedness was also determined by spectrophotometric measurement of the rates of association of single-stranded DNA. The order of relatedness between strains was similar by each method. Overall standard deviations for the DNA/DNA and DNA/RNA membrane filter techniques were +/-0.87 and +/-1.03%, respectively; that for the spectrophotometric technique was +/-4.11%. The DNA/DNA membrane technique gave higher absolute values of hybridization than did the DNA/RNA technique. R. leguminosarum and R. trifolii could not be distinguished from each other by these techniques. These results also indicated close relationships between R. lupini and R. japonicum, and (with less certainty) between R. meliloti and R. phaseoli. Of all the rhizobia tested against the A. tumefaciens 371 reference strain, the R. japonicum strains were the most unrelated. The three Agrobacterium strains used were as related to the R. lupini and R. leguminosarum references as were several rhizobium strains.

~8 Citings