STUDY ON CYSTEINE AND METHIONINE BIOSYNTHESIS PATHWAYS IN *SINORHIZOBIUM MELILOTI*

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ABSTRACT

Several cysteine and methionine auxotrophic mutants were isolated from *Sinorhizobium meliloti* strain Rmd201 by transposon Tn5 mutagenesis. Tn5 is inserted in *metA/ metZ*, *metE*, *metF* and *cysI/cysJ* genes. The study showed that the biosynthesis pathway of sulfur containing amino acids is resembled that one present in *Saccharomyces cerevicia* and *Pseudomonas aeruginosa* in some respects and the pathway that present in *Escherichia coli* in others.

INTRODUCTION

The synthesis of L-cysteine from inorganic sulfur is the predominant mechanism by which reduced sulfur is incorporated into organic compounds. In this process, inorganic sulfate, the most abundant source of utilizable sulfur in the biosphere, is taken up and reduced to sulfide by a plants and microorganisms including *Salmonella typhimurium* and *E. coli* (1).

The additional mechanisms for sulfur fixation have been described in *S. typhimurium* and *E. coli*. The first occur through the reduction of thiosulfate with O-acetyl-L-serine (2). The second mechanism involves the reaction of O-succinyl-L-homoserine with sulfid to form homocystein (2,3). However, this reaction is probably insignificant in S. *typhimurium* and *E. coli*, but it represents the major mechanism of sulfur fixation in yeast (4). Fig (1).

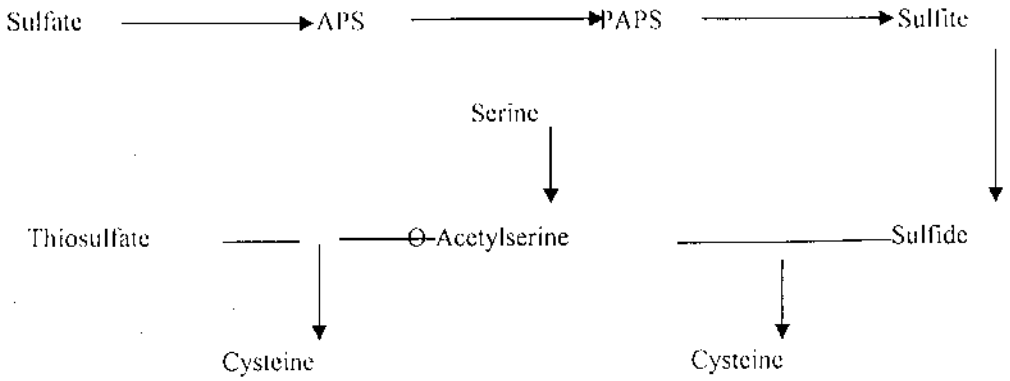

Fig. 1. The general biosynthetic pathway of cysteine in bacteria (After Kredich, 1996). Abbreviation: APS = Adenosine-5-phosphosulphate; PAPS = 3'phosphoadenosine-5'phosphosulfate.

Methionine can be synthesis from O-acylhomoseine derived from homoserine in microorganisms. Homocystein is produced from O-acylhomoserine through cystathionine or directly by Y-replacement with H_2S (5). Methionine can be synthesis directly from O-acylhomoserine and CH_3SH through methyl sulfuhydration. Homocystein is converted to methionine by two distinct pathways in microorganisms. One is the vitamin B_{12}-dependent

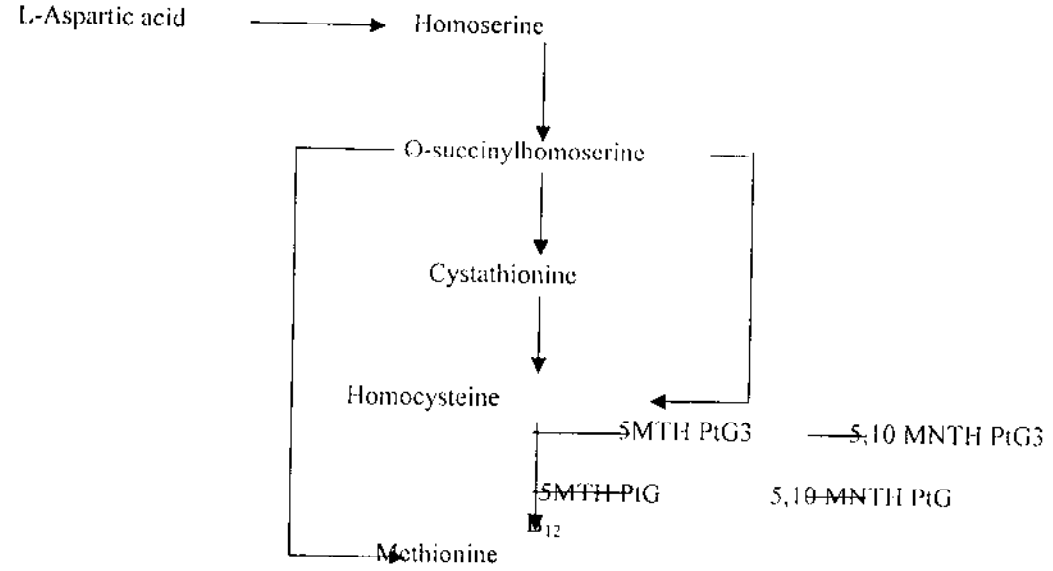

Fig. 2. Synthesis of methionine and its derivatives in microorganisms. (After Green *et al*, 1973; Soda, 1987). Abbriviation: MNTH PtG = Methylenetetrahydrofolate; MTH PtG = Methyltetrahydrofolate.

The biosynthesis of sulfur amino acids in *S. meliloti* and related genera is not fully understood. Sulfate is reduced to sulfide directly, through APS and sulfite, without production of PAPS in *S. meliloti* (7). In *Rhizobium. etli,* the pathway of sulfur amino acids is resembled that of yeast rather than *E. coli* (8).

METERIALS AND METHODS

Strains and plasmids: The bacterial strains and plasmid used in this study are listed in table 1.

Media and supplements: Complete medium trypton yeast extract (TY) and minimal medium (RMM) for rhizobia have been described earlier (9). Sulfur free minimal medium used was same as mentioned previously (10). Ability of each cysteine mutant to grow on minimal medium supplemented with nitrate as a sole nitrogen source were don as described earlier (10). Stock solutions were added to the autoclaved media to make final concentrations of 50, 30 and 10 mg/ml from amino acids, nitrogen bases and vitamins, respectively.

Antibiotics used were purchased from HiMedia Laboratories. Streptomycine sulfate and kanamycine acid sulfate stock solutions were prepared in sterile distilled water. The final concentration of antibiotics used in different media were as follows: streptomycine sulfate (100 µg/ml; kanamycine acid sulfate (100 µg/ml for *E. coli* and 400 µg/ml for *S. meliloti*), Cloromphinicol (50 µg/ml), rifampicin (40 µg/ml) and tetracycline hydrochloride (15 µg/ml).

Transposon Tn5 mutagenisis: Bacterial conjugations were don according to Kondorosi *et al* (11). Random mutagenesis of *S. meliloti* strain Rmd 201 was carried out using Tn5 delivery suicide plasmid pGS9 (12). Transconjugants obtained were screened for auxotrophs by streaking them on RMM and TY+Km^{400}+Sm^{100} agar medium. The auxotrophy of each strain was determined on the modified Holliday pools (13).

Linkage of Tn5 insertion to auxotrophy: This was determined for each auxotroph as described earlier. (10,14).

Location of biochemical block in each auxotroph: The cell suspension of methionine auxotrophs and parental strain as control were streaked on minimal medial supplemented

with three intermediates (one at a time) of methionine biosynthesis pathway. For cysteine auxotrophs, sulfur free minimal medium was supplemented with four intermediates (one at a time) of biosynthetic pathway of cysteine. The growth patterns were observed after incubation at 28°C for four days. The growth of the strains in liquid medium was determined by measuring the optical densities of the cultures at 590nm (O.D.590).

RESULTS

Tn5mutagenesis and isolation of auxotrophs: Random mutagenesis of *S. meliloti* Rmd201 with transposon Tn5 produced 34 auxotrophic mutants out of which 21 were able to grow on RMM agar supplemented with cysteine or methionine and 13 grew on RMM agar supplemented with methionine only. Tn5 in all cysteine/methionine auxotrophs is inserted in same location. BA4 and BA7 mutant strains were selected for further studies. Methionine auxotrophs were located in different positions. BA8, VK29 and VK39 mutant strains were selected for further studies.

Linkage of Tn5 insertion to auxotrophy: All kanamycin resistant transconjugants showed the respective donors auxotrophic marker, hence, there was 100% co transfer of Tn5 and the auxotrophic marker.

Location of biochemical blocks and intermediate feeding studies: The growth responses of cysteine and methionine auxotrophs to supplementation of minimal agar medium and minimal liquid medium with different intermediates of cysteine and methionine biosynthesis pathways are given in table1 and figure 3 respectively.

Cysteine mutants (BA4 and BA7) grew on sulfur free minimal medium supplemented with sodium sulfide or sodium thiosulfate but were unable to grow on the above medium supplemented with sodium sulfate or sodium sulfite. Hence these mutants were sulfite reductase mutants (Fig. 4). Three genes (*cysI, cysJ* and *cysG*) control reduction of sulfite to sulfide. Since these mutants were able to grow on minimal medium supplemented with sodium nitrate, i.e. have normal nitrite reductase activity, so they have normal *cysG* gene. (1). These mutants were disignated as *cysI/cysJ* mutants. The mutants also were able to grow on RMM supplemented with cystathionine, homocysteine or methionine. This result showed that the *S. meliloti* is able to convert the mentioned chemicals into cysteine.

Methionine auxotroph (BA8) grew on RMM supplemented with either cystathionine or homocysteine but not cyanocobalamine (vitamin B_{12}). This mutant was designated as *metA/metZ* mutant. Methionine auxotroph VK29 showed growth on RMM supplemented with cyanocobalamine and designated as *metE* mutant. The methionine auxotroph VK39 did not grow on RMM supplemented with any of the above methionine intermediates and designated as *metF* mutant.

DISCUSSION

When each auxotroph containing plasmid pJB3JI was crossed with *S. meliloti* ZB557 recipient strain, 100% co-transfer of Tn5 induced kanamycin resistance and auxotrophy were observed. These results showed that each auxotrophic cell had a single Tn5 insertion which was responsible for auxotrophy.

Cysteine mutants were able to grow on minimal medium supplemented with cysteine, methionine, cystathionine or homocysteine. The isolation of auxotrophs of rhizobia which grow on minimal medium supplemented with cysteine or methionine has been reported by several workers (15,16,8). It appears that the biosynthesis of sulfur-containing amino acids in rhizobia follows the pathway in which methionine can be converted to cysteine, present in *Saccharomyces cerevisiae* (17) and *Pseudomonas aeruginosa* (18).

Table 1: Bacterial strains and plasmids used

Strain/plasmid	Relevant characteristics	source/reference
Sinorhizobium meliloti		
AK631	Nod+Fix+, compact colony varient of wild type Rm41	Adam Kondorosi
Rmd201	spontaneous Sm^r derivatives of AK631	Khanuja &kumar (1988)
PP631	AK631(pJB3JI)	Peter Putnoky
ZB557	Rm41 *Phe15 leu4 rfl sm1*	Peter Putnoky
BA4, BA7	Rmd201 cysI/cysJ::Tn5	This study
BA8	Rmd201 metA/metZ::Tn5	This study
VK29	Rmd201 metE::Tn5	Vineetha KE & Prasad CK
VK39	Rmd201 metF::Tn5	Vineetha KE & Prasad CK
Escherichia coli		
WA803 (pGS9)	Met- Thi- Cmr Kmr	Selvaraj & Iyer (1983)
Plasmids		
PGS9	IncN repP15A Cmr Kmr	Selvaraj & Iyer (1983)
PJB3JI	Kms derivative of pR68.45, capable of mobilizing genomic segments of its host, Ter Cbr Nalr	Brewin et al.(1980)

Table2: Growth responses of cysteine and methionine mutants to different intermediates of biosynthesis pathways of cysteine and methionine.

Name of the strain	RMM	RMM supplied with					SFMM	SFMM supplied with			
		Cyst-eine	Methio-nine	Cysta-Thionine	Homo-cysteine	Vitamin B12		Sodium sulfate	Sodium sulfite	Sodium sulfide	Sodium thio-sulfate
Rmd 201	+	+	+	+	+	+	+	+	+	+	+
BA4, BA7	-	+	+	+	+	-	-	-	-	+	+
BA8	-	-	-	+	+	-	-	-	-	-	-
VK36	-	-	-	-	-	+	-	-	-	-	-
VK39	-	-	-	-	-	-	-	-	-	-	-

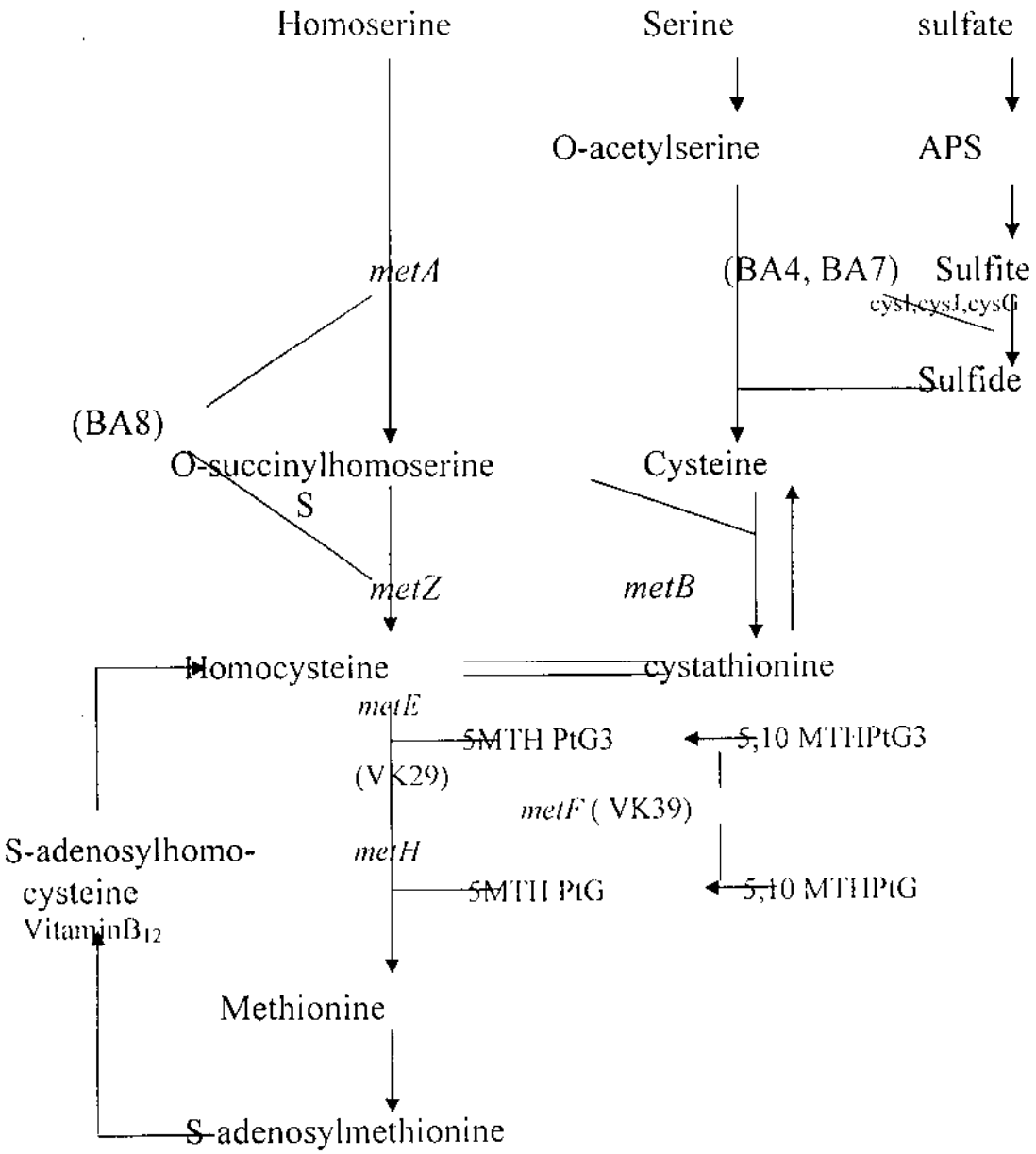

Fig. 4. The proposed pathway for biosynthesis of sulfur-containing amino acids in *Sinorhizobium meliloti*. APS= Adenosine-5'-phosphosulfate.

The *metE* mutant grew on RMM supplemented with cyanocobalamin. It appears that the final step of methionine biosynthesis pathway (methylation) in rhizobia resembles that present in *E. coli* which is catalysed by two distinct enzymes, one requiring only folic acid as a co-factor and the other requires both folic acid and vitamin B_{12} as co-factor.

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دراسة عن المسار الايضي لتصنيع الحامضين الامينيين السستين والمثيونين في بكتريا السينورايزوبيوم مليلوتي

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الخلاصة

تم عزل بعض الطافرات الخاصة بالحامض الاميني السيستين والمثيونين في بكتريا السينورايزوبيوم مليلوتي السلالة Rmd201 وقد استخدم الرتانسبوسون Tn5 المحمول على البلازميد PGS9 في عملية التطفير . لقد بينت الدراسة ان الطفرات قد حصلت في الجينات التالية met A , met Z , met E , met F و cysI / cysJ

كما اظهرت الدراسة ايضاً ان مسار تصنيع الحامضين الامينين السابقي الذكر مشابه للمسار الموجود في الخمائر (سكرومايسز سيريفسيا) وبكتريا سيدوموناس اشرشيا كولاي في اوجه اخرى.

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