Phosphoribosylpyrophosphate Synthetase of *Escherichia coli*
Identification of a Mutant Enzyme

Bjarne HOVE-JENSEN and Per NYGAARD
Enzyme Division, Institute of Biological Chemistry B, University of Copenhagen

(Received February 5/May 4, 1982)

From an *Escherichia coli* purine auxotroph a mutant defective in phosphoribosylpyrophosphate (PRib-PP) synthetase has been isolated and partially characterized. In contrast to the parental strain, the mutant was able to grow on nucleosides as purine source, whereas growth on purine bases was reduced. Kinetic analysis of the mutant PRib-PP synthetase revealed an apparent *Km* for ATP and ribose 5-phosphate of 1.0 mM and 240 μM respectively, compared to 60 μM and 45 μM respectively for the wild-type enzyme. ADP, which inhibits the wild-type enzyme at a concentration of 0.5 mM ribose 5-phosphate, stimulated the mutant enzyme. The activity of PRib-PP synthetase in crude extract was higher in the mutant than in the parent. When starved for purines an accumulation of PRib-PP was observed in the parent strain, while the pool decreased in the mutant. During pyrimidine starvation derepression of PRib-PP synthetase activity was observed in both strains, although to a lesser extent in the mutant. Our data suggest that the mutant harbors a mutation in the structural gene for PRib-PP synthetase. The mutation responsible for the altered PRib-PP synthetase was located in the *purB-hemA* region at 26 min on the recalibrated linkage map.

The rate of purine nucleotide biosynthesis in *Escherichia coli* is determined at least in part by the availability of PRib-PP. PRib-PP is synthesized from ribose 5-phosphate and ATP in a reaction which is dependent upon inorganic phosphate and magnesium, and is catalyzed by PRib-PP synthetase. PRib-PP synthetase of *Salmonella typhimurium* has been most thoroughly studied by Switzer and coworkers [1–3]. PRib-PP can be regarded as an intermediate in the biosynthesis of purine and pyrimidine nucleotides and coenzymes NAD and NADP and of the amino acids histidine and tryptophan. In addition PRib-PP is required for the utilization of adenine, hypoxanthine, guanine, xanthine and uracil for nucleotide synthesis and for the pyridine nucleotide cycle [3]. The formation of PRib-PP therefore is subjected to strict metabolic control [3]. Mutants of PRib-PP synthetase have not previously been reported in *E. coli*. However, in *S. typhimurium* two PRib-PP synthetase mutants with reduced activity have recently been isolated [4, 5]. In man inborn enzyme abnormalities have been reported with increased PRib-PP synthetase activity [6, 7] and altered sensitivity to ADP [8]. A mutant of the latter type has also been identified in heptoma cells [9]. The present paper describes isolation and characterization of an *E. coli* mutant defective in PRib-PP synthetase. Kinetic data from a partially purified enzyme indicate that the mutation resides within the structural gene for PRib-PP synthetase. The mutant genotype is designated prs.

**MATERIALS AND METHODS**

**Bacterial Strains**

The strains used in the present work are listed in Table 1.

**Growth Conditions**

AB medium [16] was used as basal salt medium. Glucose was added to 0.2%. Amino acids were added to a final concentration of 40 μg/ml, purine bases and purine nucleosides were added to a final concentration of 15 μg/ml and 30 μg/ml respectively. Thiamin was used at a concentration of 1 μg/ml; δ-aminolevulinic acid was added to minimal as well as to rich media to a final concentration of 40 μg/ml. Growth was monitored in an Eppendorf photometer at 436 nm. An absorbance of 1 (1-cm light path) correspond to 0.2 mg dry weight/ml or 2 × 10⁸ cells/ml. Bacteria were grown with shaking at 37°C. Solid media were prepared by adding 1.5% agar (Difco) to the liquid medium. In some growth experiments a low-phosphate (0.3 mM) medium was employed [17].

**Determination of Nucleotide and PRib-PP Pools**

Cells were grown exponentially for five generations in the low-phosphate medium and then labelled for two generations with [32P]orthophosphate prior to sampling. Specific activity was 33 Ci/mol. Extraction and chromatographic separation of nucleoside triphosphates and PRib-PP was performed according to Jensen et al. [17].
Table 1. Strains of Escherichia coli used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Derivation/Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO 003</td>
<td>F&quot; rpsL, relA, metB</td>
<td>defined wild type, E. coli K12 58-161</td>
</tr>
<tr>
<td>SO 446</td>
<td>F&quot; rpsL, relA, metB, purE, deoD, apt</td>
<td>[11], derived from SO 003</td>
</tr>
<tr>
<td>HO 70</td>
<td>F&quot; rpsL, relA, metB, purE, deoD, apt, pur</td>
<td>from SO 446, see Results</td>
</tr>
<tr>
<td>HO 24</td>
<td>F&quot; rpsL, relA, metB, purE, deoD, apt, pur, pyrF</td>
<td>from SO 1172, N-methyl-N'-nitro-N-nitrosoguanidine and penicillin counter selection [12]; pyrF verified by assay of orotidine 5'-monophosphate decarboxylase [13] and by mapping from HO 24 by plating on 6-nitrophenyl-thiogalactoside and isopropylthiogalactoside [14]</td>
</tr>
<tr>
<td>HO 52</td>
<td>F&quot; rpsL, relA, metB, purE, deoD, apt, pyrF, lacY</td>
<td>from SO 446, as HO 52</td>
</tr>
<tr>
<td>HO 47</td>
<td>F&quot; rpsL, relA, metB, purE, deoD, apt, lacY</td>
<td>from HO 47, N-methyl-N'-nitro-N-nitrosoguanidine and penicillin counter selection</td>
</tr>
<tr>
<td>HO 70</td>
<td>F&quot; rpsL, relA, metB, purE, deoD, apt, pyrF, lacY</td>
<td>from HO 70, transduction with P1 (HO 52), selecting Trp&quot;</td>
</tr>
<tr>
<td>HO 222</td>
<td>F&quot; rpsL, thi, his, tyrA, pur</td>
<td>from RS 3059, transduction with P1 (SO 1172), selecting Pur&quot;, Hem&quot;</td>
</tr>
</tbody>
</table>

Enzyme Assays

One unit of enzyme activity catalyses the conversion of 1 nmol of substrate/min at 37 °C. Specific activities are given as units/mg protein. Protein concentrations were determined by the method of Lowry et al. [18].

PRib-PP synthetase was assayed with [γ-32P]ATP as substrate according to Jensen et al. [17]. Purine salvage enzymes (hypoxanthine phosphoribosyltransferase, guanine phosphoribosyltransferase, purine nucleoside phosphorylase and guanosine kinase) were assayed as described previously [11, 19]. GMP reductase was assayed as described by Mager and Magasanik [20]. PRib-PP amidotransferase was assayed with [U-14C]glutamine as substrate, essentially as described by Messenger and Zalkin [21]. Uracil phosphoribosyltransferase was assayed according to Molloy and Finch [22]. Orotate phosphoribosyltransferase was assayed as described by Schwartz and Neuhard [13].

Transductions

These were performed with Plvir according to Miller [12].

Partial Purification of PRib-PP Synthetase

The purification is based on a previously published procedure [1]. 10 g (wet weight) of cells were suspended in 20 ml of 50 mM phosphate buffer, pH 7.5. Cells were homogenized in a French pressure cell at 41 Mpa. The homogenate was treated for 1 h at 0–4 °C with DNase (Worthington) and RNase A (Sigma) (1 μg/ml of each) and centrifuged for 1 h at 40000 rev./min in a Beckman L2 65B ultracentrifuge, rotor 50.1. The supernatant was 35% saturated with solid ammonium sulfate and stirred for 2 h, a precipitate was collected by centrifugation and redissolved in a tenth the original volume 50 mM phosphate buffer, pH 7.5. Finally the extract was dialyzed against the same buffer and stored at 4 °C.

Purine Starvation Experiments

Exponential cultures were grown in low-phosphate medium supplemented with hypoxanthine (15 μg/ml). The cultures were then diluted into fresh medium with limiting hypoxanthine (5 μg/ml), sufficient to give a cell density corresponding to 0.2 mg dry weight/ml. The determination of aminoimidazolerosiboside in the culture fluids were performed as described [23].

Pyrimidine Starvation

Exponential cultures were grown in low-phosphate medium supplemented with hypoxanthine (15 μg/ml) and uracil (10 μg/ml). The cultures were then diluted into fresh medium containing excess hypoxanthine (30 μg/ml) in order to avoid purine starvation and limiting uracil (4 μg/ml), sufficient to give a cell density corresponding to 0.2 μg dry weight/ml.

RESULTS

Isolation of the PRib-PP Synthetase Mutant

In Salmonella typhimurium and in Escherichia coli it has been shown that purine auxotrophs, mutated in purE (encoding phosphoribosylaminimidazole carboxylase) and also lacking purine nucleoside phosphorylase activity (encoded by deoD) are unable to grow on guanosine as the sole purine source [11, 24], though the enzymes needed for guanosine utilization (Fig.1), guanosine kinase and GMP reductase, are present [25]. The reason for this behaviour is not known. In an attempt to study this we set up a selection process for guanosine-growing mutants.

Strain SO 446 was submitted to ultraviolet irradiation to give a survival of 0.1%, and cultured overnight in minimal medium containing hypoxanthine and glyceral (0.04%). 109 cells were plated on glucose minimal agar with guanosine required for growth. Colonies were picked and purified on the same medium. Purine prototrophs and purine nucleoside phosphorylase revertants were discarded. A number of different mutants were obtained in this selection. Amongst them was a mutant with a partially defective PRib-PP synthetase (SQ 1172). Other mutants, to be described elsewhere, had increased guanosine kinase activity or an additional mutation in the purine de novo pathway, e.g. purF or purI mutants.

Growth Properties of SO 1172, prs

Early observations of the mutant revealed that purine de novo synthesis was decreased, as examined by ejection of aminoimidazolerosiboside, when grown in minimal medium.
Compared to the parental strain SO 446, growth of SO 1172 on purine bases was reduced, while enhanced growth was seen on nucleosides (Table 2). Addition of compounds which reduce the need for PRib-PP synthesis, revealed that tryptophan and histidine increased the growth rate of the mutant (Table 2). Addition of uridine and NAD had no effect on growth (data not given).

The possibility that the different growth patterns were related to different levels of purine salvage enzymes was also investigated. However, the activity of hypoxanthine phosphoribosyltransferase, guanine phosphoribosyltransferase, purine nucleoside phosphorylase, guanosine kinase and GMP reductase were identical in SO 446 and SO 1172 (data not given).

PRib-PP Synthetase from SO 1172

Measurement of PRib-PP synthetase activity in crude extracts revealed that the level was elevated in the mutant, compared to the parental strain (Table 3). In order to study the enzyme in more detail a partial purification of the mutant and the parent PRib-PP synthetase was performed. Results from kinetic analyses are shown in Table 3. It appears that $K_m$ for both ATP and ribose 5-phosphate is significantly enhanced in the mutant enzyme.

It is known that ADP is an inhibitor of PRib-PP synthetase \cite{2,26}. The wild-type enzyme was inhibited by ADP at high and low concentrations of ribose 5-phosphate. In contrast the mutant enzyme was stimulated by ADP at low ribose 5-phosphate and slightly inhibited at high ribose 5-phosphate (Fig. 2). The kinetic data indicate an altered regulation of PRib-PP synthesis in the mutant.

Heat-inactivation experiments were performed on the partially purified enzymes at a concentration of 4 mg protein/ml in 50 mM phosphate buffer, pH 7.5. Both enzymes lost 50–60% of their activity during a 10-min treatment at 55 °C.

Effect of Purine Starvation

When E. coli and S. typhimurium purine auxotrophs are grown with limited purine source, PRib-PP and intermediary compounds of the purine de novo pathway prior to the blocks accumulate \cite{4,27}. Furthermore a derepression of the synthesis of purine de novo enzymes occurs [24,28]. Nucleotide pool analysis in SO 1172 (prs) and SO 446 (prs') revealed no significant differences between the two strains during exponential growth on hypoxanthine, but the pool of PRib-PP was higher in the mutant than in the parent (Table 4). When starved for purine, the PRib-PP pool increased in SO 446, whereas it decreased in SO 1172. Aminoimidazolobisoderibose accumulated in both strains, most drastically in SO 446.

The data suggest that the synthesis of PRib-PP was reduced in SO 1172, when subjected to purine starvation.

---

Table 1. Biosynthesis of purine nucleotides in Escherichia coli, de novo and salvage pathways. Abbreviations: RibSP, ribose 5-phosphate; PRib-PP, phosphoribosylpyrophosphate; PRibN, phosphoribosylamine; GlyRib, glycineamidoribotide; fGlyRib, formylglycineamidoribotide; fGlyNRib, formylglycineamidoribotide; NLmRib, aminoimidazolobisoderibotide; cNLmRib, carboxyaminoimidazolobisoderibotide; Suc-AMP, succinyl-AMP; IMP, inosine 5’-monophosphate; XMP, xanthosine 5’-monophosphate. The de novo pathways have been extended to include PRib-PP synthetase. Gene symbols are given for the de novo pathway. Salvage enzymes, gene designations are given in parentheses: 1, purine nucleoside phosphorylase (deoD); 2, guanosine kinase (guaC); 3, GMP reductase (guaC); 4, adenosine phosphoribosyltransferase (apt); 5, hypoxanthine phosphoribosyltransferase (hpt); 6, guanine (xanthine) phosphoribosyltransferase (gpt); 7, adenosine deaminase (add).

---

Table 2. Growth characteristics of SO 446, prs" and SO 1172, prs on different purine sources

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Generation time with additions of</th>
<th>guanosine</th>
<th>guanine or xanthine</th>
<th>inosine or adenosine</th>
<th>hypoxanthine</th>
<th>hypoxanthine, histidine, tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO 446</td>
<td>purE, deoD, apt</td>
<td>min</td>
<td>&gt;600</td>
<td>&gt;250</td>
<td>220</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>SO 1172</td>
<td>purE, deoD, apt, prs</td>
<td>min</td>
<td>58</td>
<td>600</td>
<td>89</td>
<td>82</td>
<td>66</td>
</tr>
</tbody>
</table>

---

Table 3. Some kinetic properties of wild-type and mutant PRib-PP synthetase of Escherichia coli

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Specific activity of crude extract</th>
<th>Approx. yield</th>
<th>$K_m$ for RibSP</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme fraction</td>
<td>units/mg protein</td>
<td>%</td>
<td>µM</td>
<td></td>
</tr>
<tr>
<td>SO 466 prs&quot;</td>
<td>40</td>
<td>457</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>SO 1172 prs</td>
<td>90</td>
<td>1040</td>
<td>240</td>
<td>1005</td>
</tr>
</tbody>
</table>
Enzymatic analysis revealed that no significant changes occurred in response to purine starvation. However, synthesis of PRib-PP amidotransferase was derepressed in SQ 446, but not in SQ 1172 (Table 4).

**Effect of Pyrimidine Starvation**

In *S. typhimurium* it is known that the synthesis of PRib-PP synthetase [29] and of the pyrimidine de novo enzymes [13] are derepressed during conditions of pyrimidine starvation and that the pools of PRib-PP, UTP and CTP decline [27]. This was also observed in the present study both with SO 446 (prs") and SO 1172 (prs), but the derepression of the synthesis of PRib-PP synthetase was less pronounced in the mutant strain (Table 5).

**Transfer of the Genetic Lesion Causing the Defective PRib-PP Synthetase to an Independent Genetic Background**

We obtained a preliminary map position of the prs gene at approximately 26 min on the recalibrated linkage map [10], by conjugation. Among the suitable genetic markers in this region of the chromosome, pyrC, purB, hemA and trp were examined for cotransduction with the *K*<sub>m</sub> defect of PRib-PP synthetase. Selected recombinants were scored for the PRib-PP synthetase phenotype by assay at different substrate concentrations. The data given in Table 6 reveal a linkage of the *K*<sub>m</sub> defect with purB and hemA, as 92% of the selected purB<sup>B</sup>, hemA<sup>A</sup> transductants inherited the prs<sup>+</sup> allele from the donor strain. No linkage with pyrC or trp was found.

PRib-PP synthetase was examined in one such purB<sup>B</sup>, hemA<sup>A</sup>, prs<sup>+</sup> transductant, HO 222, together with the recipient strain, RS 3059, purB, hemA, prs<sup>+</sup>. The data shown in Fig. 3 reveal that the transductant strain HO 222 harbors a PRib-PP synthetase with kinetic properties identical to those of PRib-PP synthetase of strain SO 1172, i.e. a considerable increase in *K*<sub>m</sub> for both the substrates, ATP and ribose 5-phosphate. A decrease in activity of wild-type PRib-PP synthetase at

![Fig. 2. The effect of ADP on P-Rib-PP synthetase activity.](image)

**Table 4. Nucleotide, PRib-PP and aminoimidazolriboside pools and purine enzymes in exponential and in purine-starved cultures**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth condition</th>
<th>Pool size of ATP</th>
<th>GTP</th>
<th>CTP</th>
<th>UTP</th>
<th>PRib-PP</th>
<th>NImRib*</th>
<th>Specific activity of PRib-PP synthetase</th>
<th>Prib-PP amidotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO 446</td>
<td>exponential</td>
<td>5.4</td>
<td>3.1</td>
<td>1.2</td>
<td>1.7</td>
<td>0.7</td>
<td>&lt;6</td>
<td>42</td>
<td>7</td>
</tr>
<tr>
<td>prs&lt;sup&gt;-&lt;/sup&gt;</td>
<td>starved</td>
<td>0.2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>1.2</td>
<td>114</td>
<td>39</td>
<td>19</td>
</tr>
<tr>
<td>SO 1172</td>
<td>exponential</td>
<td>5.0</td>
<td>2.6</td>
<td>1.1</td>
<td>1.5</td>
<td>1.2</td>
<td>&lt;6</td>
<td>87</td>
<td>9</td>
</tr>
<tr>
<td>prs&lt;sup&gt;-&lt;/sup&gt;</td>
<td>starved</td>
<td>0.7</td>
<td>0.9</td>
<td>1.7</td>
<td>1.8</td>
<td>0.4</td>
<td>26</td>
<td>77</td>
<td>7</td>
</tr>
</tbody>
</table>

* Aminoimidazolriboside.

**Table 5. Nucleotide and PRib-PP pools and pyrimidine enzymes in exponential and in pyrimidine-starved cultures**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth condition</th>
<th>Pool size of ATP</th>
<th>GTP</th>
<th>CTP</th>
<th>UTP</th>
<th>PRib&lt;sup&gt;-&lt;/sup&gt;-PP</th>
<th>Specific activity of Prib-PP synthetase</th>
<th>Orotate phosphoribosyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO 82</td>
<td>exponential</td>
<td>7.5</td>
<td>3.9</td>
<td>1.2</td>
<td>1.8</td>
<td>0.9</td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td>prs&lt;sup&gt;-&lt;/sup&gt;</td>
<td>starved</td>
<td>21.2</td>
<td>11.7</td>
<td>0.9</td>
<td>0.04</td>
<td>0.6</td>
<td>118</td>
<td>90</td>
</tr>
<tr>
<td>HO 52</td>
<td>exponential</td>
<td>5.4</td>
<td>3.6</td>
<td>1.0</td>
<td>1.5</td>
<td>0.6</td>
<td>122</td>
<td>13</td>
</tr>
<tr>
<td>prs&lt;sup&gt;-&lt;/sup&gt;</td>
<td>starved</td>
<td>29.0</td>
<td>17.9</td>
<td>0.2</td>
<td>&lt;0.04</td>
<td>&lt;0.1</td>
<td>179</td>
<td>56</td>
</tr>
</tbody>
</table>
concentrations of ribose 5-phosphate above 0.5 mM is seen in crude extract (Fig. 3A). This is most likely due to substrate inhibition in the presence of ADP, since ADP is readily formed from ATP in crude cell extracts. With the purified enzyme, no decrease in activity is seen at higher concentrations of ribose 5-phosphate, unless ADP is added. The mutant enzyme is less sensitive to ADP (cf. Fig. 2) and no inhibition is observed in such extracts (Fig. 3A). The activity of PRib-PP synthetase was essentially the same at 5, 10 and 20 mM ribose 5-phosphate (not shown).

The specific activity of PRib-PP synthetase in crude extract was 95 units/mg protein for strain HO 222 (prs), whereas for strain RS 3059 (prs <sup>+</sup>) it was 40 units/mg protein. Apparently, the increased specific activity of mutant PRib-PP synthetase is genetically linked with the $K_m$ defect.

**DISCUSSION**

The present study reports the isolation and partial characterization of an *Escherichia coli* mutant with a defective PRib-PP synthetase. The selection procedure employed here, i.e. the isolation of guanosine growing mutants in a *purE, deoD* genetic background, has previously been used in *Salmonella typhimurium*, resulting in a variety of different mutants. Thus mutants were isolated which showed increased activity of guanosine kinase or GMP reductase. Others had an additional block in the purine *de novo* pathways, namely *purF* and *purG* mutants [24]. In *E. coli* Kochian et al. [30] and Livshis and Sukhodolets [31, 32] isolated guanosine-generating mutants in a *purD, deoD* strain. One of the mutations mapped in the 10–15-min interval on the chromosome. This mutant may have increased guanosine kinase activity. The mutants resulting from our selection are similar to those mentioned above.

Although we cannot at present offer an adequate explanation for correlation of the selective conditions, i.e. growth on guanosine as sole purine source, with the mutant phenotype, i.e. partially defective PRib-PP synthetase, it should be emphasized that the selection protocol employed has yielded PRib-PP synthetase mutants in *E. coli* as well as in *S. typhimurium*. However, the fact that mutants with secondary purine blocks appear in the selection may suggest that a (yet unidentified) component of the purine *de novo* pathway may interfere with the utilization of guanosine in vivo. By regarding PRib-PP synthetase as the very first enzyme in the purine *de novo* pathway, a *purE, prs* mutant would mimic the double mutants mentioned above (e.g. a *purE, purF* mutant) (cf. Fig. 1).

The enzymatic properties of PRib-PP synthetase of our mutant were greatly altered. $K_m$ for both substrates, ATP and ribose 5-phosphate, was increased (Table 3) and the mutant enzyme was less sensitive to inhibition by ADP (Fig. 2). The kinetic properties of the defective enzyme suggest that the mutation resides within the structural gene for PRib-PP synthetase. This is supported by analysis of a transductant strain (HO 222) which inherited the PRib-PP synthetase with mutant properties from the *prs* donor (Fig. 3). The increased activity of PRib-PP synthetase of the mutant (Table 3) was linked to the $K_m$ defect by transduction and may represent a mechanism that compensates for a less-efficient enzyme. In addition, we found less derepression of enzyme synthesis in the mutant strain during pyrimidine starvation (Table 5).

The altered kinetic properties of the mutant enzyme were also expressed during purine starvation. In contrast to the wild-type strain, the PRib-PP pool of the mutant decreased (Table 4). This decrease may be a consequence of a malfunctioning PRib-PP synthetase at the low ATP concentration. Furthermore the synthesis of PRib-PP amidotransferase was derepressed under purine starvation, but only in the wild type. How this lack of derepression in the mutant is related to the PRib-PP pool is not known at present but it is in accordance with the reduced aminoimidazoleriboside formation observed (Table 4).

We have preliminarily mapped the *prs* gene in the *purB-hemA* region of the chromosome (Table 6). In *S. typhimurium* a different situation is found. The two mutations which confer altered cellular level of PRib-PP synthetase activity have not been fully characterized. The mutation which causes unmeasurable PRib-PP synthetase activity in crude extracts is located at 47 min on the *S. typhimurium* linkage map [5]. The other mutant which was identified in our laboratory was
isolated by the same procedure as we used [4]. This mutant has low but measurable enzymatic activity in crude extracts. Genetically it is found that a locus close to 7 min on the linkage map is related to PRib-PP synthetase or guanosine growth.

We are currently working on a more elaborate genetic characterization of strain S($2172, specifically with respect to correlation of guanosine growth with the functioning of PRib-PP.

We would like to thank Jenny Steno Christensen and Inge Skibshøj for their expert technical assistance.

REFERENCES
