Production of L-Serine by a Methanol-utilizing Bacterium, *Arthrobacter globiformis* SK-200

Yoshiki Tani, Takahiro Kanagawa, Aran Hanpongkittikun, Koichi Ogata & Hideaki Yamada

To cite this article: Yoshiki Tani, Takahiro Kanagawa, Aran Hanpongkittikun, Koichi Ogata & Hideaki Yamada (1978) Production of L-Serine by a Methanol-utilizing Bacterium, *Arthrobacter globiformis* SK-200, Agricultural and Biological Chemistry, 42:12, 2275-2279, DOI: 10.1080/00021369.1978.10863349

To link to this article: https://doi.org/10.1080/00021369.1978.10863349

Published online: 09 Sep 2014.
Production of L-Serine by a Methanol-utilizing Bacterium, 
*Arthrobacter globiformis* SK–200t

Yoshiki Tani, Takahiro Kanagawa, Aran Hanpongkittikun,∗
Koichi Ogata** and Hideaki Yamada

Department of Agricultural Chemistry, Kyoto University,
Kyoto 606, Japan
Received June 2, 1978

A methanol-utilizing bacterium, strain SK–200, which was isolated as an L-serine producer, was identified as *Arthrobacter globiformis*. The strain excreted a considerable amount of L-serine when glycine and glucose were added in a methanol medium. Cultural conditions for L-serine production were studied. Derivation of mutants increased the productivity of L-serine. A methionine auxotroph produced 5.2 mg per ml of L-serine.

In previous papers, we reported the utilization of methanol for the fermentative production of branched-chain amino acids by *Methylomonas aminofaciens*1,2 and FAD by methanol-utilizing yeasts.3 Methylo trophs assimilate methanol through the serine pathway or the ribulose monophosphate pathway. In the principal part of the serine pathway, C1-compound reacts with glycine to form L-serine.

Although the fermentative production of L-serine from glycine4,5 and others6 was reported with several microorganisms, the yield of L-serine is still insufficient.

The present paper describes the production of L-serine from glycine by the cultivation of a methanol-utilizing bacterium having the serine pathway.

**MATERIALS AND METHODS**

*Medium and cultivation.* The basal medium consisted of 0.6 g of NH4NO3, 0.5 g of KH2PO4, 0.1 g of NaCl, 0.02 g of MgSO4·7H2O, 10 μg of thiamine·HCl, 20 μg of nicotinic acid, 20 μg of pyridoxine·HCl, 10 μg of p-aminobenzoic acid, 20 μg of riboflavin, 20 μg of calcium pantothenate, 1 μg of biotin and 500 μg of folic acid in 100 ml of tap water, pH 7.0. Media M and MG were prepared by adding 1.6 g of methanol, and 1.6 g of methanol plus 0.3 g of glycine, respectively, to 100 ml of the basal medium. Medium MGG consisted of 1.2 g of methanol, 2 g of glycine, 2 g of glucose, 0.6 g of NH4NO3, 2.8 mmol of potassium phosphate buffer (pH 8.0), 0.1 g of NaCl and 0.02 g of MgSO4·7H2O in 100 ml of tap water. The pH of medium MGG was around 6.0 after autoclaving. To slant and plate cultures, agar was added in a proportion of 1.5% (w/v). Organisms were preserved on MG or MGG slant. The cell suspension prepared from the slant culture or the liquid culture was inoculated into a test tube (diameter: 16.5 mm) containing 5 ml of the medium. Cultivation was aerobically carried out at 28°C for 72 hr.

*Analytical methods.* The ninhydrin and microbiological methods to determine the amount of L-serine in the culture filtrate were principally the same as described previously.1) The solvent system for paper chromatography was tert-butanol–methyl ethyl ketone–water–28% ammonium hydroxide (40: 30: 20: 10, by volume). Cell growth was turbidimetrically measured at 610 nm.

*Derivation of mutants.* The procedure to derive methionine auxotroph was as follows: The overnight culture, 4 ml, was inoculated into 100 ml of medium MGG. After shaking for 6 hr at 28°C, the cells were suspended in 20 ml of physiological saline at a concentration of about 106 cells per ml and were exposed to UV irradiation to 99.5% kill under continuous stirring. The irradiated suspension was mixed with 20 ml of double-strength medium MGG containing 25 μg of l-methionine per ml, and was cultivated for 48 hr at 28°C. The cells were collected and washed by centrifugation, and then suspended in 10 ml of physiolo-
gical saline. One milliliter of this suspension was poured into 50 ml of medium MGG and was cultivated at 28°C. After 2.5 hr, penicillin G was added to the culture at a final concentration of 100 units per ml, and the cultivation was continued for 5 hr. The cells were collected and washed by centrifugation, and then suspended in 1 ml of physiological saline. The suspension, in a quantity of 0.2 ml, was spread on an agar plate of MGG containing 25 μg of L-methionine per ml. Methionine auxotroph on the plate was determined and purified by a replica plating method.

To derive an L-serine-unutilizable or a D-serine-resistant mutant, the basal medium containing 1% L-serine or medium MGG containing 20 mg of D-serine per ml was used in appropriate parts of the procedure for methionine auxotroph.

**Chemicals.** All chemicals used in this experiment were obtained from commercial sources and were used without further purification.

**RESULTS**

**Isolation of L-serine producer**

Methanol-utilizing bacteria were isolated from soils and other samples by an enrichment culture method using medium M. Each isolate was transferred to the agar plate of medium MG. Glycine inhibited the growth of many isolates, especially that of the bacteria having red to pink pigments. About 200 strains which could grow in the presence of 0.3% glycine were selected and inoculated into medium MG. After cultivation, the amount of serine in the culture filtrate was determined by the ninhydrin method. However, no strains accumulated a detectable amount of serine in the medium. One-third of the bacteria produced about 0.05 mg of serine per ml with the increase of glycine concentration to 1%. Strain SK–200 was the best producer. The following experiments concern the serine production by this strain.

**Identification of strain SK–200**

Strain SK–200 was isolated from the soil of a field in Iwakura, Kyoto. Taxonomical characteristics of the strain were as follows; Cells were rods (0.5~1.0 by 1.0~4.0 μm) and sometimes coccoid (0.8~1.2 μm in diameter). Non-motile. Gram-positive. Not acid-fast. Sugars, organic acids, amino acids and alcohols were used as carbon sources. Little acid was produced from sugars. Catalase-positive. Oxidase-negative. Cell walls contained lysine. No special growth factor was required. Gelatin and starch were hydrolyzed. Nitrate was reduced to nitrite. The G+C content of DNA was 63.9 moles %. Optimum temperature for growth was 25~30°C. From these characteristics, strain SK–200 was identified as *Arthrobacter globiformis* on the basis of Bergey’s Manual of Determinative Bacteriology, 8th ed.
TABLE I. EFFECT OF CARBOHYDRATE ON THE BACTERIAL GROWTH AND L-SERINE PRODUCTION

Medium MG containing 2% glycine was used.

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Growth (OD₆₅₀)</th>
<th>L-Serine formed (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.30</td>
<td>0.01</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>0.56</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.96</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>0.74</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>0.61</td>
<td>0.16</td>
</tr>
<tr>
<td>8.0</td>
<td>0.44</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.55</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.96</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>1.01</td>
<td>0.17</td>
</tr>
<tr>
<td>4.0</td>
<td>1.11</td>
<td>0.24</td>
</tr>
<tr>
<td>8.0</td>
<td>1.32</td>
<td>0.24</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>1.43</td>
<td>0.61</td>
</tr>
<tr>
<td>1.0</td>
<td>1.37</td>
<td>1.10</td>
</tr>
<tr>
<td>2.0</td>
<td>1.62</td>
<td>1.60</td>
</tr>
<tr>
<td>4.0</td>
<td>1.74</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of Methanol and Glycerol Concentrations on L-Serine Production.

Medium MGG was used in the variation of concentration of methanol for (A) or glycine for (B). In experiment B, sterilized 20% glycine was added to medium MGG, which contained initially 0.5% glycine and was sterilized, in indicated concentrations. ○, pH; △, growth; ●, L-serine.

The bacterial growth and L-serine production registered their respective maxima when the initial pH of the medium was 6.0. The final pH of the medium was always 8.0 to 8.6 when L-serine was produced in a high yield. However, the maintenance of pH at 6, 7 and 8 during fermentation had no significant effect on L-serine production.

The use of organic nitrogen compounds such as peptone and urea as nitrogen source resulted in the decrease of L-serine production.

Time course of L-serine production

The amount of L-serine in the culture filtrate reached its maximum in a late exponential phase of L-serine in the culture filtrate. Table I shows the effect of glucose, sucrose and glycerol on the growth and L-serine production when a medium with high concentrations of methanol and glycine was used. Increases in amount of glucose added stimulated both L-serine production and bacterial growth. Figure 2 shows how increased concentrations of methanol and glycerol contributed to L-serine production when glucose was added.

By the autoclaving of the medium containing glucose and other components, especially glycine, brownish color compounds, possibly melanoidines were formed. The concentration of the brownish color compounds in the medium was changed by mixing two kinds of medium MGG which were autoclaved with or without the addition of glucose. The increase of the brownish color compounds restrained the bacterial growth but stimulated L-serine production.

The bacterial growth and L-serine production registered their respective maxima when the initial pH of the medium was 6.0. The final pH of the medium was always 8.0 to 8.6 when L-serine was produced in a high yield. However, the maintenance of pH at 6, 7 and 8 during fermentation had no significant effect on L-serine production.

The use of organic nitrogen compounds such as peptone and urea as nitrogen source resulted in the decrease of L-serine production.

Time course of L-serine production

The amount of L-serine in the culture filtrate reached its maximum in a late exponential phase of

Fig. 3. Time Course of L-Serine Production.

Overnight culture in a medium, prepared by excluding glycine and methanol from medium MGG, in a quantity of 3 ml, was inoculated into a 500-ml shaking flask containing 100 ml of medium MGG. ○, pH; △, growth; ●, L-serine.
growth phase and then decreased rapidly (Fig. 3). A. globiformis SK-200 grew well in medium MGG containing 1 % L-serine instead of glycine. Eighty per cent of L-serine in the medium was consumed after 2-day culture. A strong activity of L-serine dehydratase of A. globiformis has been reported. 

**Addition of several compounds**

The effects of compounds, which may affect the metabolism of glycine and L-serine, on L-serine production were examined (Table II). L-Tryptophan, L-threonine and glyoxylate increased the amount of L-serine produced. L-Methionine strongly inhibited the production of L-serine. Folate, which is a precursor of the cofactor of serine transhydroxymethylase, showed a little stimulative effect when added in a higher concentration. Another precursor of the cofactor of the enzyme, pyridoxine, was inhibitory.

An addition of surfactants or antibiotics did not stimulate L-serine production. Some metal ions such as Zn²⁺ and Co²⁺ showed an effect to slightly increase L-serine production.

**L-Serine production by mutants**

The inhibition of L-serine production by L-methionine (Table II) suggested that a regulation system of serine transhydroxymethylase operated as reported with *Escherichia coli.* We obtained L-methionine auxotrophs as described in METHODS AND MATERIALS. Some of them, Met 117, 124 and 125, produced higher amounts of L-serine than that of the parent strain (Table III). The highest value of L-serine formed was 5.2 mg per ml when an auxotroph, Met 125, was cultured in the presence of 2 mg per ml of L-methionine.

**DISCUSSION**

The peculiar metabolic pathway of methylotrophs should be useful for the fermentative production of metabolites. L-Serine production by a methanol-utilizing bacterium having the serine pathway has been reported.¹⁰
Serine Production by a Methanol Utilizer

The inhibition of growth by methanol is a common obstacle to the utilization of methylotroph when methylotroph is cultured in a higher concentration of methanol. Several fed-batch culture methods were employed to increase the production of cells and metabolites. During the course of the present study, feeding of methanol was also attempted to result in a little increase in L-serine production. Another problem to the fermentative production of L-serine from glycine is the toxicity of glycine to the growth of microorganism. Keune et al. added glycine at the end of the exponential growth phase. In this case, the cells which had already grown in the culture medium converted glycine into L-serine. We isolated methylotrophs which are tolerant to glycine. Furthermore we found that an addition of glucose to the medium counteracted the growth inhibition which was caused by the higher concentrations of glycine and methanol.

Methionine auxotrophs produced higher amounts of L-serine, when L-methionine was added in an amount of 2 mg per ml. This amount of L-methionine added was excessive as this mutant showed its full growth in the presence of 20 μg per ml of L-methionine. Wild type strain lost sometimes the sensitivity in L-serine production against L-methionine by spontaneous mutation. The increased L-serine productivity of the mutant may be attributable to the lack of intracellular L-methionine biosynthesis and the decrease in sensitivity to extracellular L-methionine.

The strong activity of hydroxypyruvate reductase, a key enzyme of the serine pathway, was found in the cell-free extract of cells grown on the medium containing methanol and glycine but not that on glucose medium. This suggests that L-serine is produced from glycine by the catalysis of serine transhydroxymethylase.

Acknowledgment. We are grateful to the Central Research Laboratories of Ajinomoto Co., Inc., for their part in the taxonomical studies.

REFERENCES