ELSEVIER

FEMS Microbiology Letters 221 (2003) 187–190

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Inhibitory effect of L-pyroglutamate on extremophiles: correlation with growth temperature and pH

Chan Beum Park [a,1], Dewey D.Y. Ryu [b], Sun Bok Lee [a,*]

[a] *Department of Chemical Engineering, Pohang University of Science and Technology, San 31, Hyoja-Dong, Pohang 790-784, South Korea*
[b] *Department of Chemical Engineering and Materials Science, University of California, Davis, CA 95616, USA*

Received 28 October 2002; received in revised form 17 February 2003; accepted 21 February 2003

First published online 1 April 2003

Abstract

L-Pyroglutamate (PGA) is naturally occurring from L-glutamate solution with accelerated formation rate under high temperature and low pH. Even though PGA has been identified as a neurotoxic agent on brain cells, the effect of PGA on the growth of microorganisms is rarely known. Here various kinds of microorganisms differing in their optimal growth temperature, pH, phylogeny, and isolated biotope were investigated for the effect of PGA. We found that growth of thermoacidophiles, including both archaea and bacteria, was seriously inhibited by the presence of PGA, and the extent of the inhibitory effect was closely related with growth temperature and pH. Interestingly, only microbes that grow at high temperature and low pH are inhibited by PGA, while this compound may stimulate growth rates of organisms that live at neutral pH and low temperature.
© 2003 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: L-Pyroglutamate; Extremophile; Growth inhibition; L-Glutamate

1. Introduction

L-Pyroglutamic acid (2-pyrrolidone-5-carboxylic acid, PGA) forms spontaneously from L-glutamate by the cyclization of internal amide, and its conversion rate is highly dependent on the temperature and pH of the L-glutamate solution [1]. Since its identification, the 'forgotten' amino acid has been reported to possess a strong neurotoxic effect on brain cell by reducing L-glutamate-binding on neuron [2–5]. Competitive interaction between PGA and L-glutamate with glutamate receptors was ascribed to their structural similarity [3,5]. In contrast to mammalian cells, little information is available on the effect of PGA on microorganisms to date.

Recently, we reported that PGA is a potent inhibitor for hyperthermophilic archaeon *Sulfolobus solfataricus* [6]. According to this study, the growth rate of *S. solfataricus* was reduced to one half by the presence of 5.1 mM of PGA in the growth medium, and no growth was observed in the presence of 15.5 mM PGA. It was noteworthy that L-glutamate present in a complex medium such as yeast extract was naturally converted to PGA during the cultivation, and the toxic effect of high yeast extract concentration on *S. solfataricus* [7] was considered to be at least in part caused by the formation of PGA in the culture medium.

During the last few decades, many kinds of extremophiles capable of growing under extreme conditions of temperature, pH, salt concentration, etc., have been isolated [8,9]. Taking into account the accelerated rate of L-glutamate dehydration to PGA under extreme conditions of temperature and pH [1,6], the information about the effect of PGA on the wide spectrum of microorganisms comprising extremophiles should be useful in the design of optimal growth medium and high cell-density cultivation. Here we investigated many microorganisms differing in optimal growth temperature (24–93°C), pH (3.0–7.6), phylogeny (Archaea, Bacteria, and Eucarya), and isolated biotope (terrestrial and marine): *Aeropyrum pernix* JCM-9820, *Rhodothermus obamensis* JCM9785, *Metallosphaera sedula* DSM5348, *Thermus aquaticus* ATCC31674, *Thermoplasma acidophilum* DSM1728, *Bacillus acidocaldarius* KCTC4135, *S. solfataricus* DSM1617, *Escherichia coli*

* Corresponding author. Tel.: +82 (54) 279 2268; Fax: +82 (54) 279 2699.
E-mail address: sblee@postech.ac.kr (S.B. Lee).

[1] Present address: Department of Chemical and Materials Engineering, Arizona State University, Tempe, AZ 85287, USA.

0378-1097/03/$22.00 © 2003 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-1097(03)00213-1

Table 1
Growth media for microorganisms investigated in this study

Microorganism	Growth medium	Reference
A. pernix	tryptone 4.0 g l^{-1}, $Na_2S_2O_3$ 0.612 g l^{-1}, ASNIII salts	[17]
S. solfataricus	glucose 3.0 g l^{-1}, modified Allen's salts	[7]
R. obamensis	tryptone 4.0 g l^{-1}, MJ salts, MJ trace metals	[18]
M. sedula	yeast extract 1.0 g l^{-1}, glucose 3.0 g l^{-1}, sulfur 1.0 g l^{-1}, modified Allen's salts	[19]
T. aquaticus	tryptone 1.0 g l^{-1}, Castenholz salts, Nitsch's trace elements	[20]
T. acidophilum	glucose 3.0 g l^{-1}, modified Allen's salts	[10]
B. acidocaldarius	glucose 3.0 g l^{-1}, modified Allen's salts	–
E. coli	glucose 3.0 g l^{-1}, modified Allen's salts	–
T. thiooxidans	sulfur 10.0 g l^{-1}, modified Allen's salts, $FeSO_4 \cdot 7H_2O$ 0.01 g l^{-1}	[21]
P. putida	glucose 3.0 g l^{-1}, modified Allen's salts	–
S. cerevisiae	yeast extract 1.0 g l^{-1}, glucose 3.0 g l^{-1}, modified Allen's salts	–

DH5α, *Thiobacillus thiooxidans* JCM3866, *Pseudomonas putida* KCTC2406, *Saccharomyces cerevisiae* Y190. We found that PGA seriously affected the growth of thermoacidophiles and the extent of inhibition increased with higher growth temperature.

2. Materials and methods

Control growth media used in this work are listed in Table 1. Glucose minimal medium, composed of 3.0 g l^{-1} glucose and modified Allen's salts, was basically used as a control medium for microorganisms which grow without supplementation of other nutrients. Otherwise, the composition of the control medium was modified for better cell growth as recommended for each culture collection (Table 1). All the strains were cultivated in a 500 ml screw-cap flask (working volume 50 ml) at each temperature specified in Table 2 in a shaking water bath with agitation speed of 100 rpm. The pH of growth media was adjusted with H_2SO_4 (2.0 N) before cell inoculation.

Growth of each strain was observed by turbidity measurements at 540 nm (*T. aquaticus*, *S. solfataricus*), 600 nm (*M. sedula*, *T. acidophilum*, *B. acidocaldarius*, *E. coli*, *P. putida*, *S. cerevisiae*, *T. thiooxidans*), and 660 nm (*A. pernix*, *R. obamensis*). The specific growth rate was calculated from the slope of a straight line on a semi-log plot in which the logarithm of cell concentration was plotted against the culture time. The relative growth rate of each strain was determined by normalizing the maximum growth rate in each medium, obtained from a time profile of growth, to those in the control medium. The I_{50} value of PGA was evaluated by determining the concentration of PGA at which the growth rate of microorganisms is reduced to half of that in the medium without PGA. For this purpose, the specific growth rate for each microorganism was plotted against PGA concentration and the I_{50} value was obtained by interpolation of the data points.

3. Results and discussion

We examined the growth of each species in medium with or without PGA (23.2 mM). As shown in Table 2, there was a large difference in the effect of PGA depending on organism. Thermophiles growing at neutral pH, such

Table 2
Effect of PGA (23.2 mM) on growth of various microorganisms

Microorganism	Temp. (°C)	pH	Phylogeny[a]	Biotope	Specific growth rate (h^{-1})	
					w/o PGA	w/ PGA
A. pernix	93	7.0	archaea	marine	0.095	0.093
S. solfataricus	78	3.0	archaea	terrestrial	0.025	ng[b]
R. obamensis	72	7.0	bacteria	marine	0.23	0.22
M. sedula	70	3.0	archaea	terrestrial	0.021	ng[b]
T. aquaticus	65	7.6	bacteria	marine	0.42	0.43
T. acidophilum	60	3.0	archaea	terrestrial	0.27	0.14
B. acidocaldarius	50	3.0	bacteria	terrestrial	0.19	0.15
E. coli	37	7.0	bacteria	terrestrial	0.37	0.40
T. thiooxidans	30	3.0	bacteria	terrestrial	0.024	0.023
P. putida	26	7.2	bacteria	terrestrial	0.20	0.29
S. cerevisiae	24	7.0	eucarya	terrestrial	0.33	0.35

[a]Based on universal phylogenetic tree [22].
[b]ng: no appreciable cell growth during the cultivation period of 100 h.

as *A. pernix*, *R. obamensis*, and *T. aquaticus*, were not affected by the presence of PGA, while there was significant growth inhibition by PGA for thermoacidophiles, such as *S. solfataricus*, *M. sedula*, *T. acidophilum*, and *B. acidocaldarius*. Regardless of pH, PGA was not toxic to microorganisms growing at ambient temperature. In case of *P. putida*, growth was greatly enhanced by the addition of PGA. When the ratio of specific growth rate with PGA to that without PGA was plotted, there was significant correlation in the effect of PGA to growth temperature and pH regardless of phylogeny and biotope (Fig. 1). The extent of growth inhibition by PGA on thermoacidophiles increased with higher growth temperature. The I_{50} value of PGA for growth of thermoacidophiles was analyzed by growing them in media containing different amounts of PGA. As shown in Fig. 2, the I_{50} value for the thermoacidophiles also had a close relationship with growth temperature.

The toxicity of yeast extract on the growth of *T. acidophilum* and *S. solfataricus* can be partly explained by the inhibitory effect of PGA on the growth of thermoacidophiles [7,10]. It is noteworthy that L-glutamate has been used as an important component for cell culture, especially for thermophiles, and is the most abundant amino acid in complex media such as yeast extract.

The present results show that the inhibitory effect of PGA was closely related to both growth temperature and pH of microorganisms. The intracellular pH of thermoacidophiles is usually close to neutral [11], which indicates the presence of an efficient proton extrusion mechanism against high proton concentration in growth medium. It is also known that an increase of growth temperature changes the composition of membrane affecting proton permeability [11]. In this connection, L-glutamate

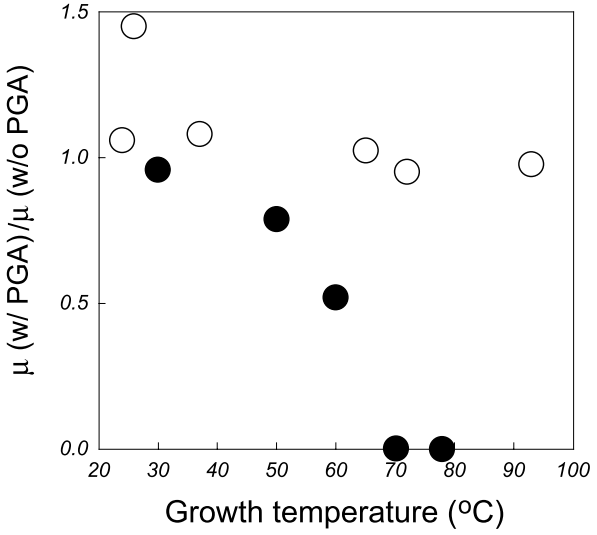

Fig. 1. Effect of growth temperature on the ratio of specific growth rate (μ) in the presence of PGA (23.2 mM) to μ in the absence of PGA for species grown at pH 3.0 (●) and species grown around pH 7.0–7.6 (○).

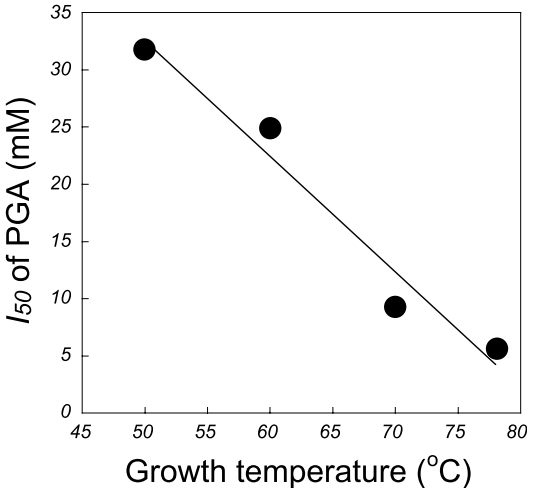

Fig. 2. Dependence of the I_{50} value of PGA on growth temperature of thermoacidophiles, which include *B. acidocaldarius* (50°C), *T. acidophilum* (60°C), *M. sedula* (70°C), and *S. solfataricus* (78°C). The I_{50} value of PGA was evaluated by determining the concentration of PGA at which the growth rate of microorganisms is reduced to half of that in the medium without PGA.

has been identified as one of the key components involved in the maintenance of the high proton concentration gradient. According to the study by Jack et al. [12], extremely thermoacidophilic *Picrophilus oshimae*, which grows at an optimum pH of 0.7 at 60°C and belongs to the same order as *T. acidophilum*, metabolized L-glutamate in correlation with cytoplasmic membrane ΔpH. While the transport rate of L-glutamate into cytoplasm was the highest among amino acids for *P. oshimae*, it was strongly inhibited by the presence of L-aspartate. In nerve cells, PGA inhibits L-glutamate transport by competitively binding at the L-glutamate receptor [3,5]. These evidences suggest a possible failure in efficient proton transport in thermoacidophiles by the presence of PGA as a structural analog of L-glutamate. In addition, it is also possible that PGA acts as a protonophore under acidic conditions, which would result in the acidification of the cytoplasm especially at high concentrations. It remains to be seen how PGA specifically affects transport of L-glutamate and/or proton across the cell membrane.

In any event, the present study illustrates that the amount of PGA should be maintained at a minimum value in the cultivation of thermoacidophiles. Despite increased interest in extremophiles, information on the reason why they cannot grow to cell densities as high as mesophiles and which factors constrain their growth is as yet scarce in the literature [13–16]. Considering that the formation of PGA from L-glutamate is highly accelerated under culture conditions of thermoacidophiles [1,6], minimization of PGA in growth medium by controlling the level of L-glutamate or complex nutrients should be an important strategy in growing thermoacidophiles to higher cell density.

Acknowledgements

This work was supported by the Korean Ministry of Science and Technology (21C Frontier Microbial Genomics and Applications Program) and in-house grants from the Pohang University of Science and Technology. The authors thank an anonymous reviewer who suggested the possible role of PGA as a protonophore.

References

[1] Wilson, H. and Cannan, R.K. (1937) The glutamic acid-pyrrolidonecarboxylic acid system. J. Biol. Chem. 119, 309–331.
[2] Borg, J., Stenger, A. and Toazara, J. (1986) Uptake and metabolism of L-[^{3}H] pyroglutamic acid in neuronal and glial cells in culture. Neurochem. Int. 8, 397–402.
[3] De Mello, C.F., De La Vega, D.D., Pizutti, L.T., Lopes, F.P., Rubin, M.A., Homerich, J.G., Melo, C.R., Somer, J.E., Souza, D.O. and Wajner, M. (1995) Neurochemical effects of L-pyroglutamic acid. Neurochem. Res. 20, 1437–1441.
[4] Dusticier, N., Kerkerian, L., Errami, M. and Nieoullon, A. (1985) Effects of pyroglutamic acid on corticostriatal glutamatergic transmission. Neuropharmacology 24, 903–908.
[5] Mornet, C. and Briley, M. (1988) The 'forgotten' amino acid pyroglutamate. Trends Pharmacol. Sci. 9, 278–279.
[6] Park, C.B., Lee, S.B. and Ryu, D.D.Y. (2001) L-Pyroglutamate spontaneously formed from L-glutamate inhibits growth of the hyperthermophilic archaeon *Sulfolobus solfataricus*. Appl. Environ. Microbiol. 67, 3650–3654.
[7] Brock, T.D., Brock, K.M., Belly, R.T. and Weiss, R.L. (1972) *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch. Mikrobiol. 84, 54–68.
[8] Huber, R., Huber, H. and Stetter, K.O. (2000) Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties. FEMS Microbiol. Rev. 24, 615–623.
[9] Herbert, R.A. (1992) A perspective on the biotechnological potential of extremophiles. Trends Biotechnol. 10, 395–401.
[10] Belly, R.T., Bohlool, B.B. and Brock, T.D. (1973) The genus *Thermoplasma*. Ann. N.Y. Acad. Sci. 225, 94–107.
[11] Jack, L.C.M., De Vossenberg, V., Driessen, A.J.M. and Konings, W.N. (1998) The essence of being extremophilic: the role of the unique archaeal membrane lipids. Extremophiles 2, 163–170.
[12] Jack, L.C.M., De Vossenberg, V., Driessen, A.J.M., Zillig, W. and Konings, W.N. (1998) Bioenergetics and cytoplasmic membrane stability of the extremely acidophilic, thermophilic archaeon *Picrophilus oshimae*. Extremophiles 2, 67–74.
[13] Park, C.B. and Lee, S.B. (1998) Ammonia production from yeast extract and its effect on growth of *Sulfolobus solfataricus*. Biotechnol. Bioprocess Eng. 3, 115–118.
[14] Park, C.B. and Lee, S.B. (1999) Inhibitory effect of mineral ion accumulation on high density growth of the hyperthermophilic archaeon *Sulfolobus solfataricus*. J. Biosci. Bioeng. 87, 315–319.
[15] Park, C.B. and Lee, S.B. (1999) Cultivation of the hyperthermophilic archaeon *Sulfolobus solfataricus* in low-salt media. Biotechnol. Bioprocess Eng. 4, 21–25.
[16] Park, C.B. and Lee, S.B. (2000) Effect of exogenous compatible solutes on growth of the hyperthermophilic archaeon *Sulfolobus solfataricus*. J. Biosci. Bioeng. 89, 318–322.
[17] Sako, Y., Nomura, N., Uchida, A., Ishida, Y., Morii, H., Koga, Y., Hoaki, T. and Maruyama, T. (1996) *Aeropyrum pernix* gen. nov., sp. nov., a novel aerobic hyperthermophilic archaeon growing at temperatures up to 100°C. Int. J. Syst. Bacteriol. 46, 1070–1077.
[18] Sako, Y., Takai, K., Ishida, Y., Uchida, A. and Katayama, Y. (1996) *Rhodothermus obamensis* sp. nov., a modern lineage of extremely thermophilic marine bacteria. Int. J. Syst. Bacteriol. 46, 1099–1104.
[19] Huber, G., Spinnler, C., Cambacorta, A. and Stetter, K.O. (1989) *Metallosphaera sedula* gen. and sp. nov. represents a new genus of aerobic, metal-mobilizing, thermoacidophilic archaebacteria. Syst. Appl. Microbiol. 12, 38–47.
[20] Brock, T.D. and Freeze, H. (1969) *Thermus aquaticus* gen. n., a nonsporulating extreme thermophile. J. Bacteriol. 98, 289–297.
[21] Butler, R.G. (1975) Pyruvate inhibition of the carbon dioxide fixation of the strict chemolithotroph *Thiobacillus thiooxidans*. Can. J. Microbiol. 21, 2089–2093.
[22] Woese, C.R., Kandler, O. and Wheelis, W.L. (1990) Towards a natural system of organisms. Proposal for the domains *Archaea*, *Bacteria*, and *Eucarya*. Proc. Natl. Acad. Sci. USA 87, 4576–4579.