

REFERENCES

- Abee, T., Palmen, R., Hellingwerf, K.J. and Konings, W.N. 1990. Osmoregulation in *Rhodobacter sphaeroides*. **J. Bacteriol.** 172: 149-154.
- Anderson, P.A., Kaasen, I., Styrvold, O.B., Boulnois, G., and Strom, A.R. 1988. Molecular cloning, physical mapping and expression of the *bet* genes governing the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. **J. Gen. Microbiol.** 134: 1737-1746.
- Barrett, M.C., and Dawson, A.P. 1975. Essentiality of ubiquinone for choline oxidation in rat liver mitochondria. **Biochem. J.** 148: 595-597.
- Boch, J., Kempf, B. and Bremer, E. 1994. Osmoregulation in *Bacillus subtilis*: synthesis of the osmoprotectant glycine betaine from exogenously provided choline. **J. Bacteriol.** 176: 5364-5371.
- Boch, J., Kempf, B., Schmid, R., and Bremer, E. 1996. Synthesis of the osmoprotectant glycine betaine in *Bacillus subtilis*: characterization of the *gbsAB* genes. **J. Bacteriol.** 178: 5121-5159.
- Borowitzka, M.A., Borowitzka, L.J., and Kessly, D. 1990. Effect of salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*. **J. Appl. Phycol.** 2: 111-119.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein in utilizing the principle of protein-dye binding. **Anal. Biochem.** 72: 248-254.

- Brouquisse, R., Wiegel, P., Rhodes, D., Yocom, C.F., and Hanson, A.D. 1989. Evidence for a ferridoxin-dependent choline monooxygenase from spinach chloroplast stroma. **Plant Physiol.** 90: 322-329.
- Brown, A.D. 1976. Microbiol water stress. **Bacterol Rev.** 401: 803-846.
- Canovas, D., Vagas, C., Csonka, L. N., Ventosa, A. and Nieto, J.J. 1996. Osmoprotectant in *Halomonas elongata*: high-affinity betaine transport system and choline-betaine. **J. Bacteriol.** 178: 7221-7226.
- Canovas, D., Vagas, C., Csonka, L. N., Ventosa, A. and Nieto, J.J. 1998. Synthesis of glycine betaine from exogenous choline in the moderately halophilic bacterium *Halomonas elongata*. **Appl. Environ. Microbiol.** 64: 4095-4097.
- Cayley, S., Lewis, B.A., and Record, T.M. Jr. 1992. Origin of osmoprotective properties of betaine and proline in *Escherichia coli* K-12. **J. Bacteriol.** 174: 1786-1595.
- Chi-shi, L., and Ru-dan, W. 1986. Choline oxidation and choline dehydrogenase. **J. Prot. Chem.** 5: 193-200.
- Choquet, C.G., Ahonkhai, I., Klein, M., and Kushner D.J. 1991. Formation and role of glycine betaine in the moderately halophile *Vibrio costicola*. **Arch. Microbiol.** 155: 153-158.
- Conka, L.N., and Hanson, A.D. 1991. Prokaryotic osmoregulation: genetics and physiology. **Annu. Rev. Microbiol.** 45: 569-606.
- Deana, D.M., Rose, A.C. and Mary, F.R. 1999. Osmoadaptation in Archaea. **Appl. Environ. Microbiol.** 65: 828-833.
- Dragolovich, J., and Pierce, S.K. 1994. Characterization of partially purified betaine aldehyde dehydrogenase from Horseshoe Crab (*Limulus polyphemus*) cardiac mitochondria. **J. Exp. Zool.** 270: 417-4252.

- Farwick, M., Siewe, R.M. and Kramer, R. 1995. Glycine betaine uptake after hyperosmotic shift in *Corynebacterium glutamicum*. **J. Bacteriol.** 177: 4690-4695.
- Fougere, F., and Le Rudulier, D. 1990. Glycine betaine biosynthesis and catabolism in bacteroids of *Rhizobium meliloti*; effect of salt stress. **J. Gen. Microbiol.** 136: 2503-2510.
- Galinski, E.A. and Truper, H.G. 1982. Betaine, a compatible solute in the extremely halophilic phototrophic bacterium *Ectothiorhodospira halochloris*. **FEMS Microbiol. Lett.** 13: 357-360.
- Galinski, E.A. 1995. Osmoadaptation in bacteria. **Adv. Microb. Physiol.** 37: 273-328.
- Galinski, E.A., and Herzog, R.M. 1990. The role of trehalose as a substitute for nitrogen-containing compatible solutes (*Ectothiorhodospira halochloris*). **Arch. Microbiol.** 153: 607-613.
- Galinski, E.A. and Truper, H.G. 1994. Microbial Behaviour in salt stressed ecosystems. **FEMS Microbiol. Rev.** 15: 95-108.
- Garlick, S., Oven, A., and Padan, A. 1977 Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. **J. Bacteriol.** 129: 623-629.
- Hanson, A.D., May, A.M., Grumet, R., Bode, J., Jamieson, G.C., and Rhodes, D. 1985. Betaine synthesis in chenopods: localization in chloroplasts. **Proc. Natl. Acad. Sci. USA** 82: 3678-3682.
- Haubrich, D.R., and Gerber, N.H. 1981. Choline dehydrogenase assay, properties and inhibitors. **Biochem. Pharmacol.** 30: 2993-3000.

- Hosaka, K. and Yamashita, S. 1980. Choline transport in *Saccharomyces cerevisiae*. **J. Bacteriol.**: 176-181.
- Hutchins, R. W., Ellefson, W.L., and Kashket, E.R. 1987. Betaine transport imparts osmotolerance on a strain of *Lactobacillus acidophilus*. **Appl. Environ. Microbiol.** 53: 2275-2281.
- Ikuta, SD., Imamura, S., Misaki, H. and Horiuti, Y. 1997. Purification and characterization of choline oxidase from *Arthrobacter globiformis*. **J. Biochem.** 82: 1741-1749.
- Imhoff, J.F., Tiemann, B. 1991. Influence of salt concentrationand temperature on the fatty acid composition of Ectotiorhodospira and other halophilic phototrophic purple bacteria. **Arch. Microbiol.** 156: 370-375.
- Incharoensakdi, A., and Kum-arb, U. 1996. Osmoregulation in a halophilic cyanobacterium *Aphanothecce halophytica*. **Second Thai-French Symposium on Plant Molecular Biology**. 283-292 .
- Ishitani, M., Nakamura, T., Han, S.Y., and Takabe, T. 1995. Expression of the betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. **Plant Mol. Biol.** 27: 307-315.
- Kaenjak, A., Graham , J.E. and Wilkinson, B.J. 1993 . Choline transport activity in *Staphylococcus aureus* induced by osmotic stress and low phosphate concentrations. **J. Bacteriol.** 175: 2400-2406 .
- Kiene, R.P. 1998. Uptake of choline and its conversion to glycine betaine by bacteria in esturine waters. **Appl. Environ. Microbiol.** 64: 1045-1051.
- Kimura, T., and Singer, T.P. 1962. Choline dehydrogenase from rat liver. **Meth. Enzymol.** 5: 562-570.

- Knowles, C.J. and Smith, L. 1971. Effect of osmotic pressure of the medium on volume of intact cells of *Azotobacter vinelandii* and on the rate of respiration. **Biochem. Biophys. Acta.** 234: 144-152.
- Ko, R., Smith, L.T., and Smith, G.M. 1994. Glycine betaine confers enhanced osmotolerance and cryotolerance on *Listeria monocytogenes*. **J. Bacteriol.** 176: 426-431.
- Koch, A.L. 1984. Shrinkage of growing *Escherichia coli* cells by osmotic challenge. **J. Bacteriol.** 159: 919-924.
- Lamark, T., Rokenes, T.P., McDougall, J., and Strom, A. 1996. The complex *bet* promoters of *Escherichia coli* regulation on oxygen (ArcA), choline (BetI), and osmotic stress. **J. Bacteriol.** 178: 1655-1662.
- Lanfald, B. and Strom, A.R. 1986. Choline-lysine betaine pathway confers a high level of osmotic tolerance in *Escherichia coli*. **J. Bacteriol.** 165: 849-855.
- Le Rudulier, D., and Bouillard, L. 1983. Glycine betaine, an osmotic effector in *Klebsiella pneumoniae* and other members of the *Enterobacteriaceae*. **Appl. Environ. Microbiol.** 46: 152-159.
- Le Rudulier, D., Strom, A.R., Dandekar, A.M., Smith, L.T., Valentine, R.C. 1984. Molecular biology of osmoregulation. **Science.** 224: 1064-1068.
- Lin, C.S., and Wu, R.D. 1986. Choline oxidation and choline dehydrogenase. **J. Prot. Chem.** 5: 193-200.
- Lowe, S.E. Jain, M.K. Zeikus, J.P. 1993. Biology, ecology and biotechnological application of anaerobic bacteria adapted to environmental stresses in temperature, pH salinity, or substrates. **Microbiol. Rev.** 57: 451-509.
- Mackay, M.A., Norton, R.S., and Borowitzka, L.J. 1984. Organic osmoregulatory solutes in cyanobacteria. **J. Gen. Microbiol.** 130: 2177-2191.

- Mohammad, F.A.A., Reed, R.H., and Stewart, W.D.P. 1983. The halophilic cyanobacterium *Synechocystis* DUN 52 and its osmotic responses. **FEMS Microbiol. Lett.** 16: 287-290.
- Moor, D.J., Reed, R.H., and Stewart, W.D.P. 1987. A glycine betaine transport system in *Aphanothecce halophytica* and other glycine betaine-synthesising cyanobacteria. **Ach. Microbiol.** 147: 399-405.
- Mori, N., Kawakami, B., Hayakutome, K., Tani, Y., and Yamada, H. 1980. Characterization of betaine aldehyde dehydrogenase from *Cylindrocarpus didenum M-1*. **Argri. Biol. Chem.** 44: 3015-3016.
- Nagasawa, T., Mori, N., Tani, Y., and Ogata, K. 1976. Characterization of choline dehydrogenase from *Pseudomonas aerugenosa* A-16. **Argri. Biol. Chem.** 40: 2077-2084.
- Nomura, M., Ishitani, M., Takabe, T., Rai, A.K., and Takabe, T. 1995. *Synechococcus* sp. PCC 7491 transformed with *Escherichia coli bet* genes produces glycine betaine from choline and acquires resistance to salt stress. **Plant physiol.** 107: 703-708.
- Ohta- Fukuyama, M., Miyake, Y., Emi, S., and Yamano, T. 1980. Identification and properties of the prosthetic group of choline oxidase from *Alcaligenes* sp. **J. Biochem.** 88: 197-203.
- Oren, A. 1999. Bioenergetic aspect of halophilism. **Microbiol. Mol. Biol. Rev.** 63: 334-348.
- Pan, S.M. 1988. Betaine aldehyde dehydrogenase in spinach. **Bot. Bull. Acad. Sin.** 29: 255-263.

- Panfili, G., Manzi, P., Compagnone, D., Scaciglia, L. and Palleschi, G. 2000. Rapidly assay of choline in foods using microwave hydrolysis and choline biosensor. **J. Agric. Food. Chem.** 48: 3403-3407.
- Parente, A.M. and Silva, M.T. 1984. Ultrastructural aspects of autolysis of *Pseudomonas fluorescens* induced by osmotic shock. **J Gen Microbiol.** 130: 1453-1470.
- Perroud, B., and Le Rudire, D. 1985. Glycine betaine transport in *Escherichia coli*: osmotic modulation. **J. Bacteriol.** 161: 393-401.
- Pocard, J.A., Benard, T., Smith, L.T., and Rudulier, D.L. 1989. Characterization of three choline transport activities in *Rhizobium meliloti*: modulation by choline and osmotic stress. **J. Bacteriol.** 171: 531-537.
- Porter, R.K., Scott, J.M., and Brand, M.D. 1992. Choline transport into rat liver mitochondria characterization and kinetics of a specific transporter. **J. Biol. Chem.** 267: 14637-14646.
- Pourkomailian, B., and Booth, I.R. 1994. Glycine betaine transport by *Staphylococcus aureus*: evidence for feedback regulation of the activity of the two transport systems. **Microbiol.** 140: 3131-3138.
- Reed, R.H., Chudek, J.A., Foster, R., and Stewart, W.D.P. 1984. Osmotic adjustment in cyanobacteria from hypersaline environments. **Arch. Microbiol.** 138: 333-337.
- Reed, R.H., Richardson, D.L., Stewart, W.D.P. 1985. Na^+ uptake and extrusion in the cyanobacterium *Synechocystis* PCC 6714 in response to hypersaline treatment. Evidence for transient changes in plasmalemma Na^+ permeability. **Biochem. Biophys. Acta.** 814: 347-355.

- Rendina, G., and Singer, T.P. 1959. Extraction in soluble form assay and some properties of the enzyme. **Biochem. Biophys. Acta.** 30: 441-447.
- Rendina, G., and Singer, T.P. 1959. Studies on choline dehydrogenase. **J. Biol. Chem.** 234: 1605-1610.
- Robinson, S.P., and Jones, G.P. 1986. Accumulation of glycine betaine in chloroplast provides osmotic adjustment during salt stress. **Aust. J. Plant Physiol.** 13: 659-668.
- Robertson, D.E., Noll, D., Roberts, J.A., Menaia, G.F., and Boone, D.R. 1990. Detection of the osmoregulator betaine in methanogens. **Appl. Environ. Microbiol.** 56: 563-565.
- Rokenes, T.P., Lamark, T., and Storm, A.R. 1996. DNA-binding properties of the BetI repressor protein of *Escherichia coli*: the inducer choline stimulates BetI-DNA complex formation. **J. Bacteriol.** 178: 1663-1670.
- Rothschild, H.A., and Barron, E.S.G. 1954. The oxidation of betaine aldehyde by betaine aldehyde dehydrogenase. **J. Biol. Chem.** 209: 511-523.
- Rosentein, R., Futter-Bryniok, D., and Gotz, F. 1999. The choline-converting pathway in *Staphylococcus xylosus* C2A: genetic and physiological characterization. **J. Bacteriol.** 181: 2273-2278.
- Russell, R., and Scopes, R.K. 1994. Use of hydrophobic chromatography for purification of the membrane-located choline dehydrogenase from a *Pseudomonas* strain. **Bioseparation.** 4: 279-284.
- Sakaguchi, K. 1960. Betaine as a growth factor for *Pediococcus soyae* VIII studies on the activitise of bacteria in soy sauce brewing. **Bull. Agri. Chem. Soc. Jpn.** 24: 489-496.

- Skjerdal, O.T., Sletta, H., Flenstad, S.G., Josefson, K.D., Levine, D.W. and Ellingsen, T.E. 1995. Changes in cell volume, growth and respiration rate in response to hyperosmotic stress of NaCl, sucrose and glutamic acid in *Brevibacterium lactofermentum* and *Corynebacterium glutamicum*. **Appl. Microbiol. Biotechnol.**, 43: 1099-1106.
- Smith, L.T., Pocrad, J.A., Berward, T., and Le Rudulier, D. 1988. Osmotic control of glycine betaine biosynthesis and degradation in *Rhizobium meliloti*. **J. Bacteriol.** 170: 3142-3149.
- Streumer-Svobodova, Z., and Drahota, Z. 1977. The development of oxidative enzymes in rat liver mitochondria. **Physiol. Bohemoslov.** 26: 525-534.
- Strom, A.R. and Kaasen, I. 1993. Trehalose metabolism in *Escherichia coli*: stress protection and stress regulation of gene expression. **Mol. Microbiol.** 29: 285-296.
- Styrvold, O.B., Falkenberg, P., Lanfald, B., Eshoo, M.W., Bjornsen, T., and Strom, A.R. 1986. Selection, mapping, and characterization of osmoregulatory mutants of *Escherichia coli* blocked in the choline-glycine betaine pathway. **J. Bacteriol.** 165: 856-863.
- Thieman, B., Imhoff, J.F. 1991. The effect of salt on the lipid composition of *Ectothiorodospira*. **Arch. Microbiol.** 156: 376-384.
- Tsuge, H., Nagano, Y., Onishi, H., Futamura, Y., and Ohashi, K. 1980. A novel purification and some properties of rat liver mitochondrial choline dehydrogenase. **Biochem. Biophys. Acta.** 614 : 274-284.
- Ventosa, A., Nieto, J.J., Oren, A. 1998. Biology of moderately halophilic aerobic bacteria. **Microbiol. Mol. Biol. Rev.** 62: 504-544.

- Vonshak, A., Kancharaksa, N., Bunnag, B., and Tanticharoen, M. 1996. Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. **J. Appl. Phycol.** 8: 119-124.
- Weigel, P., Weretilnyk, E.A., and Hanson, A.D. 1986. Betaine aldehyde oxidation by spinach chloroplasts. **Plant Physiol.** 82: 753-759.
- Wilken, D.R. 1970. Estimation of choline and betaine aldehyde dehydrogenase activities in rat liver mitochondria by three independent methods. **Anal. Biochem.** 36: 323-331.
- Wilken, D.R., McMacken, M.L., and Rodiguez, A. 1970. Choline and betaine aldehyde oxidation by rat liver mitochondria. **Biocim. Biophys. Acta.** 216: 305-317.
- Williams, J.N., and Screenivasan, A. 1953. Preparation of soluble choline dehydrogenase from liver mitochondria. **J. Biol. Chem.** 203: 899-906.
- Wirthensohn, G., and Guder, W.G. 1982. Studies on renal choline metabolism and phosphatidylcholine synthesis. **Biochem. of kidney function.**, edited by F. Morel. Amsterdam: Elsevier. 119-128.
- Wyn Jones, R.G., Storey, R., Leigh, R.A. Ahmad, N., and Pollard, A. 1977. A hypothesis on cytoplasmic osmoregulation. In **Regulation of cell membrane activities in higher plants**, edited by E. Marre and C. Ciferri. Elsevier, North Holland, Amsterdam. P. 121-136.
- Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. and Somero, G.N. 1982. Living with water stress: evolution of osmolyte systems. **Science.** 217: 1214-1222.
- Zahran, H.H. 1997. Diversity, adaptation and activity of the bacterial flora in saline environment. **Biol. Fertil. Soils.** 25: 211-223.
- Zeisel, S.H. 2000. Choline: an essential nutrient for humans. **Nutrition.** 16: 669-671.

- Zeisel, S.H., and Blusztajn, J.K. 1994. Choline and human nutrition. *Annu. Rev. Nutr.* 14: 269
- Zhang, J., Blusztajn, J.K., and Zeisel, S.H. 1992. Measurement of the formation of betaine aldehyde and betaine in rat liver mitochondria by a high pressure liquid chromatography-radioenzymatic assay. *Biochem. Biophys. Acta.* 1117: 333 – 339.

APPENDICES

Appendix 1

Turk Island Salt Solution + modified BG₁₁ medium contained the following components:

1. Preparation of Turk Island Salt Solution

Stock Solution A: KCl 33.3 g

MgCl₂.6H₂O 275.0 g

CaCl₂.2H₂O 73.3 g

and made up to 5 litres with distilled water

Stock Solution B: MgSO₄.7H₂O 347.0 g

and then made up to 5 litres with distilled water

To make Turk Island Salt Solution, 500 ml of Stock Solution A was added to 500 ml of Stock Solution B. To this mixture 140.8 g of NaCl was added and the final volume was made to 5 litres distilled water.

2. Composition of modified BG₁₁ medium (BG₁₁ medium + NaNO₃ solution)

NaNO ₃	(75 g/500 ml)	50 ml
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KH ₂ PO ₄	(8 g/200 ml)	5 ml
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MgSO ₄ .7H ₂ O	(15 g/200 ml)	5 ml
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CaCl ₂ .2H ₂ O	(7.2 g/200 ml)	5 ml
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Na ₂ CO ₃	(4 g/200 ml)	5 ml
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Citric acid	(1.2 g/200 ml)	5 ml
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EDTA.Na ₂	(0.2 g/200 ml)	5 ml
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FeSO ₄ .7H ₂ O	(1.2 g/200 ml)	5 ml
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*Trace element A5 Solution + Co	5 ml
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*Trace element A5 Solution + Co contained the following component in gram per litre H₃PO₄: 2.86; ZnSO₄.7H₂O: 0.2; CuSO₄.5H₂O: 0.08; MnCl₂.4H₂O: 1.81; Na₂MnO₄.2H₂O: 0.39; Co(NO₃)₂.6H₂O: 0.049

Culture medium of *Aphanethece halophytica* was prepare by adding all solution of item 2 at indicated volume to 5 litres of Turk Island Salt Solution and the pH was adjusted to 7.6 by slowly adding 2 M NaOH. The medium was sterilized by autoclaving at 15 lb/in² for 15 minutes.

Appendix 2

Scintillation fluid (1,000 ml) as follows:

Dissolve 5.5 g PPO (2,5-diphenyloxazole) and 0.1 g POPOP [1,4-bis (5-phenyloxazole-2-yl) benzene] in 1,000 ml of a solution composed of 667 ml Toluene and 333 ml Triton X-100. Make certain that the contents are completely dissolved before the solution is used. The solution should be stored in a brown bottle in a cool dark place.

Appendix 3

Preparation of polyacrylamide gel electrophoresis:

1. Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100 ml

acrylamide	29.2 g
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N,N'-methylene-bis-acrylamide	0.8 g
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Adjusted volume to 100 ml with distilled water.

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)- aminomethane	18.2 g
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Adjusted pH to 8.8 with 1 M HCL and adjusted volume to 100 ml with distilled water.

2 M Tris-HCl pH 8.8

Tris (hydroxymethyl)- aminomethane	24.2 g
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Adjusted pH to 8.8 with 1 M HCL and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)- aminomethane	6.1 g
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Adjusted pH to 8.8 with 1 M HCL and adjusted volume to 100 ml with distilled water.

1 M Tris-HC pH 6.8

Tris (hydroxymethyl)- aminomethane	12.1 g
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Adjusted pH to 8.8 with 1 M HCL and adjusted volume to 100 ml with distilled water.

Solution B (SDS-PAGE)

2 M Tris-HCl pH 8.8	75	ml
10% SDS	4	ml
distilled water	21	ml

Solution C (SDS-PAGE)

1 M Tris-HCl	50	ml
10% SDS	4	ml

2. Non-denaturing PAGE**12.0 % Separating gel**

30% acrylamide solution	4.17	ml
1.5 M Tris-HCl pH 8.8	2.50	ml
distilled water	3.33	ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50	μl
TEMED	5	μl

5.0% stacking gel

30% acrylamide solution	1.67	ml
0.5 M Tris-HCl pH 8.8	2.50	ml
distilled water	5.80	ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50	μl
TEMED	5	μl

Sample buffer

0.5 M Tris-HCl pH 6.8	1.0	ml
glycerol	0.8	ml
0.5% bromophenol blue	0.5	ml

distilled water	5.8 ml
Electrophoresis buffer, 1 litre (25 mM Tris, 192 mM glycine)	
Tris (hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g
Dissolved in distilled water to 1 litre. Do not adjust pH with acid or base (final pH should be 8.3).	

3. SDS-PAGE

12.0% separating gel

30% acrylamide solution	4.17 ml
solution B	2.50 ml
distilled water	3.33 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μl
TEMED	5 μl

5.0% stacking gel

30% acrylamide solution	1.67 ml
solution C	2.50 ml
distilled water	5.80 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μl
TEMED	5 μl

Sample buffer

1 M Tris-HCl pH 6.8	0.6 ml
glycerol	5.0 ml
10% SDS	2.0 ml

2-mercaptoethanol	0.5 ml
1% bromophenol blue	0.5 ml
distilled water	5.8 ml

One part of sample buffer was added to four parts of sample. The mixture was heated 5 minutes in boiling water before loading to the gel.

Electrophoresis buffer, 1 litre

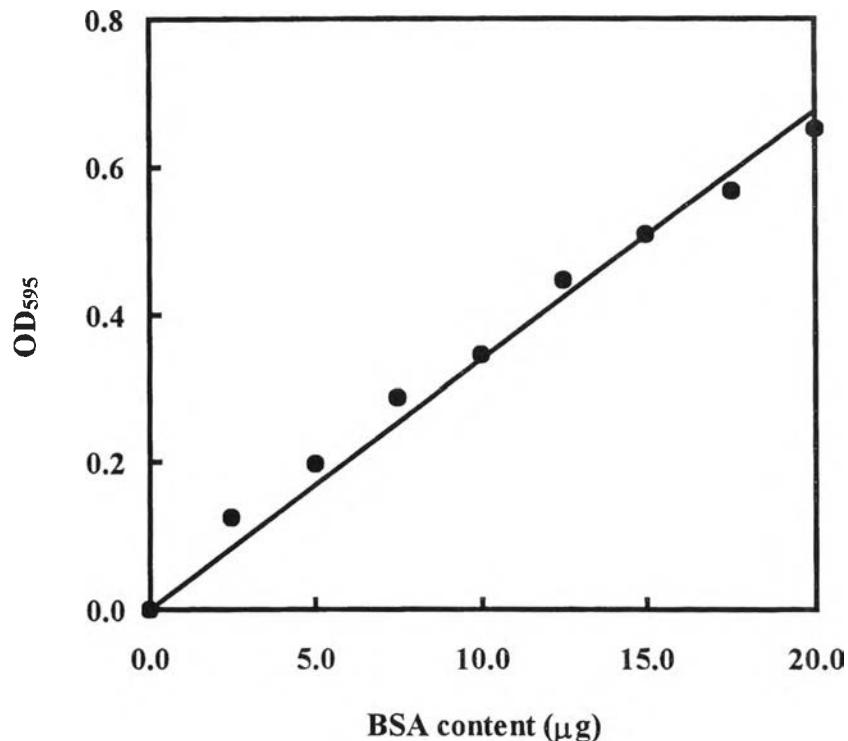
(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g
SDS	1.0 g

Dissolved in distilled water to 1 litre. Do not adjust pH with acid or base (final pH should be 8.3).

Appendix 4

Standard curve of BSA by Bradford assay



BIOGRAPHY

Mr. Aphichart Thartdee was born on August 24, 1976 in Bangkok, Thailand. He graduated with a Bachelor Degree of Science in Chemistry from Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand in 1998 and studied for a Master Degree in Biochemistry program since 1999.

