APPENDIX

Appendix A List of chemicals and materials used in this study.

The chemicals used were analytical grade unless specified.

Enzymes and restriction enzymes

Sal I	Takara Biomedicals, Japan
*Xho*I	Takara Biomedicals, Japan
*BamH*I	Takara Biomedicals, Japan
T4 DNA ligase	Promega corporation, USA
Taq polymerase	Promega corporation, USA
Ampli*Taq* Gold DNA polymerase	Promega corporation, USA
Pfx platinum DNA polymerase	GibcoBRL, USA
DNase free RNase	Takara Biomedicals, Japan

Primers

LFNF	Takara Biomedicals, Japan
LFND	Nippon Gene, Japan
LFCD	Takara Biomedicals, Japan
PVSEQ	Nippon Gene, Japan
LFC	Nippon Gene, Japan
nanH LF F	Nippon Gene, Japan
nanH Rev LF	Nippon Gene, Japan
LFF nanH	Nippon Gene, Japan
LFC Sal	Nippon Gene, Japan

Gel purification and DNA sequencing

SEAKEM agarose gel	FMC Bioproduct, USA
Nusieve agarose gel	FMC Bioproduct, USA
Quiagen® DNA purification kit (From Gel)	Quiagen, Japan
Quiagen® DNA purification kit (From PCR)	Quiagen, Japan
PRISM® DNA sequencing reagent	Perkins Elmer co., NJ, USA
Centri-SEP® columns	Princeton separation Inc., USA

Simple chemicals

Acrylamide	Wako Pure Chemical Industries, Japan
Agar	Wako Pure Chemical Industries, Japan
Ampicilin sulphate	Wako Pure Chemical Industries, Japan
APS	Wako Pure Chemical Industries, Japan
β-glucutonidase from pancrease	Sigma-Aldich Chemical Co., USA
Bovine lactoferrin	Wako Pure Chemical Industries, Japan
Bromophenol blue	Wako Pure Chemical Industries, Japan
CSPD	Boehringer Mannheim, German
Erythromycin	Wako Pure Chemical Industries, Japan
Ethidium bromide	Wako Pure Chemical Industries, Japan
Ethylenediamine tetraacetic acid	Dojindo, Japan
Formaldehyde	Wako Pure Chemical Industries, Japan
Formamide	Wako Pure Chemical Industries, Japan
GAM broth	Nissui Pharmaceutical, Japan
Gentamycin	Wako Pure Chemical Industries, Japan
Glycerol	Wako Pure Chemical Industries, Japan
Goat anti rabbit IgG alkaline phosphatase conjugate	Bio-Rad Laboratories, USA
Human lactoferrin	Sigma-Aldich Chemical Co., USA

Hybridizing Buffer	Amersham International plc, England
Lysozyme	Wako Pure Chemical Industries, Japan
MOPS	Wako Pure Chemical Industries, Japan
N'N'-bis-methylene acrylamide	Wako Pure Chemical Industries, Japan
Nutrient Oxoid No2	Difco, USA
p-nitrophenol	Wako Pure Chemical Industries, Japan
p-nitrophenyl-β-D-glucuronide	Sigma-Aldich Chemical Co., USA
Polypeptone	Nihon Seiyaku, Japan
PVDF membrane	Bio-Rad Laboratories, USA
Rabbit anti human lactoferrin antisera	ICN Pharmaceutical Inc., USA
Sodium acetate	Wako Pure Chemical Industries, Japan
Sodium citrate	Wako Pure Chemical Industries, Japan
Sodium dodesyl sulphate	Wako Pure Chemical Industries, Japan
TEMED	Amtesco, USA
Tetracyclin	Wako Pure Chemical Industries, Japan
Trimetroprim	Wako Pure Chemical Industries, Japan
Tris-Base	Sigma-Aldich Chemical Co., USA
Tween-20	Sigma-Aldich Chemical Co., USA
X-ray film	Fuji photo film Co., Tokyo, Japan
Xylenecyanol	Wako Pure Chemical Industries, Japan
Yeast extract	Difco, USA

Appendix B List of instruments used in this study

Instrument -model	**Source**
Lab top centrifuge	Tommy, USA
(High speed microcentrifuge)	
Refrigerated microcentrifuge	Tommy, USA
Ultra centrifuge	Hitachi Koki, Tokyo, Japan
PCR system	Perkins Elmer co., NJ, USA
Block incubator	Astec, USA
Multi-shaker oven	Taitec, Japan
UV-cress linker-CL-1000	Upland, USA
Minigel Electrophoresis system-Mupid-2	CosmoBio, Tokyo, Japan
Sequencing analyzer-PRISM®	Perkins Elmer co., NJ, USA
Kodak digital camera	Kodak, Japan
Electro blot apparatus	Bio-Rad Laboratories, USA
UV-Vis spectrophotometer-UV1200	Shimadzu, Japan
RNA/DNA calculator-GeneQuant II	Pharmacia Biotech, England
PAGE apparatus & power supply	Bio-Rad Laboratories, USA
-80 o refrigerator	Nihong freezer, Japan
-20 oC refrigerator	Nihong freezer, Japan
Shaker water bath	Ikemoto, Japan
High pressure steam sterilized	Tommy, USA
Vacuum concentrator	Tommy, USA
Sonicator-250 sonifier	Branson Sonic Power, USA

Appendix C Reagents and buffers preparation

1M tris HCl

Tris-base	12.1 g

Dissolve in 80 ml distilled water, adjust pH to 8.0 by concHCl

Reach volume to	100 ml

0.5 M EDTA

EDTA	18.6 g

Dissolved in distilled water

Adjust pH to 8.0 by NaOH

Total volume	100 ml

TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0)

1M Tris-HCl	1 ml
0.5 M EDTA	0.2 ml
Add distilled water to	100 ml

Solution I

Glucose	0.9 g
1M Tris-HCl	2.5 ml
0.5 M EDTA	2.0 ml
dH_2O	100 ml

Autoclave 121 $^{\circ}C$ for 20 min to sterilize and keep as stock

Complete Solution I (Freshly prepare)

Solution I	0.9 ml
10 mg/ml lyzozyme	0.1 ml
DNase free RNase	2 µl

Solution II (freshly prepare)

10% SDS	0.5ml
10N NaOH	0.1 ml
dH_2O	4.4 ml

Solution III (cool on ice before use)

5M sodium acetate	60.0 ml
Glacial acetic acid	11.5 ml
dH_2O	28.5 ml
Total	100 ml

5X TBE buffer

Tris-Base	54.0 g
Borate	27.5 g
EDTA	4.15 g

Dissolve in distilled water and adjust volume to 1000 ml

Dilute to 0.5x TBE buffer (1 liter) for running buffer with adding 0.1 ml of 5 mg/ml ethidium bromide for 1000 ml

Agarose gel for electophoresis (for 2 large size and 4 small size)

Gel density	0.7%	3%
Agarose gel (SEAKEM)	1.12 g	-
Agarose gel (NuSieve)	-	4.8 g
0.5X TBE with EtBr	160 ml	160 ml

Heat by autoclave 121 °C 5 min

Stand in RT until warm and pour on miniprep apparatus

DNase digestion buffer (20 mM Tris-HCl, 10 mM MgCl2)

1M Tris-HCl	2.0 ml
1M MgCl2	1.0 ml
dH_2O	97.0 ml

Gram negative lysis buffer

1 M Tris-HCl, pH 8.0	1.0 ml
1M NaCl	1.0 ml
500 mM Sodium citrate	1.0 ml
10% SDS	10.0 ml
dH_2O	87.0 ml

Protoplasting buffer

1 M Tris-HCl, pH 8.0	1.5 ml
4.5 M Sucrose	10.0 ml
0.5 M EDTA	1.6 ml
dH_2O	86.9 ml

20X MOPS buffer (0.4 M MOPS, 100 mM sodium acetate, 10 mM EDTA)

MOPS	87.3 g

Dissolved in distilled water 800 ml

Sodium acetate	13.6 g
EDTA. 2Na	7.45 g

Adjust pH to 7.0 by 10N NaOH

Adjust volume to	1000 ml

Autoclave: Dilute to 1x MOPS for running buffer

Gel loading buffer

Glycerol	5.0 ml
0.5 M EDTA	20 µl
Bromophenol blue	25 mg
Xylelecyanol	25 mg
dH_2O	5.0 ml

20X SSC (3M NaCl, 0.3 M sodium citrate)

Sodium chloride	175.3 g
Sodium citrate	88.2 g
Adjust total volume by distilled water	1000 ml

Dilute to 2x SSC for immersion membrane before blotting

1% Denatured agarose gel for RNA electrophoresis

Agarose gel	1.0 g
20x MOPS	5.0 ml
dH_2O	77 ml

Heat 100 °C 5 min

Formaldehyde	18.0 ml

Stand in RT until warm and pour on miniprep apparatus

Acrylamide/Bis solution

Acrylamide	29.2 g
N'N'-bis-methylene acrylamide	1.0 g
Total volume	100 ml

1.5 M Tris-HCl, pH 8.8

Tris-Base	27.23 g
Dissolve in distilled water	

Adjust pH to 8.8 by conc HCl

Adjust volume to	150 ml

0.5 M Tris-HCl, pH 6.8

Tris-Base	6.0 g
Dissolve in distilled water	

Adjust pH to 6.8 by conc HCl

Adjust volume to	100 ml

10% Sodium dodesyl sulphate

SDS	10 g
Distilled water	100 ml

Warm to increase solubilization

Sampling buffer

Water	3.8 ml
0.5 M Tris HCl, pH 6.8	1.0 ml
Glycerol	0.8 ml
10% SDS	1.6 ml
2-mercaptoethanol	0.4 ml
1% bromophenolblue (BPB)	0.4 ml
Total	8.0 ml

Running buffer, pH 8.3 (5X)

(1X: 15 mM Tris, 192 mM Glycine)

Tris Base	15.0 g
Glycine	72.0 g
SDS	5.0 g

Adjust pH to 8.3 by conc HCl

Adjust volume to	1000 ml

1X Running buffer

5x Running buffer	100 ml
Distilled water	400 ml
Total	500 ml

Slab gel preparation	**8%Running gel**	**10% Running gel**	**Stacking gel**
Distilled water	6.9 ml	5.9 ml	3.4 ml
Acrylamide/Bis	4.0 ml	5.0 ml	0.83 ml
1.5M Tris-HCl, pH 8.8	3.8 ml	3.8 ml	0.63 ml
10% SDS	0.15 ml	0.15 ml	0.05 ml
10% APS*	0.15 ml	0.15 ml	0.05 ml
TEMED	0.02 ml	0.02 ml	0.01 ml

*Dissolved 100 mg of ammonium peroxide sulfate in 1 ml of distilled water and stored at 4°C

Completely set the gel packing apparatus before adding APS and TEMED

Blotting buffer

Tris-Base	5.82 g
Glycine	2.93 g
Dissolved in total volume	800 ml
Methanol	200 ml
Total	1000 ml

Buffer for alkaline phosphatase

Tris-Base	6.06 g
NaCl	2.92 g
$MgCl_2 . 6H_2O$	5.08 g

Dissolved in distilled water

Adjust pH to 9.5 by HCl

Adjust volume to	500 ml

Luria-broth

Polypeptone	1.0 g
Yeast extract	0.5 g
NaCl	0.5 g
Total volume	100 ml

Autoclave 121 °C for 20 min

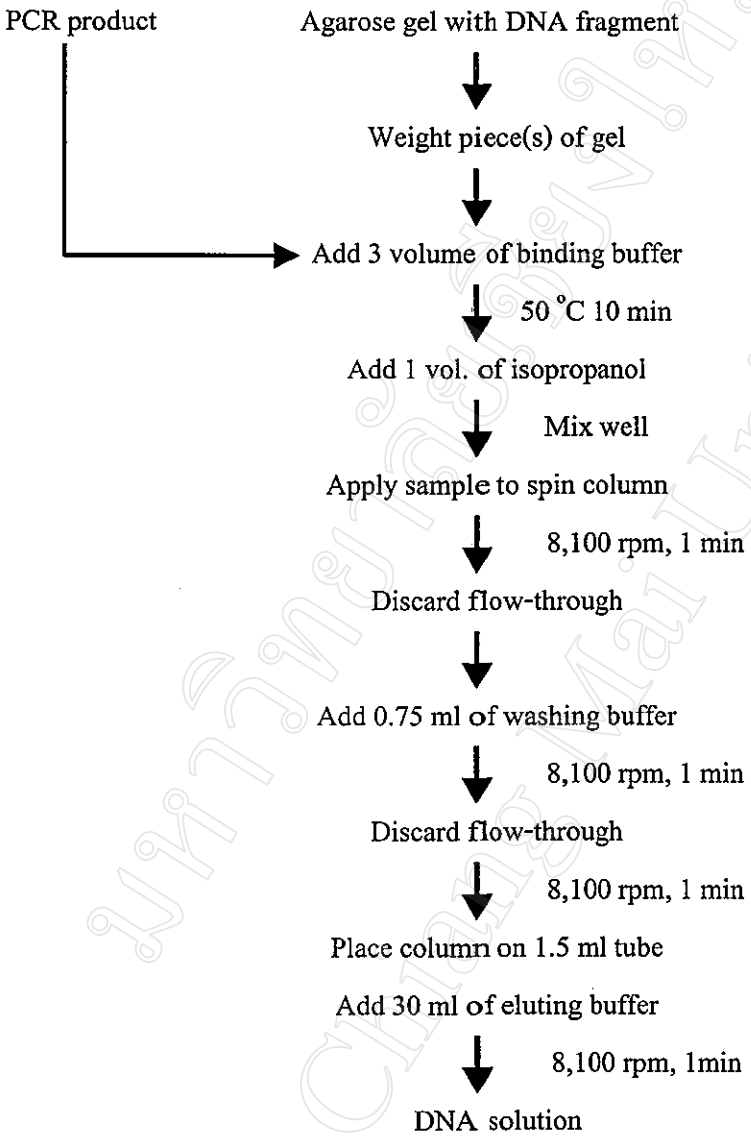
LB agar

L-Broth	100 ml
Agar	1.5 g

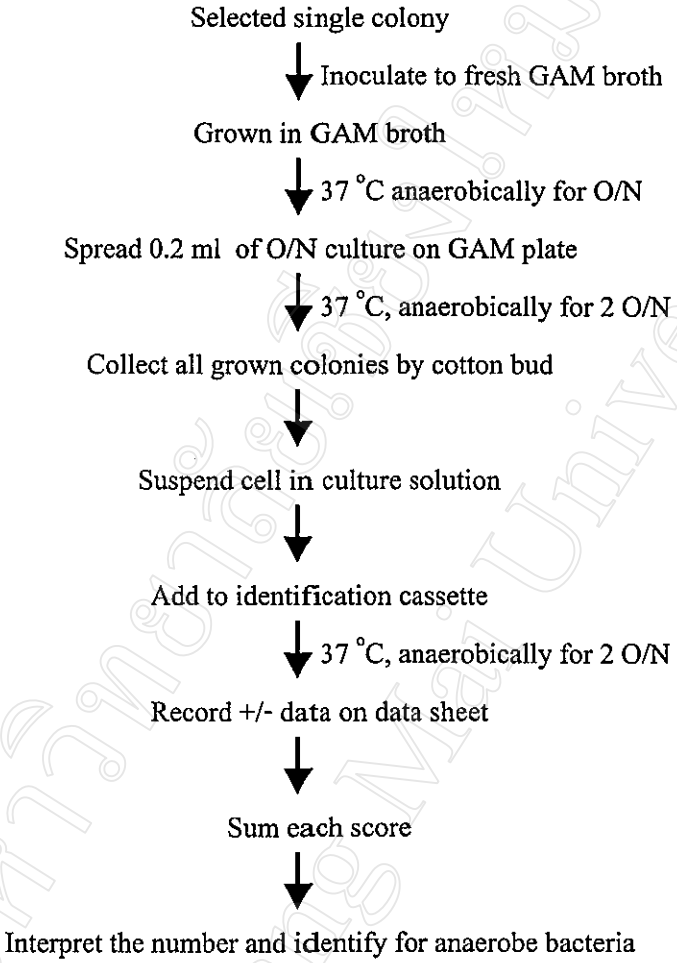
Autoclave 121 °C for 20 min

Pour onto bacteria culture plate (25 ml each)

Appendix D Isolation and purification of DNA fragment by QIAquick® Gel Extraction Kit or PCR Purification Kit



Appendix E Api-20A® Anaerobes Identification Kit



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Chewonarin T., Kuwahara T., Arimochi H., Kataoka K., Nakayama H., Yu D. Y., Tsuda H., Vinitketkumnuen U., and Ohnishi Y. (2001) The production of lactoferrin-producing *Bacteroides uniformis*. *Chiang Mai Med Bull* **40**(4): 187-194.

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CURRICULUM VITAE

Name	Teera Chewonarin
Sex	Male
Nationality	Thai
Marital status	Single
Date of birth	June 7, 1970
Home address	38 Changmoi Rd. Tumbol Changmoi, Muang district, Chiang Mai 50300 Thailand
Education	
1976-1981	Primary school at The Prince Royal' s Collage, Chiang Mai, Thailand
1982-1987	Junior high school and high school at The Prince Royal' s Collage, Chiang Mai, Thailand
1989-1993	B.Sc. (Biochemistry and Biochemical technology) Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
1993-1996	M.Sc. (Biochemistry) Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
1997-2001	Ph.D. (Biochemistry) Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Publications

Chewonarin T., Kinouchi T., Kataoka K., Arimochi H., Kuwahara T., Vinitketkumnuen U., Ohnishi Y. (1999)Effects of roselle (*Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in F344 rats. *Food Chem Toxicol* **37**(6):591-601

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Chewonarin T., Kuwahara T., Arimochi H., Kataoka K., Nakayama H., Yu D. Y., Tsuda H., Vinitketkumnuen U., and Ohnishi Y. (2001) Expression of human lactoferrin in *Bacteroides uniformis* and its effect on azoxymethane-induced aberrant crypt focus formation in the rat colon. *Anaerobe* **7**: 247-253.

Research experiences

May–December 1996

The effect of Thai medicinal plant on the formation of aberrant crypt foci and DNA-adduct in carcinogen treated rats. In department of Bacteriology school of Medicine, The University of Tokushima, Tokushima 770-8503, Japan.

October 1998-March 2000

The production of lactoferrin-producing *Bacteroides uniformis* and its effect on carcinogen-induced colon carcinogenesis. In department of Bacteriology school of Medicine, The University of Tokushima, Tokushima 770-8503, Japan.

May 2001-July 2001

The immunomolating and anti-carcinogenic effects of lactoferrin and lactoferrin-producing *Bacteroides uniformis*. In department of Bacteriology school of Medicine, The University of Tokushima, Tokushima 770-8503, Japan.