

HANDBOOK OF MICROBIOLOGICAL
MEDIA FOR THE EXAMINATION OF
FOOD

238

238

H A N D B O O K O F

Microbiological
Media
FOR THE
Examination
OF
Food

RONALD M. ATLAS
University of Louisville



CRC Press

Boca Raton Ann Arbor London Tokyo



Library of Congress Cataloging-in-Publication Data

Atlas, Ronald M., 1946-

Handbook of microbiological media for the examination of food / by Ronald M. Atlas. p. cm.

Includes bibliographical references and index.

ISBN 0-8493-2704-0

1. Food--Microbiology--Laboratory manuals. 2. Culture media (Biology)--Laboratory manuals. I. Title.

QR115.A84 1995

576'.163'0287--dc20

94-44498

CIP

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Reasonable efforts have been made to publish reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage or retrieval system, without prior permission in writing from the publisher.

CRC Press, Inc.'s consent does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained in writing from CRC Press for such copying.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

© 1995 by CRC Press, Inc.

No claim to original U.S. Government works

International Standard Book Number 0-8493-2704-0

Library of Congress Card Number 94- 44498

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

Table of Contents

Introduction to Food Microbiology	1	Anaerobic TVLS Medium	25
Food- and Waterborne Pathogens	1	Andersen's Pork Pea Agar	26
Food Spoilage	5	Andrade's Broth	26
Microbiological Production of Food	7	Anthranilic Acid Medium, Revised	27
Alphabetical Listing of Media	11	Antibiotic Media 1-21	27-31
A 1 Broth	11	Antibiotic Sulfonamide Sensitivity Test Agar	31
ABY Agar	11	AOAC Letheen Broth	32
AC Agar and Broth	11	Apple Juice Yeast Extract Medium	32
ACC Medium	11	APT Agar and Broth	32
Acetamide Agar and Broth	12	Arginine Glucose Slants	33
Acetate Agar	12	Armstrong Fusarium Medium	33
Acetate Differential Agar	13	Ascospore Agar	33
Acetobacter Medium	13	ASLA Agar	34
Acetobacter xylinum Medium	13	Asparagine Broth	34
Achromobacter Choline Medium	13	Aspergillus Differential Medium	34
Achromobacter Medium	13, 14	AT5N Medium	34
Achromobacter pestifer Medium	14	Aureomycin® Rose Bengal Glucose Peptone Agar	34
Acid Broth	14	Azide Blood Agar	35
Acid Egg Medium	14	Azide Blood Agar with Crystal Violet	35
Acid Products Test Broth	14	Azide Broth	35
Acidic Tomato Medium for <i>Leuconostoc</i>	14	Azide Broth, Rothe	36
Acidophilic <i>Bacillus stearothermophilus</i> Agar	15	Azide Citrate Broth	36
Acidophilic <i>Bacillus stearothermophilus</i> Broth	15	Azide Medium	36
Actidione® Agar	15	B ₁₂ Assay Medium	36
Actinomyces Agar and Broth	15, 16	B ₁₂ Culture Agar, USP	37
Actinomyces Isolation Agar	16	B ₁₂ Inoculum Broth, USP	37
Actinomycete Growth Medium	16	<i>Bacillus</i> Agar and Broth	37, 38
Actinomycete Isolation Agar	17	<i>Bacillus</i> Agar and Broth 1/4 Strength	37, 38
AE Sporulation Medium, Modified	17	<i>Bacillus cereus</i> Medium	38
<i>Aeromonas</i> Differential Agar	17	<i>Bacillus cereus</i> Selective Agar Base	38
<i>Aeromonas hydrophila</i> Medium	18	<i>Bacillus coagulans</i> Medium	39
<i>Aeromonas</i> Medium	18	<i>Bacillus fastidiosus</i> Medium	39
AFPA	18	<i>Bacillus</i> Medium	39, 40
Agar Medium for Differential Enumeration of Lactic Streptococci	19	<i>Bacillus stearothermophilus</i> Broth	40
Agar Medium P	19	<i>Bacillus stearothermophilus</i> Sporulation Broth	41
AK Agar No. 2	20	<i>Bacillus</i> Xylose Salts	41
AKI Medium	20	BAGG Broth	41
Alcal Mannose Medium	20	Baird-Parker Agar	42
<i>Alcaligenes</i> Agar	20	Balamuth Medium	43
<i>Alcaligenes</i> Medium	20	Basal Synthetic Medium	43
<i>Alcaligenes</i> NB YE Agar and Broth	21	Base Layer Agar with Nutrient Overlay Agar	43
Alginate Utilization Medium	21	BC Motility Medium	44
Alkaline <i>Bacillus</i> Medium	22	BCP Azide Broth	44
Alkaline Peptone Agar	22	BCPD Agar	44
Alkaline Peptone Salt Broth	22	BCPDCLS Agar	45
Alkaline Peptone Water	22	BCYE Agar	45
Amino Acid Assay Medium	22	BCYE α with Alb	45
Anacker and Ordal Medium	23	BCYE α without L-Cysteine	46
Anaerobe Agar	23	BCYE Differential Agar	46
Anaerobic Agar and Broth	24	BCYE Selective Agar with CCVC	47
Anaerobic CNA Agar	24	BCYE Selective Agar with GPVA	47
Anaerobic Egg Yolk Agar	25	BCYE Selective Agar with GVPC	48

BCYE Selective Agar with PAC	48	<i>Campylobacter fetus</i> Selective Medium	69
BCYE Selective Agar with PAV	49	<i>Campylobacter</i> Isolation Agars A and B	70
Beef Extract Agar and Broth	49, 50	<i>Campylobacter</i> Selective Medium, Blaser-Wang	71
Beef Extract V	50	<i>Campylobacter</i> Selective Medium, Butzler's	71, 72
Beef Infusion Agar and Broth	50	<i>Campylobacter</i> Selective Medium, Karmali's	72
Beef Liver Medium for Anaerobes	50	<i>Campylobacter</i> Selective Medium, Preston's	73
BG Sulfa Agar	50	Carbohydrate Fermentation Broth	73
Bicarbonate Agar	51	Carbon Assimilation Medium	73
<i>Bifidobacterium</i> Medium	51	Casamino Acids Yeast Extract Broth	74
Bile Esculin Agar	51	Casamino Acids Yeast Extract Salts Broth, Gorbach	74
Bile Oxalate Sorbose Broth	51	Casein Agar	75
Bile Salts Brilliant Green Starch Agar	52	CCVC Medium	75
Bile Salt Gelatin Agar	53	Cellobiose Polymyxin Colistin Agar	75
Biotin Assay Medium	53	Cellobiose Polymyxin B Colistin Agar, Modified	76
Bismuth Sulfite Agar and Broth	53	Cereal Agar	76
BMPA- α Medium	54	Cetrimide Agar, USP and Non-USP	76
Bouillon Medium	54	Chapman Stone Agar	77
Bovine Albumin Tween TM 80 Medium, Ellinghausen and McCullough, Modified	55	Cheese Agar	77
Bovine Serum Albumin Tween TM 80 Agar and Broth	56	China Blue Lactose Agar	77
Bovine Serum Albumin Tween TM 80 Soft Agar	56	CHO Medium Base	77
Brain Heart Infusion	57	Cholera Medium TCBS	78
Brain Heart Infusion Broth	57	Chopped Liver Broth	78
<i>Brevibacterium</i> Medium	57, 58	Chopped Meat Carbohydrate Medium	78
Brewer Anaerobic Agar	58	Christensen Agar	78, 79
Brewer Thioglycollate Medium	58	Christensen Citrate Agar, Modified	79
Brilliant Green Agar	58	Christensen Citrate Sulfide Medium	79
Brilliant Green Agar with Sulfadiazine	59	Chromogenic Substrate Broth	79
Brilliant Green Agar, Modified	59	CIN Agar	80
Brilliant Green Bile Agar and Broth	59, 60	Citrate Medium, Koser's Modified	80
Brilliant Green Bile Broth with MUG	60	Clostridia Medium	80
Brilliant Green Broth	60	<i>Clostridium acidurici</i> Medium	80
Brilliant Green Phenol Red Agar	60	<i>Clostridium aerotolerans</i> Medium	81
Bromcresol Purple Broth	60	<i>Clostridium</i> Alginate Medium	81
Bromcresol Purple Dextrose Broth	61	<i>Clostridium aminobutyricum</i> Medium	81
Bromthymol Blue Agar	61	<i>Clostridium aminovalericum</i> Medium	82
<i>Brucella</i> Agar	61	<i>Clostridium botulinum</i> Isolation Agar	82
<i>Brucella</i> Agar with Vitamin K1	62	<i>Clostridium difficile</i> Agar	83
<i>Brucella</i> Anaerobic Blood Agar	62	<i>Clostridium perfringens</i> Agar, OPSP	84
<i>Brucella</i> Medium Base	62	<i>Clostridium</i> Selective Agar	84
<i>Brucella</i> Medium, Selective	62	Coagulase Agar Base	84
<i>Brucella</i> Selective Medium	63	Coagulase Mannitol Agar	84
Buffered Charcoal Yeast Extract Differential Agar	63	Coliform Medium	85
Buffered Peptone Water	63	Coliform Medium, Modified	86
Buffered S & H Agar and Broth	63, 64	Colonization Medium	86
BYEB	64	Congo Red Agar	86, 87
CAL Agar and Broth	64	Congo Red BHI Agarose Medium	88
<i>Campylobacter</i> Agar, Blaser's	65	Congo Red Magnesium Oxalate Agar	88
<i>Campylobacter</i> Agar, Skirrow's	65	Cooke Rose Bengal Agar	88
<i>Campylobacter</i> Blood-Free Selective Agar	65	Cooked Meat Medium	89
<i>Campylobacter</i> Charcoal Differential Agar	66	Cooked Meat Medium, Modified	89
<i>Campylobacter</i> Enrichment Broth	66-68	Corn Meal Agar	89
<i>Campylobacter fecalis</i> Medium	69		
<i>Campylobacter fetus</i> Medium	69		

Corn Meal Agar with Dextrose.....	90	Enrichment Broth, pH 7.3.....	108
Crossley Milk Medium.....	90	Enrichment Broth, pH 7.3 with Pyruvate.....	109
Crystal Violet Agar.....	90	Enrichment Broth for <i>Aeromonas hydrophila</i>	110
Crystal Violet Pectate Medium.....	90	<i>Entamoeba</i> Medium.....	110
CS Vitamin B ₁₂ Agar.....	90	Enteric Fermentation Base.....	110
CYE Agar.....	91	<i>Enterobacter</i> Medium.....	111
CYE Agar, Buffered.....	91	Enterococci Confirmatory Agar and Broth.....	111
Czapek Agar.....	92	Enterococci Presumptive Broth.....	111
Czapek Dox Agar with 3% Glucose.....	92	Enterococcosel™ Agar and Broth.....	112
Czapek Dox Agar, Modified.....	92	<i>Enterococcus</i> Agar.....	112
Czapek Dox Broth.....	92	Esculin Broth.....	112
Czapek Dox Liquid Medium, Modified.....	92	Esculin Iron Agar.....	113
Czapek Solution Agar.....	93	Esculin Mannitol Agar.....	113
Czapek Yeast Autolysate Agar.....	93	ETGPA.....	113
Davis and Mingioli Glucose Minimal Medium.....	93	Ethyl Violet Azide Broth.....	114
Davis and Mingioli Medium, Modified.....	93	Fay and Barry Medium.....	114
Davis and Mingioli Medium with B ₁ and Asparagine.....	94	FC Agar and Broth.....	115, 116
Davis and Mingioli Medium with Proline.....	94	Fecal Coliform Agar, Modified.....	116
Davis Supplemented Minimal Medium.....	94	Fermentation Broth.....	116
DCLS Agar.....	95	Fermentation Medium.....	117
Decarboxylase Base, Møller.....	95	F-G Agar and Broth.....	117, 118
Decarboxylase Medium Base, Falkow.....	95	F-G Agar with Selenium.....	117
Decarboxylase Medium, Ornithine Modified.....	96	fGTC Agar.....	118
Deoxycholate Agar.....	96	Fish Peptone Agar and Broth.....	119
Deoxycholate Citrate Agar.....	96, 97	<i>Flavobacterium</i> Medium.....	119, 120
Deoxycholate Citrate Agar, Hynes.....	97	<i>Flavobacterium</i> Medium M1.....	120
Dextrose Agar and Broth.....	97, 98	Fletcher Medium.....	120
Dextrose Tryptone Agar and Broth.....	98, 99	Fletcher Medium with Fluorouracil.....	120
Dichloran Glycerol Agar.....	99	Fletcher's Semisolid Medium.....	120
Differential Broth for Lactic Streptococci.....	99	FLN Medium.....	121
DNase Agar.....	100	Flo Agar.....	121
DNase Medium.....	100	Fluid Sabouraud Medium.....	121
DNase Test Agar.....	100	Fluid Thioglycollate Agar and Medium.....	121
DNase Test Agar with Methyl Green.....	100	Fluid Thioglycollate Medium with Beef Extract.....	122
DNase Test Agar with Toluidine Blue.....	100	Fluid Thioglycollate Medium without Glucose or E _h Indicator.....	122
Double-Strength Crude Medium for <i>Lactobacillus</i>	101	Fluid Thioglycollate Medium with K Agar.....	122
Doyle and Roman Enrichment Medium.....	101	Fluorescent Pectolytic Agar.....	122
DRBC Agar.....	101	FN Medium.....	123
Duncan-Strong Sporulation Medium, Modified.....	102	Folic Acid Agar.....	123
E Agar.....	102	Fraser Broth.....	123
EC Broth.....	102	Fraser Secondary Enrichment Broth.....	124
EC Broth with MUG.....	103	G Medium.....	124
EE Broth.....	103	GBNA Medium.....	124
EE Broth, Mossel.....	103	Gelatin Agar.....	125
Egg Yolk Agar.....	103	Gelatin Infusion Broth.....	125
Egg Yolk Agar, Modified.....	104	Gelatin Medium.....	125
Eijkman Lactose Medium.....	104	Gelatin Phosphate Salt Agar and Broth.....	125, 126
Elliker Agar and Broth.....	104, 105	Gelatin Salt Agar.....	126
EMB Agar.....	105	Gelatinase Test Medium.....	126
EMB Agar Base.....	105	Giolitti-Cantoni Broth.....	126
EMB Agar, Modified.....	106	Gluconate Peptone Broth.....	127
Endo Agar and Broth.....	106, 107	Glucose Broth.....	127
Endo Agar, LES.....	106, 107	Glucose Phosphate Broth.....	127
Enriched Nutrient Broth.....	107	Glucose Salt Teepol Broth.....	127
		Glucose Yeast Broth with NaCl.....	127

Glucose Yeast Extract Agar.....	128	Lactobacilli MRS Broth with Cysteine.....	144
Glucose Yeast Peptone Medium	128	Lactobacilli MRS Broth with Ethanol	144
GN Broth, Hajna.....	128	<i>Lactobacillus bifidus</i> Medium.....	144
GPVA Medium.....	128	<i>Lactobacillus bulgaricus</i> Agar.....	145
GSP Agar.....	128	<i>Lactobacillus</i> Heteroferm Screen Agar	145
Gum Tragacanth Gum Arabic Medium.....	129	<i>Lactobacillus</i> Heteroferm Screen Broth	146
H Agar and Broth.....	129	<i>Lactobacillus</i> Medium	146
H Medium	129	<i>Lactobacillus</i> Sake Medium	147
H Top Agar.....	129	<i>Lactobacillus</i> Selection Oxgall Agar	147
Halophilic Agar and Broth.....	129	Lactose Broth.....	147
Hektoen Enteric Agar.....	130	Lactose Egg Yolk Milk Agar.....	147
Hemorrhagic coli Agar.....	130	Lactose Gelatin Medium.....	148
HHD Medium	130	Lactose Ricinoleate Broth.....	148
HL Agar.....	130	Lambda Broth	148
Horie Arabinose Ethyl Violet Broth.....	131	Lauryl Sulfate Broth	148
HPC Agar.....	131	Lauryl Sulfate Broth with MUG.....	149
Hugh-Leifson's Glucose Broth.....	131	Lauryl Tryptose Mannitol Broth	
HY Agar for <i>Flavobacterium</i>	132	with Tryptophan.....	149
HY Medium for <i>Flavobacterium</i>	132	LB Agar.....	149
HYA Agar.....	132	LB Broth, Modified.....	149
Indole Medium.....	132	LB Medium.....	150
Indole Nitrite Medium	133	LB Medium for X1776	150
Infection Medium.....	133	LB Medium for X1776 with Tetracycline	
Infusion Broth	133	and Ampicillin	150
Irgasan® Ticarcillin Chlorate Broth.....	133	LB Medium with Ampicillin.....	151
Iron Agar, Lyngby	134	LB Medium with Chloramphenicol.....	151
Iron Milk Medium.....	134	LB Medium with Glucose.....	152
Iron Milk Medium, Modified.....	134	LB Medium with IPTG Medium	152
IsoVitalX® Enrichment	134	LB Medium with 25mg Kanamycin	152
J Agar and Broth	134, 135	LB Medium with Rifampicin.....	152
Jordan's Tartrate Agar	135	LB Medium with Tetracycline	152
Kanamycin Esculin Azide Agar and Broth	135	LB Medium with Tetracycline	
KC Bottom Agar	135	and Ampicillin	152, 153
KC Broth.....	136	LB Medium with TMP.....	153
KC Top Agar	136	LB Medium with TPP.....	153
KCN Broth.....	136	LB Top Agar.....	153
KF <i>Streptococcus</i> Agar and Broth	136, 137	LBE Medium	153
Kim-Goepfert Agar	137	LBS™ Agar and Broth.....	154
Kleb Agar	138	Lead Acetate Agar.....	154
<i>Klebsiella</i> Medium.....	138	Lecithin Lipase Anaerobic Agar	155
Kligler Iron Agar.....	139	Lee's Agar	155
KM Agar	139	<i>Legionella</i> Agar Base.....	155
Korthof Medium	140	<i>Legionella pneumophila</i> Medium	156
Korthof Medium, Modified.....	140	<i>Legionella</i> Selective Agar	156
Koser Citrate Medium.....	140	<i>Leptospira</i> Medium.....	157
Kranep Agar Base	141	<i>Leptospira</i> Medium, EMJH	157
L Medium.....	141	<i>Leptospira</i> Medium, Modified	157
L Medium for <i>Salmonella</i>	141	<i>Leptospira</i> Protein-Free Medium	157
L15 Medium, Modified Leibovitz.....	141	<i>Leuconostoc</i> Medium.....	158
Lab-Lemco Agar and Broth	142	Levine EMB Agar	158
Lactic Acid Bacteria Medium	142	LICNR Broth	158
Lactic Agar for Yogurt Bacteria, Modified	142	<i>Listeria</i> Enrichment Broth	159
Lactic Bacteria Broth.....	142	<i>Listeria</i> Enrichment Broth, FDA	159
Lactic Streak Agar.....	143	<i>Listeria</i> Enrichment Broth I and II,	
Lactobacilli Agar and Broth, AOAC.....	143	USDA FSIS	160
Lactobacilli MRS Broth.....	144	<i>Listeria</i> Fermentation Broth.....	161

<i>Listeria</i> Transport Enrichment Medium.....	161	Mannitol Maltose Agar	177
Litmus Lactose Agar	161	Mannitol Salt Agar and Broth	177, 178
Litmus Lactose Agar with Crystal Violet.....	161	Mannitol Yolk Polymyxin Agar	178
Litmus Milk	162	McBride Agar, Modified	178
Liver Broth.....	162	McBride <i>Listeria</i> Agar	178
Liver Broth with NaCl	162	McClung Carbon-Free Broth	179
Liver Veal Agar.....	163	McClung-Toabe Agar.....	179
Liver Veal Egg Yolk Agar	163	McClung-Toabe Agar, Modified	179, 180
Lowenstein-Gruft Medium.....	163	McClung-Toabe Egg Yolk Agar, CDC Modified	180
Lowenstein-Jensen Medium.....	164	MD Medium.....	181
Lowenstein-Jensen Medium with NaCl	164	Membrane Lauryl Sulfate Broth	182
Lowenstein-Jensen Medium without Glycerol.....	164	Methylene Blue Milk Medium.....	182
Lowenstein-Jensen Medium with Streptomycin	165	MH IH Agar	182
LPM Agar.....	165	Micro Assay Culture Agar	182
LPM Agar with Esculin and Ferric Iron	166	<i>Micrococcus</i> Medium	183
LS Differential Medium.....	166	<i>Micrococcus</i> Medium, FDA	183
Lysine Agar, Selective.....	166	MIL Medium.....	183
Lysine Arginine Iron Agar	167	Milk Agar	183
Lysine Decarboxylase Broth, Falkow	167	Minerals Modified Glutamate Agar	184
Lysine Decarboxylase Broth, Taylor Modification	167	Minerals Modified Medium	184
Lysine Decarboxylase Medium.....	168	MLCB Agar	184
Lysine Iron Agar	168	Modified Semi-Solid Rappaport Vassiliadis Medium	184
Lysine Medium	168	Møller Decarboxylase Broth.....	185
Lysine Ornithine Mannitol Agar	168	Møller KCN Broth Base	185
M Broth.....	169	Motility Indole Ornithine Medium	185
M16 Agar	169	Motility Medium	186
M17 Agar	169	Motility Medium S.....	186
M17 Broth.....	170	Motility Nitrate Agar.....	186
M56 Agar	170	Motility Nitrate Medium	186
M56 Medium	170	Motility Nitrate Medium, Buffered.....	187
MacConkey Agar	171	Motility Sulfide Medium.....	187
MacConkey Agar No. 2	171	Motility Test and Maintenance Medium	187
MacConkey Agar No. 3	171	Motility Test and Maintenance Medium, Gilardi.....	188
MacConkey Agar without Crystal Violet.....	172	Motility Test and Maintenance Medium, Tatum	188
MacConkey Agar without Salt.....	172	Motility Test Medium	188
MacConkey Agar, CS	172	Motility Test Medium, Semisolid	188
MacConkey Broth.....	172	MP 5 Medium	188
MacConkey Broth, Purple	173	MP 7 Medium	189
Magnesium Oxalate Agar	173	MPH Agar	189
Malachite Green Broth.....	173	MRS Agar and Broth	190
Maleate Medium for <i>Pseudomonas fluorescens</i>	173	MRVP Broth.....	190
Maleate Medium for <i>Pseudomonas fluorescens</i> with Glucose and Phenol Red.....	174	MRVP Medium	191
Malonate Broth	174	Mucate Broth	191
Malonate Broth, Ewing Modified	174	Mucate Control Broth	191
Malt Agar.....	174	MVTY Medium	191
Malt and Peptone Medium.....	175	MWY Medium	192
Malt Extract Agar and Broth.....	175, 176	<i>Mycobacterium</i> Medium.....	192
Malt Extract Yeast Extract 40% Glucose Agar ...	176	<i>Mycobacterium</i> Yeast Extract Medium	192
Manganese Nutrient Agar	176	Mycobactin Medium	193
Mannitol Agar	176	Mycobactosel™ Agar	193
Mannitol Egg Yolk Polymyxin Agar.....	177	Mycobactosel™ L-J Medium.....	194
		NBY Medium.....	194

Neopeptone Glucose Agar	195	Phenylalanine Agar	210
Nickels and Leesment Agar, Modified	195	Phenylalanine Malonate Broth	210
Nitrate Broth	195	Phenylethanol Agar	210
Nitrate Broth, <i>Campylobacter</i>	195	Plate Count Agar	211
Nitrate Broth, Enriched	196	Plate Count Agar, Modified	211
Nitrate Reduction Broth	196	Plate Count Agar, Special	211
Nutrient Agar	196, 197	Plate Count Agar with Antibiotic	211
Nutrient Agar, 1.5%	197	Plate Count Agar with Antibiotic-Free	
Nutrient Agar, pH 5.0	197	Skim Milk	211
Nutrient Agar, pH 6.0	197	Plate Count Broth	212
Nutrient Agar with 0.5% NaCl	197	PM Indicator Agar	212
Nutrient Agar with 0.5% NaCl and		PMP Broth	212
Sodium Citrate	198	Polypectate Gel Medium	212
Nutrient Agar with 3% NaCl	198	Polysorbate 80 Agar	212
Nutrient Agar with 10% NaCl	198	Potassium Cyanide Broth	213
Nutrient Agar with 10% NaCl and Maltose	198	Potassium Tellurite Agar	213
Nutrient Agar with Manganese	198	Potato Dextrose Agar and Broth	213, 214
Nutrient Agar with Phytone	199	Potato Dextrose Agar with Antibiotics	214
Nutrient Agar with Potato Starch	199	Potato Dextrose Agar with 2% Glucose	
Nutrient Broth	199	and 60% Sucrose	214
Nutrient Gelatin	199	Potato Extract Agar	214
NWRI Agar	200	Potato Flakes Agar	215
NZY Agar and Broth	200	Potato Malt Agar	215
Oatmeal Agar	200	Pre-Enrichment Medium	215
Orange Serum Agar	200, 201	Presence-Absence Broth	215
Orange Serum Broth Concentrate 10X	201	Preston Enrichment Broth	216
Organic Acid Medium KP	201	Pril Xylose Ampicillin Agar	216
Oxford Agar	201	Proteose Agar	216
Oxford Agar, Modified	202	Proteose No. 3 Agar	216, 217
Oxidative Fermentative Glucose Medium,		Proteose Yeast Extract Medium	218
Semisolid	202	<i>Pseudomonas aeruginosa</i> Agar	218
Oxidation Fermentation Medium	202	<i>Pseudomonas</i> Agar F	218
Oxidation Fermentation Medium,		<i>Pseudomonas</i> Agar P	218
Hugh-Leifson's	203	<i>Pseudomonas</i> Basal Mineral Medium	219
Oxidation Fermentation Medium, King's	203	<i>Pseudomonas</i> CFC Agar	219
Oxidative-Fermentative Glucose Medium,		<i>Pseudomonas</i> CN Agar	219
Semisolid, with NaCl	203	<i>Pseudomonas</i> Isolation Agar	220
Oxidative-Fermentative Medium	203	Purple Agar and Broth	220
Oxidative-Fermentative Test Medium	204	Purple Lactose Agar	221
Oxytetracycline Glucose Yeast Extract Agar	204	PY Medium with Glucose	221
P Agar	205	PYEX Glucose Sali Medium	222
PAC Agar	205	PYG Broth	222
Pai Medium	205	R2A Agar	222
PALCAM Agar	205	R3A Agar	222
Park and Sanders Enrichment Broth	206	Raka-Ray Agar	223
PE2 Medium	206	Rappaport Broth, Modified	223
Pectin Medium	207	Rappaport-Vassiliadis Enrichment Broth	223
Peptone Iron Agar	207	Rappaport-Vassiliadis R10 Broth	224
Peptone Medium	207	Rappaport-Vassiliadis Soya Peptone Broth	224
Pfizer Selective <i>Enterococcus</i> Agar	207	Reinforced Clostridial Agar	224
Phenol Red Agar and Broth	207, 208	Reinforced Clostridial Medium	224
Phenol Red Glucose Broth	208	Reinforced Clostridial Medium with	
Phenol Red Lactose Agar and Broth	208, 209	Sodium Lactate	225
Phenol Red Mannitol Agar and Broth	209	Rimler-Shotts Medium	225
Phenol Red Sucrose Broth	209	Rippey-Cabelli Agar	225
Phenol Red Tartrate Agar and Broth	209, 210	Rogosa Agar	226

Rogosa SL Agar and Broth	226	Standard Methods Broth	243
Rose Bengal Chloramphenicol Agar	227	Standard Methods Caseinate Agar	243
Russell Double Sugar Agar	227	<i>Staphylococcus</i> Agar No. 110	243
SA Agar	227	<i>Staphylococcus</i> Broth	243
Sabouraud Agar, Modified	227	<i>Staphylococcus</i> Medium	244
Sabouraud Maltose Agar and Broth	228	<i>Staphylococcus</i> Medium No. 110	244
Sabouraud Medium, Fluid	228	<i>Staphylococcus-Streptococcus</i>	
Saccharolytic Clostridia Medium	228	Selective Medium	244
<i>Salmonella</i> Medium	228	Starch Agar	244
<i>Salmonella Shigella</i> Agar	229	Starch Hydrolysis Agar	245
<i>Salmonella Shigella</i> Agar, Modified	229	<i>Streptococcus</i> Medium	245
Salt Broth, Modified	229	<i>Streptococcus</i> Selective Medium	245
Salt Colistin Broth	229	<i>Streptococcus thermophilus</i> Agar	245
Salt Meat Broth	230	Stuart <i>Leptospira</i> Broth, Modified	245
Salt Polymyxin Broth	230	Sucrose Agar	246
Salt Tolerance Medium	230	Syncase Broth	246
Salt Tolerance Medium, Gilardi	231	T ₁ N ₀ Broth	246
Salt Tolerance Medium, Tatum	231	T ₁ N ₁ Agar and Broth	247
SBG Enrichment Broth	231	T ₁ N ₂ Agar	247
SBG Sulfa Enrichment	231	T ₁ N ₃ Broth	247
Schleifer-Krämer Agar	231	T ₁ N ₆ Broth	247
Sea Water Agar	232	T ₁ N ₈ Broth	247
Selenite Broth	232	T ₁ N ₁₀ Broth	247
Selenite Broth Base, Mannitol	232	T7 Agar Base	248
Selenite Cystine Broth	233	Tap Water Agar	248
Selenite F Broth	233	Tarshis Blood Agar	248
Serum Glucose Agar	233	TB Nitrate Reduction Broth	248
Serum Glucose Agar, Farrell Modified	233	TCBS Agar	248
Serum Potato Infusion Agar	234	Teepol Broth, Enriched	249
Seven H11 Agar	235	Tellurite Glycine Agar	249
Seven Hour Fecal Coliform Agar	235	Tergitol 7 Agar and Broth	249, 250
SF Broth	235	Tetrathionate Broth	250, 251
SFP Agar	236	Tetrathionate Broth, Hajna	252
<i>Shigella</i> Broth	236	Tetrathionate Broth, USA	252
SIM Medium	237	Tetrathionate Broth with Novobiocin	252
Simmons' Citrate Agar	237	Tetrathionate Crystal Violet	
Single Layer Agar	237, 238	Enhancement Broth	253
Skim Milk Agar	238	Thermoacidurans Agar	253
Skirrow <i>Brucella</i> Medium	238	Thioglycollate Broth USP, Alternative	253
SL Medium	239	Thioglycollate Medium, Brewer Modified	253
Slanetz and Bartley Medium	239	Thioglycollate Medium, USP	254
Sodium Chloride Broth, 6.5	239	Thioglycollate Medium with 20% Bile	254
Sodium Dodecyl Sulfate Polymyxin		Thioglycollate Medium without	
Sucrose Agar	239	Glucose and Indicator	254
Sodium Lactate Agar	240	Thioglycollate Medium without Indicator	254
Sodium Lactate Agar, Modified	240	TNT Medium	255
Soft Agar Gelatin Overlay	240	Toluidine Blue DNA Agar	255
Sour Dough Medium	240	Tomato Juice Agar and Broth	255, 256
Spirit Blue Agar	241	Tomato Juice Agar Special	256
Spore Strip Broth	241	Tomato Juice Medium	256
Sporulation Broth	241	Tomato Juice Yeast Extract Milk Medium	257
Spray's Fermentation Medium	241	TPEY Agar	257
SPS Agar	242	TPGY Medium	257
SS Deoxycholate Agar	242	Tributylin Agar	257
ST Holding Medium	242	Trimethylamine N-Oxide Medium	258
STAA Agar Base	242	Triple Sugar Iron Agar	258

Introduction to Food Microbiology

Food products serve not only as sources of nutrition for humans and other animals but also as substrates for the growth of microorganisms. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizable losses of food products. Some disease-causing microorganisms also contaminate foods and potable waters. Foodborne and waterborne pathogens remain a major source for the spread of disease worldwide. A major concern in food microbiology is the preservation of food products and the prevention of foodborne and waterborne spread of disease. Careful quality control in the food industry is essential for preventing outbreaks of foodborne disease and for controlling food spoilage. Many foods are routinely examined for the presence of disease-causing and food spoilage microorganisms. Many of these quality control procedures involve the cultivation of microorganisms using a variety of media.

Food- and Waterborne Pathogens

The ingestion of foods containing toxins and human pathogens can cause a variety of diseases. It is essential to prevent contamination of food products with human pathogens and to control the potential proliferation of toxin-producing microorganisms, which can result in food poisoning and the transmission of foodborne pathogens. In some cases, the growth of pathogenic microorganisms in a food is accompanied by obvious signs of spoilage, such as the production of gas or foul-smelling compounds, which give a clear indication that the product should not be eaten. In other cases, there are no obvious signs of spoilage to indicate the presence of pathogens or toxins. It is therefore necessary to carry out inspection programs aimed at detecting the possible contamination of food products with pathogenic microorganisms. As a consequence of the high quality control procedures employed in the food industry, few occurrences of foodborne disease are traced back to the food processor. Rather, most outbreaks of food poisoning are caused by improper post process handling of the food, often as a result of improper preparation and/or handling at home or in a food service setting.

Quality control laboratories in the food industry routinely perform tests to detect pathogens in food products and to ensure that the numbers and types of microorganisms associated with a food are not likely to cause serious food spoilage and/or health problems. In some cases, quality control procedures are aimed at detecting the presence of specific microorganisms, but in most cases the tests consist of exam-

ining the food for indicator organisms. For example, coliform counts are routinely performed on representative samples of many food products as an indication of possible fecal contamination, since food contaminated with fecal material has a relatively high probability of containing human pathogens. Foods such as hamburger often have 100,000 total bacteria per gram, but as long as they do not contain any *Salmonella* or other pathogens, they are considered safe for human consumption. In the United States, agencies involved in the surveillance and regulation of foods are the Department of Agriculture (USDA), the Food and Drug Administration (FDA), and state boards of health. Standards based on the presence or absence of pathogens, such as those set by Congress and regulated by the USDA and FDA, do provide the safeguards needed for assurance of the safety of food products.

Microorganisms routinely enter the gastrointestinal tract in association with ingested food and water. The large resident microbiota that develops in the human intestinal tract after birth is important for the maintenance of good health and is usually not involved in disease processes. This microbiota is normally noninvasive and is associated with the surface tissues and ingested food material. Some pathogenic microorganisms, however, possess toxigenic or invasive properties that permit them to cause disease when they enter the gastrointestinal tract.

There are two distinct processes that can initiate disease through the gastrointestinal tract. In the first type, microorganisms growing in food or water can produce toxins, and their ingestion initiates a disease process. Such diseases are classified as food poisoning or intoxication because the etiological agents of the disease need not grow within the body; that is, there is no true infectious process. Toxins absorbed through the gastrointestinal tract can cause neural damage and death in some cases, as well as localized inflammation and gastrointestinal upset in others. In the second type of disease-causing process, invasive pathogens establish an initial infection through the gastrointestinal tract and cause localized gastrointestinal upset or systemic disease symptomatology. Generally, the establishment of infection through the gastrointestinal tract requires a relatively large infectious dose; that is, a relatively large number of pathogenic microorganisms is required to successfully overcome the inherent defense mechanisms of the gastrointestinal tract. Quite different measures are required to prevent and treat infectious gastrointestinal diseases compared to those for specific microorganisms responsible for food poisoning.

Many outbreaks of gastroenteritis involve the food-borne or waterborne transmission of viruses that can replicate within the human gastrointestinal tract and cause an inflammation of the lining of the gastrointestinal tract. Contamination of food with fecal matter is an important route of transmission of viral gastroenteritis. Several different viruses cause viral gastroenteritis, including adenoviruses, coxsackieviruses, polioviruses, and members of the ECHO virus group. The Norwalk agent, a small DNA virus identified as being responsible for an outbreak of "winter vomiting disease" that occurred in Norwalk, Ohio, in 1968, appears to be an important etiological agent of various viral gastroenteritis outbreaks. Rotavirus, a large RNA virus, also appears to be a very common etiological agent of diarrhea in infants, particularly in socioeconomically depressed regions of the world. It is difficult to detect the diversity of viruses causing this disease and foods and waters are only rarely examined for their presence.

Hepatitis is a systemic viral infection that primarily affects the liver, caused by several types of hepatitis viruses, designated types A, B, C, D, and non-A, non-B. Type A hepatitis virus is normally associated with foodborne and waterborne cases of hepatitis. Type A hepatitis virus normally enters the body via the gastrointestinal tract and causes infectious hepatitis. Although there are some documented cases of foodborne hepatitis B, types B, D and non-A, non-B hepatitis viruses, these viruses normally enter the body through skin punctures and cause serum hepatitis. Hepatitis type A virus is usually transmitted by the fecal-oral route and is prevalent in areas with inadequate sewage treatment. Several outbreaks of viral hepatitis have been associated with contaminated shellfish, such as oysters, that have concentrated viruses from sewage effluents. There have also been outbreaks from vegetables contaminated with infected fecal matter used as fertilizer. Contaminated potable water supplies are probably the most common source of hepatitis A viral infections.

Polioviruses may enter the body through either the gastrointestinal or respiratory tracts. Transmission through the ingestion of food and water containing polioviruses is considered very important. Polio is effectively controlled through the use of vaccines. This has been responsible for the decline of this disease, particularly in developed nations. Global vaccination programs are aimed at eliminating this disease worldwide.

Several *Clostridium* species cause foodborne disease. Canned foods are often the sources of clostridia causing human disease because *Clostridium* species are endospore forming anaerobes.

Hence they are resistant to elevated temperatures, sometimes surviving the canning process high temperature killing, and can grow in the can because of the absence of air. Botulism, the most serious form of bacterial food poisoning, is caused by neurotoxins produced by *Clostridium botulinum*. The toxins are absorbed from the intestinal tract and transported via the circulatory system to motor nerve synapses, where their action blocks normal neural transmissions. Various strains of *C. botulinum* elaborate different toxins. Types A, B, and E toxins cause food poisoning of humans. Type E toxins are associated with the growth of *C. botulinum* in fish or fish products, and most outbreaks of botulism in Japan are caused by type E toxins because large amounts of fish are consumed there. Type A is the predominant toxin in cases of botulism in the United States, and type B toxin is most prevalent in Europe. *C. botulinum* cultures form three distinct physiological groups that can be detected by culture methods. *C. botulinum* types C and D are nonproteolytic and do not digest coagulated egg white or meat. All type A and some type B and F strains of *C. botulinum* are proteolytic whereas all type E strains and the remaining type B strains are nonproteolytic. Type G strains of *C. botulinum* show slow proteolysis. Optimum temperature for toxin production by proteolytic strains is approximately 35°C and for nonproteolytic strains is about 27°C. Nonproteolytic strains of *C. botulinum* types B, E, and F produce toxin at 4°C.

Over 90 % of the cases of botulism involve improperly home-canned food. Of 236 outbreaks of this disease in the United States between 1899 and 1974, 57 % were caused by contaminated vegetables, 15 % by contaminated fish, and 12 % by contaminated fruit. Certain canned foods provide an optimal anaerobic environment for the growth of *C. botulinum* that results in the release of toxin into the food. *C. botulinum*, though, cannot grow and produce toxin at low pH and thus is not a problem in acidic food products. Another clostridial species, *Clostridium perfringens*, is a major cause of a less severe form of food poisoning. *C. perfringens* generally accounts for over 10 % of the outbreaks of foodborne disease in the United States. The ingestion of food containing toxin produced by *C. perfringens* and the adsorption of the toxin into the cells lining the gastrointestinal tract initiate this disease. Toxin type A of *C. perfringens* is associated with most cases of clostridial food poisoning, particularly with cooked meats if a gravy is prepared with the meat. The spores of *C. perfringens* type A can survive the temperatures used in cooking many meats, and if incubated in a warm gravy, there is sufficient time for the spores to germinate and the

growing bacteria to produce enough toxin to cause this disease.

Bacillus cereus is responsible for a relatively mild form of gastroenteritis, and recovery normally occurs in less than a day. The occurrence of gastroenteritis due to *B. cereus* requires the ingestion of a large number of spores. The symptoms include abdominal pain, profuse diarrhea, and nausea. *B. cereus* foodborne disease most commonly is associated with custards, cereal products, and meat gravies.

Strains of *Staphylococcus aureus* produce a toxin that causes an inflammation of the lining of the gastrointestinal tract. *S. aureus* can reproduce within many different types of food products. Enterotoxin-producing strains of *S. aureus* often enter foods from the skin surfaces of people who handle food. Custard-filled bakery goods, dairy products, processed meats, potato salad, and various canned foods are frequently found to be the source of the toxin. The prevention of staphylococcal food poisoning depends on proper handling and preservation of food products to prevent contamination and subsequent growth of enterotoxin-producing strains of *Staphylococcus*.

Listeria monocytogenes infects cattle, sheep, and chickens. It is transmitted to humans via contaminated milk and poultry and fecally-contaminated water and vegetables. Unpasteurized milk often is the source of human infection and cheeses have been a major source of *Listeria* infections in the United States. Identification of *Listeria monocytogenes* is based upon observation of Gram-positive rods that exhibit tumbling motility, utilization of lactose, catalase positive and oxidase negative reactions, exculin and sodium hippurate hydrolysis, Methyl Red positive reaction, ammonia production from arginine, and negative reactions for hydrogen sulfide production, nitrate reduction, gelatin liquefaction, starch hydrolysis, and urea hydrolysis.

Various *Salmonella* species, including *S. cholerae-suis* and especially the numerous serotypes of *S. enteritidis*, are commonly the etiological agents of salmonellosis. Like many enteropathogenic bacteria, *Salmonella* species have pili that enable them to adhere to the lining of the gastrointestinal tract. Although *Salmonella* species are able to reproduce within the intestines, causing inflammation, they do not normally penetrate the mucosal lining and enter the bloodstream; in some cases, however, *Salmonella* species can gain access to the circulatory system, causing bacteremia. For example, paratyphoid fever, which is caused by strains of *S. paratyphi* and *S. typhimurium*, is characterized by gastroenteritis and a relatively high rate of bacteremia. *Salmonella*

species causing gastroenteritis are normally transmitted by ingestion of contaminated food. Birds and domestic fowl, especially ducks, turkeys, and chickens, including their eggs, are commonly identified as the sources of *Salmonella* infections. Inadequate cooking of large turkeys and the ingestion of raw eggs cause a significant number of cases of salmonellosis. Outbreaks of typhoid fever, a systemic infection caused by *Salmonella typhi*, are associated with contaminated water supplies and the handling of food products by individuals infected with this bacterium. A relatively low infectious dose is required for *S. typhi* to establish an infection. Some individuals develop a carrier state; such individuals do not develop typhoid fever but act as a source of *S. typhi* and spread the disease as in the infamous case of a cook in the early twentieth century who became known as typhoid Mary.

Shigellosis, or bacterial dysentery, is an acute inflammation of the intestinal tract caused by species of the Gram-negative genus *Shigella*, including *S. flexneri*, *S. sonnei*, and *S. dysenteriae*. Water and food supplies are involved in some outbreaks of bacterial dysentery. The severe dehydration associated with this disease can cause shock and lead to death in children, in whom the incidence of bacterial dysentery is highest.

Campylobacter fetus var. *jejuni* has been found to be the causative agent of many cases of gastroenteritis in infants. In fact, *C. fetus* may be more important in juvenile gastroenteritis than *Salmonella* species. The transmission of *C. fetus* appears to be via contaminated food or water. *C. fetus* is a Gram-negative, motile, spiral-shaped bacterium, formerly known as *Vibrio fetus*, which also causes fetal abortion in cattle and sheep.

Several *Vibrio* species, including *V. cholerae*, *V. parahaemolyticus*, *V. mimicus*, and *V. vulnificus* cause foodborne and waterborne diseases. *Vibrio* species are Gram negative straight or curved rods (single curve) and ferment glucose without producing gas. *V. vulnificus* is most often associated with seafoods as a source of foodborne infections. *V. parahaemolyticus* is responsible for many cases of gastroenteritis in Japan and perhaps in the United States. It occurs in marine environments, and the ingestion of contaminated seafood, particularly the eating of raw fish, is the main route of transmission. Gastroenteritis caused by *V. parahaemolyticus* requires the establishment of an infection within the gastrointestinal tract, rather than simple ingestion of an enterotoxin. The symptoms generally appear 12 hours after ingestion of contaminated food and include abdominal pain, diarrhea, nausea, and vomiting. Recovery from this form of gastro-

enteritis normally occurs in 2 to 5 days, and the mortality rate is very low.

In contrast, cholera, which is caused by *Vibrio cholerae*, serotypes *cholerae* and *El Tor*, often is fatal. Although we typically associate cholera with Asia, sometimes referring to the disease as *Asiatic cholera*, it also occurs in the United States, primarily in the Gulf Coast region, where cases have been traced to contaminated shellfish. Cholera is now endemic to South America where eating of contaminated raw fish is a major source of infection. Contaminated water supplies are also responsible for the geographic spread of cholera from the initial focus in Peru throughout Latin America. Cholera is a particular problem in socioeconomically depressed countries, where there is poor sanitation and inadequate sewage treatment and where medical facilities have only a limited capacity to deal with outbreaks. This disease is endemic in the Ganges delta, and there are annual epidemic outbreaks of cholera in India and Bangladesh. In these endemic areas of Asia the death rate is normally 5 to 15 %. Seasonal outbreaks of cholera often occur in Southeast Asia when monsoon rains wash sewage material into drinking water supplies. During sudden epidemics, the mortality rate may reach 75 %.

Yersinia enterocolitica and related species, such as *Y. pseudotuberculosis*, produce a severe form of enterocolitis. Outbreaks of yersiniosis are most common in Western Europe but have also been confirmed in the United States. *Y. pseudotuberculosis* is found in pork, raw milk, and various other foods. *Y. enterocolitica* is widely distributed and has been found in water, milk, fruits, vegetables, and seafoods. This organism is psychrotrophic and thus is able to reproduce within refrigerated foods, where it can multiply and reach an infectious dose. In fact, *Y. enterocolitica* grows better at 25°C than at 37°C.

Coliform bacteria, principally *Escherichia coli*, are widely used as indicators of human fecal contamination of food and water. When used in this manner *E. coli* is not screened as a pathogen but rather because it is found in high numbers in human feces and is relatively short lived in the environment. Finding *E. coli* in food or potable water indicates contamination with human fecal matter that may also carry pathogens such as *Salmonella* and *Shigella*. Some *E. coli* strains, however, are human pathogens. These are designated enterotoxigenic or enteropathogenic *E. coli* if they produce toxins or can invade the body through the gastrointestinal tract.

Enterotoxin-producing strains of *Escherichia coli* are also capable of causing both mild and severe forms of gastroenterocolitis. In most cases, entero-

toxin-producing strains of *E. coli* do not invade the body through the gastrointestinal tract; rather, toxin released by cells growing on the surface lining of the gastrointestinal tract causes diarrhea. Aside from diarrhea, abdominal cramps are normally the only other clinical symptom of this disease. Travelers from the United States to Mexico often suffer severe diarrhea as a result of ingestion of strains of *E. coli* foreign to their own microbiota and therefore generally avoid drinking the water. Many cases of severe diarrhea in children are caused by noninvasive, enterotoxin-producing strains of *E. coli*. In some cases, enteropathogenic strains of *E. coli* invade the body through the mucosa of the large intestine to cause a serious form of dysentery. Invasive strains of *E. coli* are primarily associated with contaminated food and water in Southeast Asia and South America. The ability to invade the mucosa of the large intestine depends on the presence of a specific K antigen in enteropathogenic serotypes of *E. coli*. The enterotoxins produced by *E. coli* cause a loss of fluids from intestinal tissues. With proper replacement of body fluids and maintenance of the essential electrolyte balance, infections with enterotoxic *E. coli* normally are not fatal.

E. coli O157:H7 has emerged as an important foodborne and waterborne pathogen. This strain of *E. coli* causes hemorrhagic colitis and serious kidney disease. It frequently is fatal. Outbreaks of disease caused by infections with *E. coli* O157:H7 have most often been traced to contaminated undercooked hamburger meat. The U.S. Food and Drug Administration recommends thorough cooking of hamburger meat. Improved surveillance of meats for bacterial contamination is being implemented by the FDA.

Arizona hinshawii is associated with poultry, and products containing eggs such as custards. It causes gastroenteritis that generally lasts a few days. *Aeromonas hydrophila* has been the source of some foodborne disease outbreaks in the United States. It is ubiquitous in water and can infect various animals, including frogs. *Plesiomonas shigelloides* has been implicated in several foodborne disease outbreaks involving fish and shellfish. It can grow on minimal media containing ammonium salts as the nitrogen source and glucose as the carbon source. Infections cause gastrointestinal upsets.

Tuberculosis, which is caused by *Mycobacterium tuberculosis* and related mycobacterial species, can be transmitted by the ingestion of contaminated food. Before the extensive use of pasteurization, milk contaminated with *M. tuberculosis* was associated with outbreaks of this disease. The dairy indus-

try routinely screens milk for the presence of mycobacteria, such as *M. bovis* that commonly infects cattle and can also cause human disease.

Legionella species from potable waters can cause human infections when aerosols are inhaled. Most outbreaks of Legionnaire's disease have been traced to air-conditioning cooling systems. *Legionella* bacteria multiply in the cooling system waters, which are rapidly evaporated to provide cooling, and inadvertently become airborne and circulate through the air-conditioning system. In other cases potable waters, including aerosols from dental instruments and shower heads, are the source of *Legionella* infections. There have also been outbreaks of Legionnaire's disease due to contaminated aerosols used to moisten produce and keep fruits and vegetables fresh.

Mycotoxins produced by some fungi are responsible for serious cases of food poisoning. Many mycotoxins are potent neurotoxins. Various species of mushrooms contain toxins that can be absorbed through the gastrointestinal tract, and the ingestion of poisonous mushrooms, such as *Amanita phalloides*, is normally fatal. The amatoxins and phallotoxins produced by *A. phalloides* and other species of *Amanita* cause symptoms of food poisoning 8 to 24 hours after their ingestion. Initial symptoms include vomiting and diarrhea; later, degenerative changes occur in liver and kidney cells, and death may ensue within a few days of ingesting as little as 5 to 10 mg of toxin.

Some filamentous fungi, other than mushrooms, also produce toxins that can cause human disease. *Aspergillus* species growing on peanuts and grains produce aflatoxins, which are potent carcinogens, as well as toxic. They are known to cause death in sheep and cattle and may be involved in some human disease conditions. Aflatoxins are the only known carcinogens for which the United States government has set permissible levels; all other products with carcinogenic activity are banned outright.

Ergotism, another disease caused by fungi, results from ingesting grain containing ergot alkaloids produced by *Claviceps purpurea*. The toxins of *C. purpurea* cause degeneration of the capillary blood vessels, and this type of food poisoning has a relatively high mortality rate. Symptoms of ergotism may include vomiting, diarrhea, thirst, hallucinations, convulsions, and lesions of the extremities. Various outbreaks of mass hallucinations have been traced to contamination of food with ergot alkaloids, and there are even theories that the Salem witch hunts in colonial Massachusetts were related to grain contamination and widespread ergotism.

Algae are rarely considered as the etiological agents of disease, but paralytic shellfish poisoning is caused

by toxins produced by dinoflagellate *Gonyaulax*. Blooms of *Gonyaulax* cause red tides in coastal marine environments. During such algal blooms, the algae and the toxins they produce can be concentrated in bivalve shellfish, such as clams and oysters. The ingestion of shellfish containing algal toxins can lead to symptoms that resemble those of botulism. Shellfishing is banned in areas of *Gonyaulax* blooms to prevent this form of food poisoning.

Amebic dysentery or amebiasis is caused by the protozoan *Entamoeba histolytica*. Infections with *E. histolytica* may be asymptomatic or may involve mild or severe diarrhea and abdominal pain. Amebic dysentery occurs as a result of inadequate sewage treatment and contamination of water with *E. histolytica*, whose cysts are not killed by the chlorination methods normally used to treat municipal drinking water. Infection is acquired by ingesting contaminated food or water containing cysts of *E. histolytica*.

Giardia lamblia, a flagellated protozoan, is responsible for most cases of diarrhea infection caused by protozoa. The cysts of *G. lamblia* can enter the gastrointestinal tract through contaminated water. A high incidence of giardiasis occurred among groups touring Leningrad during the 1970s as a result of contaminated water supplies. In 1973 a major outbreak of giardiasis occurred in upstate New York, with an estimated 4,800 individuals developing symptoms of the disease. The following year, giardiasis was the most common waterborne disease in the United States. *G. lamblia* can live saprophytically within the small intestine without causing any symptoms of giardiasis, and in the United States almost 4 % of the population appears to be infected by this organism. Excessive growth of the organism, however, can cause disease symptoms that include diarrhea, dehydration, mucus secretion, and flatulence.

Cryptosporidium is a newly emerging pathogen that has caused major outbreaks of waterborne disease in the United States. A 1993 outbreak of cryptosporidiosis in Milwaukee infected over 400,000 individuals. Excessive growth of this protozoan in municipal water supplies is the source of infection. New surveillance requirements are being implemented to prevent future major outbreaks of *Cryptosporidium* caused disease.

Food Spoilage

The growth of microorganisms in foods can alter the quality of the product, causing sizable economic losses to the food industry due to food spoilage. Food spoilage is a change in a food that renders it undesirable or unsafe for human consumption, and the growth of microorganisms in a food represents

only one process that may cause food to spoil. The growth of pathogenic microorganisms in a food is certainly undesirable, as it can make that food unsafe to eat, but other microbially induced changes in food, such as decreased nutritional content and altered taste, odor, color, and texture, can also make a food undesirable for human consumption. Such food spoilage is difficult to define because it depends on the culture of the consumer; for example, Eskimos bury fish to produce a stinky mess and in Britain meat should have the strong aroma of aging, but these same foods would likely be rejected as spoiled in most parts of the United States.

Foods are classified according to their susceptibility to microbial spoilage as nonperishable, semiperishable, or perishable. Perishable foods, such as meats, fish, poultry, most fruits and vegetables, eggs, and milk, readily spoil because of microbial activities and generally have short shelf lives unless steps are taken to remove, kill, or prevent the growth of associated microorganisms. Semiperishable foods, such as potatoes and apples, generally remain unspoiled for prolonged periods of time unless improperly handled. Nonperishable foods, such as sugar, flour, and numerous dry products, normally do not spoil, but even these foods can spoil under appropriate conditions. For example, if cereals, grains, and flours are stored under conditions of high moisture, various fungal and bacterial populations are able to grow and spoil the products. We have all observed the growth of fungi on bread that has been stored too long.

The changes that occur in a food during microbial spoilage depend on the particular microbial populations involved, their enzymatic activities, environmental conditions, and the nature of the food. The microorganisms involved in the spoilage process generally do not originate within the food, the inner tissues of most plants and animals being sterile, but rather come from the surface tissues or are a result of contamination during processing. Several factors controlling food spoilage are intrinsic to the food, but others are extrinsic environmental parameters. The most important extrinsic factors influencing food spoilage are (1) temperature of storage; (2) relative humidity; and (3) oxygen concentration. By controlling these parameters, it is possible to alter the rate of food spoilage. It is somewhat more difficult to control the inherent properties of animal and plant tissues that influence microbial spoilage of foods. The intrinsic moisture content, pH, and physical and chemical nature of the food, in part, control the numbers and types of microorganisms involved in the spoilage process. Fruits, vegetables, meats, poultry, seafoods, milk and dairy products, and various other food products differ in their biochemical

composition and therefore are subject to spoilage by differing microbial populations.

Fruits and vegetables are subject to rot because of the microbial degradation of pectin, the biochemical responsible for maintaining the firmness and texture of fruits and vegetables. Microbially produced pectinesterases and polygalacturonases hydrolyze pectins, resulting in the formation of soft spots in fruits and vegetables. About 20 % of the harvested crops of fruits and vegetables are lost to spoilage primarily because of the activities of bacteria and fungi. Carbohydrates, which are present in high concentrations in fruits, vegetables, and other foods, are readily degraded by numerous microorganisms, resulting in the production of various degradation products, such as low molecular weight acids and alcohols. The accumulation of such products of microbial metabolism can alter the taste of a food, generally causing it to sour. The taste of sour milk, for example, is associated with the accumulation of lactic acid from the microbial transformation of carbohydrates. The accumulation of low molecular weight acids may also alter the smell of the product; sour milk often can be recognized just by smelling it. In canned foods, production of acid and no gas is referred to as flat-sour spoilage because the food becomes sour, but the can shows no evidence of food spoilage because no gas is produced; that is, the can remains flat. When gas is produced during spoilage of canned foods, the can swells, as can be seen by examining the lids, which are designed to push outward if pressure builds within the can because of microbial action. Thermophilic microorganisms, that is, those that can withstand exposure to elevated temperatures, and especially endospore formers, are important in the spoilage of canned foods.

Meats and other proteinaceous products can be decomposed by anaerobic bacteria, resulting in putrefaction. Putrefaction of meat is the result of the breakdown of proteins by proteinases. The subsequent degradation of amino acids produces foul-smelling, low molecular weight sulfur- and nitrogen-containing compounds, such as mercaptans, hydrogen sulfide, ammonia, and amines. The evolution of noxious, odoriferous compounds from putrefying proteins renders food unacceptable for human consumption. The characteristic odor of hydrogen sulfide, for example, renders rotting eggs inedible, and the development of off odors and slime on poultry and beef reflects the presence of increased microbial populations and spoilage of the meat. In canned foods such spoilage is referred to as sulfide stinkers. Under aerobic conditions, the decomposition of proteins generally does not result in the production of compounds with obnoxious odors. The spoilage of

meat under aerobic conditions, though, can result in the accumulation of surface slime, generally because of the growth of *Pseudomonas*, *Achromobacter*, *Streptococcus*, *Leuconostoc*, *Bacillus*, *Micrococcus*, and *Lactobacillus* species. The physical state of the meat influences which microbial species may be involved in spoilage. The spoilage of fresh whole meats is normally associated with lactic acid bacteria, particularly *Lactobacillus*, *Leuconostoc*, and *Streptococcus* species, whereas the spoilage of ground beef is primarily due to the growth of *Pseudomonas*, *Achromobacter*, and *Micrococcus*.

Spoilage by microbial degradation of fats and oils produces rancidity, caused by oxidation or hydrolysis of lipids. Spoiled butter, for example, becomes rancid because of the hydrolysis of butterfat, with the production of free fatty acids and glycerol and the accompanying development of undesirable flavors. Fish with high lipid levels, such as salmon and mackerel, may also become rancid because of the hydrolysis of fats and oils.

The growth of some microorganisms can alter the color of the food. For example, the growth of *Serratia marcescens* can produce bright red bread; the combined growth of *Pseudomonas* species and *Streptococcus lactis* produces blue milk; the growth of fungi on meat surfaces can produce off colors, such as black spot caused by *Cladosporium herbarum*, white spot caused by *Sporotrichium carnis*, and green patches produced by *Penicillium* species; and the growth of heterolactic fermenters on cured meats, such as frankfurters, causes greening because of the action of peroxidases on the meat pigments. The development of unnatural colors in a food reduces its acceptability, regardless of whether there is any associated development of an off taste or smell. Microbial growth can also alter the texture of a food. For example, the growth of *Bacillus* species in products such as milk and dough can produce ropiness, a textural change resulting from the growth of encapsulated *Bacillus* species together with the hydrolysis of starch and proteins.

Microbiological Production of Food

Although microbial growth is a problem when it results in food spoilage, microorganisms are used beneficially in the food industry for food production. Many of the foods and beverages we commonly enjoy, such as wine and cheese, are the products of microbial enzymatic activity. For the most part, it is the fermentative metabolism of microorganisms that is exploited in the production of food products. The accumulation of fermentation products, such as ethanol and lactic acid, is desirable because of their characteristic flavors and other properties. Only a

few processes, such as the production of vinegar, make use of microbial oxidative metabolism. The microbial production of foods can be viewed as an exercise in harnessing microbial biochemistry to produce desired, rather than adverse, changes in food products.

The production of fermented foods requires the proper substrates, microbial populations, and environmental conditions to obtain the desired end product. Quality control is essential in food fermentation to ensure that the product is of high quality. A fermented food may require additional preservation to prevent spoilage because further uncontrolled microbial growth could render it inedible. For example, once wine is produced, it must be maintained under anaerobic conditions in order to prevent its oxidation to vinegar.

The microbial processes used in food production traditionally employ microbial enzymatic activities to transform one food into another, with the microbially produced food product having properties vastly different from those of the starting material. In addition to the use of microorganisms to produce fermented food products, microbial biomass is now considered a potential source of protein for meeting the food needs of an expanding world population. Some microorganisms, such as mushrooms, have been used as food products for centuries. The growth of bacteria, algae, and fungi as proteinaceous food, however, is a relatively new concept. Microbial biomass can be used as an animal feed supplement or may be developed as a direct source of protein for human consumption.

Numerous products are made by the microbial fermentation of milk, including buttermilk, yogurt, and many cheeses. The fermentation of milk is primarily carried out by lactic acid bacteria. The lactic acid fermentation pathway and the accumulation of lactic acid from the metabolism of the milk sugar lactose are common to the production of fermented dairy products. The accumulated lactic acid in these products acts as a natural preservative. The differences in the flavor and aroma of the various fermented dairy products are due to additional fermentation products that may be present in only relatively low concentrations.

Different fermented dairy products are produced by using different strains of lactic acid bacteria as starter cultures and different fractions of whole milk as the starting substrate. Sour cream, for example, uses *Streptococcus cremoris* or *S. lactis* for the production of lactic acid, and *Leuconostoc cremoris* or *S. lactis diacetylactis* for the production of the characteristic flavor compounds. Cream is the starting