



**Sharif University of Technology**

# **BBRC CULTURE COLLECTION**

**Biochemical and Bioenvironmental Eng. Research Center**

**Culture Collection**

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# **IN THE NAME OF GOD**



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## **Introduction**

The Biochemical and Bioenvironmental Eng. Research Center (BBRC) was established in 1970 at Sharif University of Technology, to conduct and perform research on problems of national importance in the fields of Biochemical and Bioenvironmental Engineering. BBRC (Biochemical and Bioenvironmental Eng.), BBRC strain collection is a merge of local collection located at BBRC , Sharif university of Technology ready to supply all the relevant services both to the public and private sectors. Research Center Culture collection located at Biochemical and bioenvironmental eng. Research center as a collection of industrial, biochemical and educational importance since BBRC establishment. The BBRC collection has an on-going concern with all aspects of culture collection activity and, in particular, with the encouragement of new initiatives and improvement of the quality standards of scientific services provided to the national user community **The main objectives** of BBRC culture collection are to act as a national depository, to supply authentic microbial cultures and to provide related services to the scientific community working in research institutions, universities and industries. The increasing demands on culture collection for authenticated, reliable biological material and associated information have paralleled the growth of biotechnology. At present the collection contains about 200 strains including bacteria and fungi usable for industrial and biochemical and educational services. About one third of the collection belong the strains screened and isolated from native sources.

Microbial cultures are usually supplied freeze – dried in glass ampules.

This site permits users to request strains also orders could be demanded by fax at: 982166005417.

The center focuses on basic research in the area of microbial isolation and preparation in freeze – dried samples. Our total strains number are 108 including: Bacteria, Fungi and Screened and Partially Characterized Strains.

### **Freeze Dry a Microbial Culture**

One of best ways to store a bacterial, fungal, yeast or other microorganism culture for long period of time is to use a process called lyophilization or freeze drying it. This short laboratory procedure can be used with any commercially available freeze drier, for preservation of culture collections.

**Difficulty:** Easy

**Time Required:** 3 – 24 hours not including culture growth time.

### **Method**

1. Grow the microorganism on Liquid Broth (LB) or other appropriate nutrient plate or tubes.
2. Prepare sterile crimp-cap vials by autoclaving ahead of time with the caps (rubber stopper) placed loosely on top. Place paper labels printed with the culture identification inside the tubes prior to autoclaving. Alternatively use tubes with caps designed for sterility.
3. Add 4 ml lyophilization buffer to the plate. If necessary, the cells can be suspended using a sterile glass rod.
4. Quickly transfer the culture suspension to the sterilized vials. Add approximately 1.5 ml per vial. Seal with the rubber cap.
5. Freeze the culture suspension inside the vials by placing the vials in minimum a – 20 degrees' Celsius freezer.

6. Once cultures are frozen, prepare the freeze drier by turning it on and allowing time for the appropriate temperature and vacuum conditions to stabilize. Of course this is done according to the manufacturer's instructions for the particular brand of freeze drier you are using.
7. Carefully and aseptically place the vial caps loosely on top of the vials, so moisture can escape during the freeze drying process and place the vials into a freeze drier chamber. Apply the vacuum to the chamber according to the manufacturer's instructions.
8. Allow the culture time to completely lyophilize (dry out). This may take anywhere from a couple hours to overnight depending on the volume of each sample and how many samples you have.
9. Remove the samples from the freeze drier chamber according to the manufacturer's instructions and immediately seal the vials with the rubber caps, then the crimp tops.
10. Store the lyophilized culture collection at 4°C not exposed to light.

### **What you need**

- Freeze drier
- Nutrient or other appropriate agar plates (and incubator to grow culture)
- Glass rod
- Lyophilization buffer
- Crimp – top vials with rubber stoppers (and crimper apply the caps)
- Freezer

### **Revival of cultures**

- Carefully opening the ampoules.
- About 0.4 – 0.5 ml suitable medium or physiological serum should be added to the ampoules and the content mixed.

- The suspension should be sub – cultured into a few suitable solid or liquid media.
- Subcultures should be incubated at optimum temperature.
- Resuscitated freeze – dried cultures tend to exhibit a lengthened lag period of time.

**SECTION A**

**COLLECTION OF BACTERIA**



## Fully Characterized Strains

### Bacteria

10001-*Acetobacter*

- Medium for growth: 1
- Incubation temperature: 26 °C

\*\*\*\*\*

10004 – *Bacillus cereus*

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10005 – *Bacillus coagulans*

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10006- *Bacillus licheniformis*

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10009 – *Bacillus subtilis* subsp. *spizizenii*

ATCC-6633

Deposited as *Bacillus subtilis*. Quality control strain .Used for assay of different antibiotics, media and antibacterial activity testing, bactericides and sterility testing. Produces restriction endonuclease Bsu6633I.

- Medium for growth: 1

- Incubation temperature: 30 °C

\*\*\*\*\*

10016 – *Corynebacterium glutamicum*

ATCC 13032

Type strain, production of L-glutamic acid

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10019 – *E coli*

ATCC – 11105

- Medium for growth: 4
- Incubation temperature: 37 °C

\*\*\*\*\*

10028 – *Micrococcus luteus*

ATCC – 4698

Detection of aerosols

Produces 6-aminopenicillanic acid

Produces L-Aspartyl-L-phenylalanine esters

Quality control strain

Sensitive to lysozyme

Quality control strain for BBL products

- Medium for growth: 8
- Incubation temperature: 30 °C

\*\*\*\*\*

10035 – *Pseudomonas*

- Medium for growth: 1
- Incubation temperature: 30°

\*\*\*\*\*

10036 – *Pseudomonas aeruginosa*

RPMC – B -3556

Production of lipase.

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10040 – *Serratia marcesens*

- Medium for growth: 1
- Incubation temperature: 26 °C

\*\*\*\*\*

10042 – *Streptomyces erythreus*

Type strain, production of erythromycin

- Medium for growth: 9
- Incubation temperature: 26 °C

\*\*\*\*\*

10045 – *Methylobacterium extorquens*

DSMZ – 1337

ATCC 43645

- Medium for growth: 7
- Incubation temperature: 30 °C

\*\*\*\*\*

10047 – *Geobacillus thermoleovorans*

DSMZ – 5366

ATCC- 43513

Utilizes hydrocarbons.

- Medium for growth: 1
- Incubation temperature: 60 °C

\*\*\*\*\*

10048 – *Streptomyces fradia*

DSMZ – 40705

Produces neomycin.

- Medium for growth: 10
- Incubation temperature: 28 °C

\*\*\*\*\*

10049 – *Hyphomicrobium facile* subs.*facile*

DSMZ – 1565

ATCC 27485

- Medium for growth: 5
- Incubation temperature: 30 °C

\*\*\*\*\*

10050 – *Staphylococcus aureus*

Assay of chlortetracycline aureomycin, demeclocycline ,demethylchlortetracycline, oxytetracycline, terramycin, tetracycline ,tobramycin, tylosin. Reference strain. Testing of iodophores.

- Medium for growth: 8
- Incubation temperature: 37 °C

\*\*\*\*\*

10053- *Bacillus licheniformis*

Production of protease.

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10054- *Bacillus sphaericus*

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

10055 – *Pseudomonas putida*

ATCC – 12633

Degrades aromatic acids

Hydrolyzes d,L-alpha-amino acid amides

Metabolizes 2-ketogluconate 2-ketogluconic acid

Metabolizes beta-ketoadipate

Bacteriophage host

- Medium for growth: 1
- Incubation temperature: 26 °C

\*\*\*\*\*

10056 – *Streptomyces rimasus*

ATCC – 10970

Produces Rimocidin [Reg TM]

Produces oxytetracycline terramycin

Produces tetracyclines

Transformation of uleine

- Medium for growth: 9
- Incubation temperature: 26 °C

\*\*\*\*\*

10057 – *Alcaligenes eutrophus*

ACM -1296

Derived from strain ATCC 17699, produces poly - B – hydroxy butyric acid

- Medium for growth: 2
- Incubation temperature: 28°C

\*\*\*\*\*

10058 - *Streptomyces clavuligerus*

DSMZ – 41826

Produces cephamycin C, clavulanic acid.

- Medium for growth: 10
- Incubation temperature: 28 °C

\*\*\*\*\*

10059 – *Bifidobacterium bifidum*

DSM 20456

Isolated from stool of breast-fed infant. Type strain

- Medium for growth: 3
- Incubation temperature: 37 °C

\*\*\*\*\*

10060 - *E coli*

ATCC 11303

Readily produces clonal mutants, Phage host, produces L-asparaginase, production of aspartate transaminase

- Medium for growth: 4
- Incubation temperature: 37 °C

\*\*\*\*\*

10061 – *Enterobacter cloacae*

- Medium for growth: 1
- Incubation temperature: 37 °C

\*\*\*\*\*

10062 – *Lactobacillus acidophilus*

DSM 20079

Isolated from human. Type strain. Produces hydrogen peroxide.

- Medium for growth: 6
- Incubation temperature: 37 °C

\*\*\*\*\*

10063 – *Xanthomonas campestris*

DSM- 1706

Produces xanthan, continuous fermentation

- Medium for growth: 13
- Incubation temperature: 26 °C

\*\*\*\*\*

10064 - Sulfate-Reducing Bacteria

- Medium for growth: 11
- Incubation temperature: 30 °C

\*\*\*\*\*

10065 – *Thiobacillus thioparus*

DSM – 5368

Isolated from activated sludge, Japan.

Degradation of volatile organic sulfur compounds (carbon disulfide, methane thiol ,dimethyl sulfide, dimethyl disulfide)

- Medium for growth: 12
- Incubation temperature: 30 °C

\*\*\*\*\*

10066 – *Bacillus* Sp.

DSMZ-2523

ATCC- 31006

Produces cyclodextrins (U.S. Pat. 3,923,598).

- Medium for growth: 26
- Incubation temperature: 30 °C

\*\*\*\*\*

10067 – *Lactobacillus plantarum*

DSMZ – 20205

Assay of amino acids, Arginine and tryptophan, biotin ,calcium pantothenate, niacin, nicotinic acid, pantothenic acid; AOAC methods 43.117-43.187, 1980;

- Medium for growth: 27
- Incubation temperature: 37 °C

\*\*\*\*\*

10068 – *Lactobacillus Plantarum* subsp . *Plantarum*

ATCC 14917

Isolated from pickled cabbage. Type strain. Quality control strain.



- Medium for growth: 27
- Incubation temperature: 30 °C with %5 CO<sub>2</sub>

\*\*\*\*\*

10069 – *Lactobacillus rhamnosus*

ATCC – 7469

Assay of folic acid

Assay of niacin amide nicotinamide

Assay of riboflavin vitamin B2

Produces folate transport protein

Food testing

- Medium for growth: 27
- Incubation temperature: 37 °C

\*\*\*\*\*

10070 – *Natronorubrum tibetense*

DSMZ – 13204

Isolated from sediment (pH 10, 18 °C)

- Medium for growth: 22
- Incubation temperature: 37 °C

\*\*\*\*\*

10071 – *Halorubrum vacuolatum*

DSMZ -8800

- Medium for growth: 22
- Incubation temperature: 37°C

\*\*\*\*\*

10072 – *Natronorubrum gregoryi*

DSMZ – 3393

ATCC- 43098

Isolated from solar salt works liquor

- Medium for growth: 22
- Incubation temperature: 37°C

\*\*\*\*\*

10073 – *Haloferax mediterranei*

DSMZ – 1411

ATCC -33500

Isolated from solar salt pond.

Grows with single carbon sources on defined inorganic media.

- Medium for growth: 23 and 24
- Incubation temperature: 37°C

\*\*\*\*\*

10074 – *Natrialba magadii*

DSMZ – 3394

ATCC- 43099

- Medium for growth: 22
- Incubation temperature: 37°

\*\*\*\*\*

10075 – *Natronococcus occultus*

DSMZ – 3396

ATCC- 43101

- Medium for growth: 22

- Incubation temperature: 37°C

\*\*\*\*\*

10076 – *Halobacterium salinarum*

ATCC– 33171

- Medium for growth: 25

- Incubation temperature: 35°C

\*\*\*\*\*

10077- *Gluconobacter oxydans* subsp.suboxydans ( *Acetobacter suboxydans* )

Assay of p-aminobenzoic, nicotinic, pantothenic acids, and production of sorbose

- Medium for growth: 28

- Incubation temperature: 26°C

\*\*\*\*\*

10078- *Lysinibacillus macroides*

NCBI- AJ628749

Isolated by BBRC for Sulfur Oxidation

- Medium for growth: 1

- Incubation temperature: 30°C

\*\*\*\*\*

10079- *Brevundimonas naejangsanensis*

GenBank- FJ544245

Isolated by BBRC for Sulfur Reduction

- Medium for growth: 1

- Incubation temperature: 30°C

\*\*\*\*\*

10080- *Pseudomonas aeruginosa*

Isolated by BBRC for Diesel Removal

- Medium for growth: 1

- Incubation temperature: 30°C

\*\*\*\*\*

**1. Fully Characterized Strains**  
**1.1. Bacteria**

<b>No</b>	<b>Species</b>	<b>BBRC NO</b>	<b>Cross Reference</b>
1.	<i>Acetobacter</i>	10001	-
2.	<i>Alcaligenes eutrophus</i>	10057	ACM-1296
3.	<i>Bacillus cereus</i>	10004	-
4.	<i>Bacillus coagulans</i>	10005	-
5.	<i>Bacillus licheniformis</i>	10006	-
6.	<i>Bacillus licheniformis</i>	10053	-
7.	<i>Bacillus Sp.</i>	10066	DSMZ- 2523
8.	<i>Bacillus sphaericus</i>	10054	-
9.	<i>Bacillus subtilis</i>	10009	ATCC-6633
10.	<i>Bifidobacterium bifidum</i>	10059	-
11.	<i>Corynebacterium glutamicum</i>	10016	ATCC-1532
12.	<i>E coli</i>	10019	ATCC-11105
13.	<i>E coli</i>	10060	-
14.	<i>Enterobacter cloacae</i>	10061	-
15.	<i>Geobacillus thermoleovorans</i>	10047	DSMZ-5366
16.	<i>Hyphomicrobium facile subs.facile</i>	10049	DSMZ-1565
17.	<i>Lactobacillus acidophilus</i>	10062	-

18.	<i>Lactobacillus plantarum</i>	10067	DSMZ- 20205
19.	<i>Lactobacillus plantarum subsp. plantarum</i>	10068	DSM 20174
20.	<i>Lactobacillus rhamnosus</i>	10069	ATCC -7469
21.	<i>Methylobacterium extorquens</i>	10045	DSMZ-1337
22.	<i>Micrococcus luteus</i>	10028	ATCC-4698
23.	<i>Pseudomonas</i>	10035	-
24.	<i>Pseudomonas aeruginosa</i>	10036	RPMC- B-3556
25.	<i>Pseudomonas putida</i>	10055	ATCC-12633
26.	<i>Serratia marcesens</i>	10040	-
27.	<i>Staphylococcus aureus</i>	10050	-
28.	<i>Streptomyces erythreus</i>	10042	-
29.	<i>Streptomyces fradia</i>	10048	DSMZ- 40705
30.	<i>Streptomyces rimasus</i>	10056	ATCC-10970
31.	<i>Streptomyces clavuligerus</i>	10058	DSMZ-41826
32.	<i>Sulfate-reducing bacteria</i>	10064	
33.	<i>Thiobacillus thioparus</i>	10065	DSM- 5368
34.	<i>Xanthomonas campestris</i>	10063	-
35.	<i>Gluconobacter oxydans subsp.suboxydans ( Acetobacter suboxidans )</i>	10077	-
36.	<i>Lysinibacillus macroides</i>	10078	NCBI- AJ628749

37.	<i>Brevundimonas naejangsanensis</i>	10079	Gen Bank- FJ 544245
38.	<i>Pseudomonas aeruginosa</i>	10080	Isolated by BBRC for Diesel Removal

### 1.1.2 Halophilic strains

No	Species	BBRC NO	Cross Reference	Medium
1	<i>Natronorubrum tibetense</i>	10070	DSMZ- 13204	<a href="#">Medium 371</a> , 37°C
2	<i>Halorubrum vacuolatum</i>	10071	DSMZ- 8800	<a href="#">Medium 371</a> , 37°C
3	<i>Natronobacterium gregoryi</i>	10072	DSMZ- 3393	<a href="#">Medium 371</a> , 37°C
4	<i>Haloferax mediterranei</i>	10073	DSMZ- 1411	<a href="#">Medium 97</a> , 37°C Or <a href="#">Medium 372</a> , 37°C
5	<i>Natrialba magadii</i>	10074	DSMZ- 3394	<a href="#">Medium 371</a> , 37°C
6	<i>Natronococcus occultus</i>	10075	DSMZ- 3396	<a href="#">Medium 371</a> , 37°C
7	<i>Halobacterium salinarum</i>	10076	ATCC- 33171	<a href="#">ATCC medium 217</a> , 35°C

**SECTION B**

**COLLECTION OF MOLDS AND YEASTS**



## Molds and Yeasts

20001 – *Aspergillus flavus*

Production of aflatoxin B1

- Medium for growth: 14
- Incubation temperature: 25 °C

\*\*\*\*\*

20002 – *Aspergillus foetidus*

ATCC 16878

Production of pectic enzyme.

- Medium for growth: 15
- Incubation temperature: 25 °C

\*\*\*\*\*

20003 – *Aspergillus niger*

NCIM – 548

- Medium for growth :14
- Incubation temperature: 25 °C

\*\*\*\*\*

20004 - *Aspergillus niger*

ATCC 9029

Production of glucono-delta-lactone and gluconic acid ,aldonic acid, citric acid, lincomycin sulfoxides, urease.

Degrades pulp and paper-mill wastewater

- Medium for growth: 14
- Incubation temperature: 25 °C

\*\*\*\*\*

20005 - *Aspergillus niger*

ATCC 9142

Produces citric acid, L-malic acid, gluconic acid ,hydroxylated steroids, grindelane dimers, hydroxygrindelanes, and lipase.

Degrades acronine acronycine, apple pomace ,brewery wastes, cotton wastes, nulin, and molasses.

- Medium for growth: 14
- Incubation temperature: 25 °C

\*\*\*\*\*

20006 - *Aspergillus niger*

- Medium for growth: 14
- Incubation temperature: 25 °C

\*\*\*\*\*

20009 – *Penicillium chrysogenum*

ATCC 12687

Produces high yield of nonpigmented penicillin (in submerged culture)

- Medium for growth: 18
- Incubation temperature: 26°C

\*\*\*\*\*

20011 –*Rhizopus oryzae*

ATCC 9363

Produces lactic acid, fumaric acid; NAD-dependent lactate dehydrogenase; amylolytic enzymes and ergostadietriols useful in lowering serum cholestrol levels.

Used in tempeh fermentation. Source of lactate dehydrogenase genes used in the construction of expression vectors.

- Medium for growth: 14
  - Incubation temperature: 25°C
- \*\*\*\*\*

20013 – *Trichoderma koningii*

Isolated from agricultural soil.

Cellulase production

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

20014 – *Trichoderma Longibrachiatum*

Cellulase production

- Medium for growth: 20
- Incubation temperature: 25°C

\*\*\*\*\*

20015 – *Trichoderma reesei*

ATCC 13631

Fungus resistance testing plastics

Produces endoglucanase

Produces glucan 1,3-beta-glucosidase beta-D-1,3-glucanase, exo-1,3-beta-glucanase, exo-1,3-beta-glucosidase

Produces glucose D-glucose

Produces glucose by enzymatic hydrolysis of cellulose

Produces: cell-wall lytic enzymes

- Medium for growth: 14

- Incubation temperature: 25°C

\*\*\*\*\*

20018 - *Aspergillus niger*

Singapour

- Medium for growth: 14
- Incubation temperature: 25 °C

\*\*\*\*\*

20019 – *Penicillium simplicissimum*

Singapour

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

20020 – *Phanerochaete chrysosporium*

ATCC – 24725

Bacterial resistance testing adhesives

Type culture of *Sporotrichum pulverulentum*.

Produces ligninase and peroxidase, glyoxal oxidase, lignin peroxidase, extracellular peroxidase, xylanase.

Decolorization of textile-industry waste water; UV and benomyl resistant

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

30006 – *Saccharomyces cerevisiae*

- Medium for growth: 16
- Incubation temperature: 25°C

\*\*\*\*\*

30009 – *Debaryomyces hansenii* var. *hansenii*

CBS -767

ATCC- 36239

Ferments xylose D-xylose

Produces xylitol

- Medium for growth: 17
- Incubation temperature: 25 °C

\*\*\*\*\*

30010 – *Pichia stipitis*

CBS -7126

- Medium for growth: 17
- Incubation temperature: 25°C

\*\*\*\*\*

30012 – *Zygosaccharomyces rouxii*

DSM- 2531

- Medium for growth: 21
- Incubation temperature: 25°C

\*\*\*\*\*

30013 – *Kluyveromyces marxianus*

DSM -5420

Produces miso

Transformation host

- Medium for growth: 16
  - Incubation temperature: 25°C
- \*\*\*\*\*

30014 – *Pichia guilliermondii*

DSMZ - 11947

ATCC- 90887

Assay of Itraconazole, fluconazole, amphotericin B (9251).

- Medium for growth: 19
  - Incubation temperature: 25°C
- \*\*\*\*\*

30015 – *Candida albicans*

ATCC- 10231

Used for assay of amphotericin B fungi zone, antimicrobial preservatives, haloprogin, nystatin fungicide. Quality control strain. Used for media testing, membrane filter testing, Sterility testing, Fungicide testing.

Produces D-arabinolactone oxidase, DNA topoisomerase, aspartic proteinases, aspartyl proteinases, estrogen-binding protein, lanosterol synthase, 2,3-oxidosqualene lanosterol cyclase, phenethyl alcohol, polyamine oxidase, tryptophol.

- Medium for growth: 16
  - Incubation temperature: 25 °C
- \*\*\*\*\*

30015 – *Pichia jadinii* (*Candida utilis*)

DSMZ – 2361

Produces adenosyl-2-methyl methionine, adenosyl-D-methyl methionine, yeast protein.

Degrades ribonucleic acid (RNA)

- Medium for growth: 16
- Incubation temperature: 25°C

\*\*\*\*\*

20021 - *Aspergillus niger* var. *tubingensis*

ATCC 10864

Produces saccharifying enzymes; glucoamylase, large amounts of maltase and smaller amounts of alpha-amylase. Produces ethanol from potato starch when co cultured with *saccharomyces cerevisiae* ATCC 26603, acetyesterase, acetyl-xylan esterase, mono amin oxidase, dextranase; lipase.

- Medium for growth: 18
- Incubation temperature: 25 °C

## 1.2. Molds and Yeasts

No	Species	BBRC NO	Cross Reference
1	<i>Aspergillus flavus</i>	20001	-
2	<i>Aspergillus foetidus</i>	20002	-
3	<i>Aspergillus niger</i>	20003	NCIM-548
4	<i>Aspergillus niger</i>	20004	-
5	<i>Aspergillus niger</i>	20005	-
6	<i>Aspergillus niger</i>	20006	-
7	<i>Aspergillus niger</i>	20018	سنگاپور
8	<i>Candida albicans</i>	30016	-
9	<i>Debaryomyces hansenii</i> var. <i>hansenii</i>	30009	ہلند CBS-767
10	<i>Kluyvero myces marxianus</i>	30013	-
11	<i>Penicillium chrysogenum</i>	20009	-
12	<i>Penicillium simplicissimum</i>	20019	سنگاپور
13	<i>Phanerochaete chrysosporium</i>	20020	-ATCC-24725
14	<i>Pichia guilliermondii</i>	30014	DSMZ- 11947 ATCC- 90887
15	<i>Pichia jadinii (Candida utilis )</i>	30015	- DSMZ - 2361
16	<i>Pichia stipitis</i>	30010	ہلند CBS-7126
17	<i>Rhizopus oryzae</i>	20011	-
18	<i>Saccharomyces cerevisiae</i>	30006	-
19	<i>Trichoderma koningil</i>	20013	-
20	<i>Trichoderma longibrachiatum</i>	20014	-
21	<i>Trichoderma reesei</i>	20015	-



22	<i>Zygosaccharomyces rouxii</i>	30012	-
23	<i>Aspergillus niger var. tubingensis</i>	20021	ATCC -10864

**SECTION C**

**COLLECTION OF SCREENED AND PARTIALLY  
CHARACTERIZED STRAINS**

## Screened and Partially Characterized Strains

9001 – Yeast

TOC Removal

- Medium for growth: 17
- Incubation temperature: 25°C

\*\*\*\*\*

9002 – Fungi

PAH Removal

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

9003 – Bacteria

PAH Removal

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9004 – Bacteria

Furfural Treatment

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9006 – Bacteria

Sulfur Removal

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9007 – Bacteria

Sulfur Removal

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9008 - Bacteria

Sulfur Removal

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9012 – Bacteria

Asphaltene Removal

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9013 – Bacteria

Oil Emulsifier Production

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9014 – Bacteria

Oil Emulsifier Production

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9015 - Bacteria

Oil Emulsifier Production

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9016 – Bacteria

Sulfur Removal

- Medium for growth: 1
- Incubation temperature: 45 °C

\*\*\*\*\*

9018 - Bacteria

Sulfur Removal

- Medium for growth: 1
- Incubation temperature: 30 °C

\*\*\*\*\*

9019 – Bacteria

- Medium for growth: 1
- Incubation temperature: 50°C

\*\*\*\*\*

9020 - Bacteria

- Medium for growth: 1
- Incubation temperature: 50°C

\*\*\*\*\*

9021 - Bacteria

- Medium for growth: 1
  - Incubation temperature: 50°C
- \*\*\*\*\*

#### 9022 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 50°C
- \*\*\*\*\*

#### 9023 - Bacteria

- Medium for growth: 1
  - Incubation temperature: 50°C
- \*\*\*\*\*

#### 9026 – Yeast

- Medium for growth: 16
  - Incubation temperature: 30°C
- \*\*\*\*\*

#### 9048 - Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°C
- \*\*\*\*\*

#### 9050 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 60°C
- \*\*\*\*\*

#### 9052 – Fungi

- Medium for growth: 14
- Incubation temperature: 30°C

\*\*\*\*\*

9056 – Bacteria

- Medium for growth: 1
- Incubation temperature: 37°C

\*\*\*\*\*

9057 – Fungi

- Medium for growth: 14
- Incubation temperature: 45°C

\*\*\*\*\*

9058 - Fungi

- Medium for growth :14
- Incubation temperature: 45°C

\*\*\*\*\*

9059 – Bacteria

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9060 – Bacteria

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9061 – Bacteria

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9062 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°
- \*\*\*\*\*

9063 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°
- \*\*\*\*\*

9064 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°
- \*\*\*\*\*

9065 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°
- \*\*\*\*\*

9066 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 30 °C °
- \*\*\*\*\*

9067 – Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°C
- \*\*\*\*\*

9068 – Bacteria

- Medium for growth: 1
- Incubation temperature: 30°C



\*\*\*\*\*

9069 – Bacteria

- Medium for growth: 1
- Incubation temperature: 30°C

\*\*\*\*\*

9070 - Fungi

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

9071- Fungi

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

9072- Fungi

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

9073- Fungi

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

9074- Fungi

- Medium for growth: 14
- Incubation temperature: 25°C

\*\*\*\*\*

9075- Fungi

- Medium for growth: 14
  - Incubation temperature: 25°C
- \*\*\*\*\*

#### 9077- Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°C
- \*\*\*\*\*

#### 9078- Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°C
- \*\*\*\*\*

#### 9079- Bacteria

- Medium for growth: 1
  - Incubation temperature: 30°C
- \*\*\*\*\*

## **2. Screened and Partially Characterized Strains**

<b>No</b>	<b>Species</b>	<b>BBRC NO</b>	<b>Cross Reference</b>
1	Yeast	9001	TOC Removal
2	Fungi	9002	PAH Removal
3	Bacteria	9003	PAH Removal
4	Bacteria	9004	Furfural Treatment
5	Bacteria	9006	Sulfur Removal
6	Bacteria	9007	Sulfur Removal
7	Bacteria	9008	Sulfur Removal
8	Bacteria	9012	Asphalthene Removal
9	Bacteria	9013	Oil Emulsifier production
10	Bacteria	9014	Oil Emulsifier production
11	Bacteria	9015	Oil Emulsifier production
12	Bacteria	9016	Sulfur removal
13	Bacteria	9018	Sulfur removal
14	Bacteria	9019	Oil Emulsifier production
15	Bacteria	9020	Oil Emulsifier production
16	Bacteria	9021	Oil Emulsifier production
17	Bacteria	9022	Oil Emulsifier production
18	Bacteria	9023	Oil Emulsifier production

19	Yeast	9026	PAH removal
20	Bacteria	9048	Sulfur removal
21	Bacteria	9050	Sulfur removal
22	Fungi	9052	Sulfur removal
23	Bacteria	9056	Petroleum Oil removal
24	Fungi	9057	Thermophylic Fungi for oil removal
25	Fungi	9058	Thermophylic Fungi for oil removal
26	Bacteria	9059	Diesel Removal
27	Bacteria	9060	Diesel Removal
28	Bacteria	9061	Diesel Removal
29	Bacteria	9062	Diesel Removal
30	Bacteria	9063	Diesel Removal
31	Bacteria	9064	Diesel Removal
32	Bacteria	9065	Diesel Removal
33	Bacteria	9066	Diesel Removal
34	Bacteria	9067	Diesel Removal
35	Bacteria	9068	Diesel Removal
36	Bacteria	9069	Diesel Removal
37	Fungi	9070	Lignolytic Enzyme producers Fungi
38	Fungi	9071	Lignolytic Fungi
39	Fungi	9072	Lignolytic Fungi
40	Fungi	9073	Lignolytic Fungi

41	Fungi	9074	Lignolytic Fungi
42	Fungi	9075	Lignolytic Fungi
43	Bacteria	9077	Cyanide Removal
44	Bacteria	9078	Diesel Removal
45	Bacteria	9079	Cyanide Removal From Soil

## **SECTION D**

# **NUMERICAL INDEX OF BBRC STRAINS**

## NUMERICAL INDEX OF BBRC STRAINS

### Bacteria

10001	<i>Aceto bacter</i>
10004	<i>Bacillus cereus</i>
10005	<i>Bacillus coagulans</i>
10006	<i>Bacillus licheniformis</i>
10009	<i>Bacillus subtilis</i>
10016	<i>Corynebacterium glutamicum</i>
10019	<i>E coil</i>
10028	<i>Micrococcus luteus</i>
10035	<i>Pseudomonas</i>
10036	<i>Pseudomonas aeruginosa</i>
10040	<i>Serratia marcesens</i>
10042	<i>Streptomyces erythreus</i>
10045	<i>Methylobacterium extorquens</i>
10047	<i>Geobacillus thermoleovorans</i>
10048	<i>Streptomyces fradia</i>
10049	<i>Hyphomicrobium facile subs.facile</i>
10050	<i>Staphylococcus aureus</i>
10053	<i>Bacillus licheniformis</i>

- 10054 *Bacillus sphaericus*
- 10055 *Pseudomonas putida*
- 10056 *Streptomyces rimasus*
- 10057 *Alcaligenes eutrophus*
- 10058 *Streptomyces clavuligerus*
- 10059 *Bifidobacterium bifidum*
- 10060 *E coil*
- 10061 *Enterobacter cloacae*
- 10062 *Lactobacillus acidophilus*
- 10063 *Xanthomonas campestris*
- 10064 *Sulfate – reducing bacteria*
- 10065 *Thiobacillus thioparus*
- 10066 *Bacillus Sp.*
- 10067 *Lactobacillus plantarum*
- 10068 *Lactobacillus plantarum* subsp *plantarum*
- 10069 *Lactobacillus rhamnosus*
- 10070 *Natronorubrum tibetense*
- 10071 Halorubrum vacuolatum
- 10072 *Natronorubrum gregoryi*
- 10073 *Haloferax mediterranei*



- 10074 *Natrialba magadii*
- 10075 *Natronococcus occultus*
- 10076 *Halobacterium salinarum*
- 10077 *Gluconobacter oxydans* subsp. suboxydans ( *Acetobacter suboxydans* )
- 10078 *Lysinibacillus macroides*
- 10079 *Brevundimonas naejangsanensis*
- 10080 *Pseudomonas aeruginosa*

### **Molds**

- 20001 *Aspergillus flavus*
- 20002 *Aspergillus foetidus*
- 20003 *Aspergillus niger*
- 20004 *Aspergillus niger*
- 20005 *Aspergillus niger*
- 20006 *Aspergillus niger*
- 20009 *Penicillium chrysogenum*
- 20011 *Rhizopus oryzae*
- 20013 *Trichoderma koningii*
- 20014 *Trichoderma Longibrachiatum*
- 20015 *Trichoderma reesei*

- 20018 *Aspergillus niger*  
20019 *Penicillium simplicissimum*  
20020 *Phanerochaete chrysosporium*  
20021 *Aspergillus niger*

**Yeast**

- 30006 *Saccharomyces cerevisiae*  
30009 *Debaryomyces hansenii* var. *hansenii*  
30010 *Pichia stipitis*  
30012 *Zygosaccharomyces rouxii*  
30013 *Kluyveromyces marxianus*  
30014 *Pichia guilliermondii*  
30015 *Pichia jadinii* (*Candida utilis*)  
30016 *Candida albicans*

**SECTION E**

**STRAINS LISTED BY APPLICATION**

## LIST OF STRAIN WITH SPECIAL APPLICATION (Native Strains)

<u>APPLICATION</u>	<u>STRAIN</u>	<u>BBRC NO.</u>
TOC Removal	Yeast	9001
PAH Removal	Fungi	9002
PAH Removal	Bacteria	9003
Furfural Treatment	Bacteria	9004
Sulfur Removal	Bacteria	9006
Sulfur Removal	Bacteria	9007
Sulfur Removal	Bacteria	9008
Asphalthene Removal	Bacteria	9012
Oil Emulsifier Production	Bacteria	9013
Oil Emulsifier Production	Bacteria	9014
Oil Emulsifier Production	Bacteria	9015
Sulfur Removal	Bacteria	9016
Sulfur Removal	Bacteria	9018
Oil Emulsifier Production	Bacteria	9019
Oil Emulsifier Production	Bacteria	9020
Oil Emulsifier Production	Bacteria	9021
Oil Emulsifier Production	Bacteria	9022
Oil Emulsifier Production	Bacteria	9023

PAH Removal	Yeast	9026
Sulfur Removal	Bacteria	9048
Sulfur Removal	Bacteria	9050
Sulfur Removal	Fungi	9052
Petroleum Oil Removal	Bacteria	9056
Thermophylic Fungi for oil Removal	Fungi	9057
Thermophylic Fungi for oil Removal	Fungi	9058
Diesel Removal	Bacteria	9059
Diesel Removal	Bacteria	9060
Diesel Removal	Bacteria	9061
Diesel Removal	Bacteria	9062
Diesel Removal	Bacteria	9063
Diesel Removal	Bacteria	9064
Diesel Removal	Bacteria	9065
Diesel Removal	Bacteria	9066
Diesel Removal	Bacteria	9067
Diesel Removal	Bacteria	9068
Diesel Removal	Bacteria	9069
Lignolytic Enzyme Producers Fungi	Fungi	9070
Lignolytic Fungi	Fungi	9071

Lignolytic Fungi	Fungi	9072
Lignolytic Fungi	Fungi	9073
Lignolytic Fungi	Fungi	9074
Lignolytic Fungi	Fungi	9075
Cyanide Removal	Bacteria	9077
Diesel Removal	Bacteria	9078
Cyanide Removal	Bacteria	9079

**SECTION F**

**LIST OF CULTURE MEDIA**

### **Medium1**

#### **NUTRIENT AGAR**

Peptone	5.0g
Meat extract	3.0g
Agar	15.0g
Distilled water	1.0L

Adjust pH to 7.0 for bacillus strains the addition of 10.0 mg  $\text{MnSO}_4 \times \text{H}_2\text{O}$  is recommended for sporulation

### **Medium2**

#### **LB AGAR**

Peptone	10.0g
Yeast extract	5.0g
NaCl	5.0g
Distilled water	1.0 L

Adjust pH to 7.2 add 1.5% agar

### **Medium3**

#### **BIFIDOBACTERIUM MEDIUM**

Casein peptone, tryptic digest	10.0g
Yeast extract	5.0g
Meat extract	5.0g
Glucose	10.0g



K <sub>2</sub> HPO <sub>4</sub>	3.0g
Tween 80	1.0ml
Tap water	1.0 L

Adjust pH to 6.8

After sterilization, aseptically add solutions of sodium ascorbate and cysteine - HCL to a final concentration of 1.0% and 0.05% respectively. Medium not freshly prepared should be heated in a steamer for 10 minutes before addition of reducing substances.

#### **Medium4**

##### **NUTRIENT AGAR WITH 0.5% Na CL**

Agar	15.0g
NaCl	5.0g
Pancreatic digest of gelation	5.0g
Beef extract	3.0g
DW	1.0L

pH 6.8 ±0.2 at 25°C

#### **Medium5**

##### **HYPHOMICROBIUM MEDIUM**

KH <sub>2</sub> PO <sub>4</sub>	1.36g
Na <sub>2</sub> HPO <sub>4</sub>	2.13g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.50g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.20g

CaCl <sub>2</sub> x H <sub>2</sub> O	6.00mg
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00mg
MnSO <sub>4</sub> x H <sub>2</sub> O	1.00mg
NaMoO <sub>4</sub> x2 H <sub>2</sub> O	1.50mg
Methylamine hydrochloride	3.38g
Yeast extract	0.10gr
Distilled water	1000.00ml

Adjust pH to 7.4 with NaOH. Autoclave 20 min at 121°C ,final pH: 7.2

### **Medium6**

#### **MRS MEDIUM WITH CYSTEINE**

Casein peptone, tryptic digest	10.0g
Meat extract	10.0g
Yeast extract	5.0g
Glucose	20.0g
Tween 80	1.0g
K <sub>2</sub> HPO <sub>4</sub>	2.0g
Sodium acetate	5.0g
Diammonium citrate	2.0g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2g
MnSO <sub>4</sub> .H <sub>2</sub> O	0.05gr

Add 0.05% cysteine – hydrochloride.

Distilled water 1.0L

Adjust pH 6.2 – 6.5

Lactobacilli MRS Broth (Difco 288130)

Note: Cystein – hydrochloride should be filter sterilized

### **MEDIUM7**

Medium 1 + 1% methanol

### **Medium8**

#### **TRYPTICASE SOY AGAR**

Pancreatic digest of casein 17.0g

NaCl 5.0g

Papaic digest of soybean meal 3.0g

K<sub>2</sub>HPO<sub>4</sub> 2.5g

Glucose 2.5g

Agar 15.0g

Distilled water 1.0L

pH 7.3±0.2 at 25°C

### **Medium9**

#### **SPRULATION AGAR**

Yeast extract 1.0g

Beef extract	1.0g
Tryptose	2.0g
FeSO <sub>4</sub>	Trace
Glucose	10.0g
Agar	15.0g
Distilled water	1.0L

Adjust pH to 7.2; for broth ,eliminate agar and reduce concentration to 1/3 of given quantities.

### **Medium10**

#### **GYM STREPTOMYCES MEDIUM**

GYM Streptomyces Medium

Glucose	4.0 g
Yeast Extract	4.0g
Malt Extract	10.0g
CaCO <sub>3</sub>	2.0g
Agar	12.0g
Distilled water	1000.0 ml

Adjust pH to 7.2 with KOH before adding agar (use pH indicator paper). Delete CaCO<sub>3</sub> if liquid medium is used.

### **Medium11**

#### **SRB MEDIUM**

KH <sub>2</sub> PO <sub>4</sub>	0.5g
NH <sub>4</sub> CL	1g

Na <sub>2</sub> SO <sub>4</sub>	1g
CaCl <sub>2</sub> .6H <sub>2</sub> O	1g
MgSO <sub>4</sub> .7H <sub>2</sub> O	2g
Sodium Lactate	3.5g
Yeast Extract	1g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.5g
Ascorbic acid	0.1g
Thioglycolic Acid	0.1g
Distilled water	1000ml

Note: FeSO<sub>4</sub>.7H<sub>2</sub>O should be filter sterilized. After sterilization aseptically add solution of FeSO<sub>4</sub>.7H<sub>2</sub>O Adjust pH to 7.5 with NaOH

## Medium 12

### THIOBACILLUS THIOPARUS II MEDIUM

KH <sub>2</sub> PO <sub>4</sub>	2.0g
K <sub>2</sub> HPO <sub>4</sub>	2.0g
NH <sub>4</sub> Cl	0.4g
Na <sub>2</sub> CO <sub>3</sub>	0.4g
MgCl <sub>2</sub> .6HO <sub>2</sub>	0.2g
Vitamin solution (see medium 98)	3ml
Trace metal solution (see medium 61)	1.0ml
Bromocresol purple (sat.aqu.solution)	2.0ml

Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> .5H <sub>2</sub> O	5.0g
Agar ,if necessary	15.0g
Distilled water	1000.0 ml

Sterilize the phosphates separately in 1/10 of medium volume and mix with the other salts when cool.

Add filter-sterilized vitamin solution. Medium pH 7.1.

### **Medium13**

#### **GLUCOSE YEAST EXTRACT AGAR**

Glucose	20.0g
Yeast extract	10.0g
CaCO <sub>3</sub> (light precipitate)	20.0g
Agar	17.0g
Distilled water	1000.0ml

### **Medium14**

#### **Potato Dextrose Agar (PDA)**

Diced Potatoes	300.0g
Glucose	20.0g
Agar	15.0g
DW	1.0L

Boil finely diced potatoes in 500 ml of water until thoroughly cooked. Filter through cheese – cloth and add water to filtrate to 1.0 l. The agar is dissolved in the filtrate by heating, and Glucose is added prior to sterilization.

## **Medium15**

### **Czapek Agar**

NaNO <sub>3</sub>	3.0g
K <sub>2</sub> HPO <sub>4</sub>	1.0g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5g
KCL	0.5g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.01g
Agar	15.0g
DI Water	900ml

Autoclave at 121°C

Let cool and add following filter sterilized solution:

Sucrose (commercial grad)	30g
DI Water	100ml

Final pH 7.3±0.2 at 25°C

## **Medium16**

### **Yeast Mold Agar**

Agar	20.0g
Glucose	10.0g
Peptone	5.0g
Yeast extract	3.0g
Malt extract	3.0g

Distilled water        1.0L

Adjust pH to 6.2±0.2

Omit agar, for making YM Broth

YM Agar (Difco 271120)

YM Broth (Difco 271210)

### **Medium17**

#### **GPYA Medium**

Glucose                40.0g

Peptone                5.0g

Yeast extract        5.0g

Agar                    15.0g

Distilled water       1.0L

### **Medium18**

#### **Malt Extract Agar (Blakeslees formula)**

Malt extract        20.0g

Glucose               20.0g

Peptone               1.0g

Agar                    20.0g

DW                      1.0L

Add glucose prior to sterilization.



### **Medium19**

#### **Universal Medium for Yeasts (YM)**

Yeast extract	3.0g
Malt extract	3.0g
Peptone from soybeans	5.0g
Glucose	10.0g
Agar	15.0g
Distilled water	1000.0 ml

### **Medium 20**

#### **Oat Meal Agar**

Agar	5.0gr
Oatmeal, instant for babies	40.0 g

Add agar to distilled water and bring volume to 500.0 ml Mix thoroughly. Gently heat and bring to boiling. Add instant oatmeal for babies to distilled water and bring volume to 250.0 ml. Mix thoroughly Autoclave for 15 min at 15 psi pressure 121°C

### **Medium21**

#### **Medium for Osmophilic Fungi (Harrold's) (M 40 Y)**

Sucrose	400.0g
Malt extract	20.0g
Yeast extract	5.0g

Agar	20.0g
Distilled water	1000.0 ml

## **Medium22**

### **Natronobacreria Medium**

KH <sub>2</sub> PO <sub>4</sub>	1.00g
KCL	1.00g
NH <sub>4</sub> CL	1.00g
MgSO <sub>4</sub> × 7 H <sub>2</sub> O	0.24g
CaSSo <sub>4</sub> × 2 H <sub>2</sub> O	0.17g
Trace element solution SL – 10	1.00ml
Agar, if necessary	20.00g
Nacl	200.00 g
Na <sub>2</sub> – glutamate	1.00g
Yeast extract	5.00g
Casamino acids	5.00g
Na <sub>2</sub> CO <sub>3</sub>	5.00g

Add distilled water to give a final volume of 1000 ml

Adjust pH to 6.5 before autoclaving.

Sterilize Na<sub>2</sub>CO<sub>3</sub> separate from medium add after cooling.

Check final pH to be 9.0 – 9.5.

If agar medium is prepared, heat and dissolve agar before adding sodium chloride.

Trace element solution SL – 10:

HCl (25% 7.7 M)	10.00 ml
FeCl <sub>2</sub> × 4 H <sub>2</sub> O	1.50 g
ZnCl <sub>2</sub>	70.00 mg
MnCl <sub>2</sub> × 4 H <sub>2</sub> O	100.00 mg
H <sub>3</sub> Bo <sub>3</sub>	6.00 mg
CoCl <sub>2</sub> × 6 H <sub>2</sub> O	190.00mg
CuCl <sub>2</sub> × 2 H <sub>2</sub> O	2.00 mg
NiCl <sub>2</sub> ×6 H <sub>2</sub> O	24.00 mg
Na <sub>2</sub> MoO <sub>4</sub> × 2 H <sub>2</sub> O	36.00 mg
Distilled water	990.00ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water ,add and dissolve the other salts.

Finally make up to 1000.0 ml

### **Medium23**

#### **Halobacterium Medium**

Casamino acids	7.50g
Yeast extract	10.00g
Na <sub>3</sub> – citrate	3.00g
KCl	2.00g
MgSO <sub>4</sub> × 7 H <sub>2</sub> O	20.00g
FeSO <sub>4</sub> × 7 H <sub>2</sub> O	0.05g
MnSO <sub>4</sub> × H <sub>2</sub> O	0.20g

NaCl	250.00g
Agar	20.00g
Distilled water	1000.00ml

Adjust pH to 7.4. Add the agar after dissolving all ingredients in the water and adjustment of pH.

## **Medium24**

### **Halobacterium Medium**

Yeast extract	5.00g
Casamino acids	5.00g
Na – glutamate	1.00g
KCl	2.00g
Na <sub>3</sub> – citrate	3.00g
MgSO <sub>4</sub> × 7 H <sub>2</sub> O	20.00g
NaCl	200.00g
FeCl <sub>2</sub> × 4 H <sub>2</sub> O	36.00mg
MnCl <sub>2</sub> × 4 H <sub>2</sub> O	0.36mg
Agar	20.00g

Add distilled water to give a final volume of 1000 ml.

Adjust pH 7.0 – 7.2

## **Medium25**

### **Van Niel's Yeast Agar with 25% NaCl**

ATCC Medium 112: (see below) with 25% NaCl

K <sub>2</sub> HPO <sub>4</sub>	1.0g
MgSO <sub>4</sub>	0.5g
Yeast extract	10.0g
Agar	20.0g
Tap water	1.0L

Adjust pH to 7.0 – 7.2

Autoclave at 121°C for 15 minutes.

### **Medium26**

The same as medium 1. After sterilization add sterile 1M Na-sesquicarbonate solution (1ml in 10 ml) to achieve a pH of 9.7.

Na-sesquicarbonate solution:

NaHCO <sub>3</sub>	4.2 g
Na <sub>2</sub> CO <sub>3</sub> anhydrous	5.3 g
Distilled water	100ml

### **Medium27**

#### **MRS Broth Medium**

Casein peptone, tryptic digest	10 g
Meat extract	10 g
Yeast extract	5.0 g
Glucose	20 g
Tween 80	1.0 g

K <sub>2</sub> HPO <sub>4</sub>	2.0 g
Sodium acetate	5.0 g
Diammonium citrate	2.0 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2 g
MnSO <sub>4</sub> ·H <sub>2</sub> O	0.05 g
Distilled water	1.0 L

Adjust pH to: 6.2-6.5

Lactobacilli MRS Broth (Difco 288130)

### **Medium 28**

#### MANITOL AGAR

Yeast extract	5.0 g
Peptone	3.0 g
Mannitol	25.0 g
Agar	15.0 g
DW	1000.0 ml

Autoclave at 121°C

## **Section G**

# **Persian Information**

## مقدمه

مرکز مهندسی بیوشیمی محیط زیست از سال ۱۳۴۹ (۱۹۷۰) در دانشگاه صنعتی شریف آغاز به فعالیت نمود. هدف از تاسیس این مرکز انجام تحقیقات و پژوهش در زمینه فرایندهای زیستی در حوزه های مختلف صنعتی تعریف گردید. برای انجام فرایندهای زیستی نیاز به میکروبها با تولید های خاص می باشد. بر حسب نوع فرایند و تولید خاص، نوع میکروارگانیسم و اطلاعات علمی دقیق از شرایط رشد و تولید آن گونه میکروبی از الزامات تحقیق می باشد. از بدو تاسیس مرکز مهندسی بیوشیمی - محیط زیست، مطالعات تئوری و تجربی جدا سازی میکروبها از منابع مختلف و همچنین سفارش این میکروبها از کلکسیون های بین المللی از جمله ATCC آغاز گردید. البته نمونه های جدا سازی شده با استفاده از امکانات موجود و خدمات سایر ارگانها بصورت خشک انجمادی تهیه و در کلکسیون مرکز مهندسی بیوشیمی ذخیره شده است.

در کشور های مختلف مراکز متعدد ملی و بین المللی کلکسیون میکروبی موجود است که هدف اصلی آنها جمع آوری میکروارگانیسم های مورد نیاز صنعتی و تشخیصی می باشد. مرکزیت دادن به مراکز کلکسیون در سال ۱۹۴۷ در گرد هم آیی Common wealth specialist conference پیشنهاد شد و در سال ۱۹۷۰ بمنظور هم آهنگی، فدراسیون جهانی کلکسیون میکروارگانیسم ها World collection for culture تاسیس شد.

با شناخت بیوتکنولوژی جدید و مهندسی ژنتیک، میکروارگانیسم های با خصوصیات مورد نظر و از طریق فرایندهای ژنتیکی و اصلاح ژنتیکی در فراوری گونه های نو ترکیب برای تولیدات با اهمیت دارویی از جمله انسولین، هورمون رشد و داروهای خاص مد نظر واقع گردید.

مراکز کلکسیون همواره باید با فدراسیون جهانی کلکسیون میکروارگانیسم ها در تماس دائم بوده و با روشهای جدید نگهداری میکروارگانیسم ها آشنا می شوند.



کلکسیون میکروبی (BBRC) Biochemical and Bioenvironmental Eng. Research Center که از سال ۱۳۴۹ به صورت ملی در مرکز مهندسی بیوشیمی محیط زیست فعالیت خود را آغاز نمود در حال حاضر ۱۰۸ نمونه میکروبی شامل باکتری و قارچ نگهداری می نماید که حدود یک سوم از این میکروارگانیسم ها بومی ایران هستند. این کلکسیون بطور مداوم با نمونه هایی از میکروارگانیسم های بومی و سفارش شده از خارج و داخل غنی تر می شود. از سال ۱۳۹۰ پس از تکمیل امکانات فرایند جهت تهیه نمونه های خشک انجمادی، تلاش های متعدد برای ثبت کلکسیون در داخل و ثبت بین الملل در دست اقدام می باشد.

## خدمات مرکز کلکسیون BBRC

۱. تهیه نمونه ای میکروبی بصورت لیوفیلیزه ( خشک انجماد ی) بر طبق روشهای استاندارد بین المللی.
۲. جدا سازی نمونه های میکروبی جدید از منابع داخلی، شناسائی اولیه ان ها، تشخیص مقدماتی و آماده سازی جهت نگهداری.
۳. ارائه خدمات به سایر ارگان های آموزشی پژوهشی و دانشگاهها از لحاظ ارسال نمونه ها یا آموزش تهیه و نگهداری سوشها به محققین.
۴. ارائه و دریافت اطلاعات از طریق سایت مرکز مهندسی بیوشیمی محیط زیست.
۵. ارائه سفارش از طریق سایت و یا تلفکس ۶۶۰۰۵۴۱۷ امکان پذیر است

## تهیه نمونه های لیوفیلیزه و کشت آمپول ها

آپول های مخصوص لیوفیلیزه با دترجنت شسته شده و سپس با آب شیر و در انتها با آب خالص شستشو شود. آپول ها در  $121^{\circ}\text{C}$  برای مدت ۲۰ دقیقه استریل شده و در دمای اطاق نگهداری می شوند. قبل از استفاده، آپول ها بر چسب زنی می شوند. مراحل تهیه نمونه به ترتیب شامل:

- محیط سوسپانسیون. معمولا از Skim milk ۱۰ درصد و یا ۱۰ درصد Skim milk و ۵ درصد گلو تامات بصورت استریل در آب مقطر استفاده می شود

- کشت هوازی یا بی هوازی بر اساس بروشور آماده نموده و بصورت جامد یا مایع تهیه می شوند. کشت های مایع را می توان بصورت استریل در ۴۰۰۰g و ۳۰ دقیقه سانتی رفوژ نمود تا تعداد کافی سلول موجود باشد.

- کشت های جامد توسط محیط سوسپانسیون به میزان ۴ میلی لیتر جدا شده و در صورت لزوم از میله مخصوص برای جدا کردن استفاده می شود.

- با سرعت سوسپانسیون کشت به ظروف (آپول های) استریل وارد که معمولا ۱/۵ میلی لیتر به هر آپول، کشت سلولی وارد می شود.

- سر ظروف یا آپولها بصورت نیمه بسته (loose) آماده می شود و آپول ها در فریزر حداقل  $20^{\circ}\text{C}$  - قرار می گیرد.

- پس از فریز نمودن آپول ها، مرحله خشک انجمادی با قرار دادن آپول ها در خشک کن انجمادی و تنظیم شرایط خشک کن انجمادی انجام می شود.

- باید در نظر داشت که سر ظروف یا آپول ها بصورت loose و عاری از میکروب بسته شود تا خروج رطوبت از آنها تسهیل شود.

- معمولا زمان خشک انجمادی بر حسب حجم نمونه متغیر (۲۴ - ۳) ساعت و طولانی است.

- پس از خشک کردن، نمونه ها از دستگاه بر طبق دستور خارج می شوند .
- نمونه های خشک کن انجمادی می توانند در دمای  $4^{\circ}\text{C}$  در شرایط تاریک نگهداری شوند .

### تجدید کشت نمونه های لیوفیلیزه

- برای تهیه و کشت میکروارگانیزم های لیوفیلیزه ، توسط پی پت پاستور بطور استریل به هر آمپول  $0/5 - 0/4$  میلی لیتر از محیط کشت مایع یا سرم فیریولوژی اضافه نموده و کاملاً آنرا مخلوط کرده، مجموعه محتویات آمپول را به محیط کشت مایع انتقال داده و دو سه قطره آن را به محیط کشت جامد منتقل نمایید. مشخصات محیط جامد و یا مایع مورد نیاز برای هر سوش میکروبی در بروشور کلکسیون موجود است . کشت ها را در شرایط بهینه رشد در انکو باتور قراردادده و زمان لازم برابر زمان عادی رشد در شرایط عادی می باشد .
- آمپول های لیوفیلیزه تا هنگام مصرف باید در یخچال ( $4^{\circ}\text{C}$ ) و عدم دسترسی به نور مستقیم قرار گیرد.
- تجدید کشت نمونه های لیوفیلیزه به زمان بیشتری نسبت به نمونه های عادی نیازمند است.



نشانی: تهران - خیابان آزادی - دانشگاه صنعتی شریف - صندوق پستی ۱۳۹۹-۱۱۵۵

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وبسایت:



**کلیسیون میکروبی**  
**مرکز مهندسی بیوشیمی**  
**محیط زیست**

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