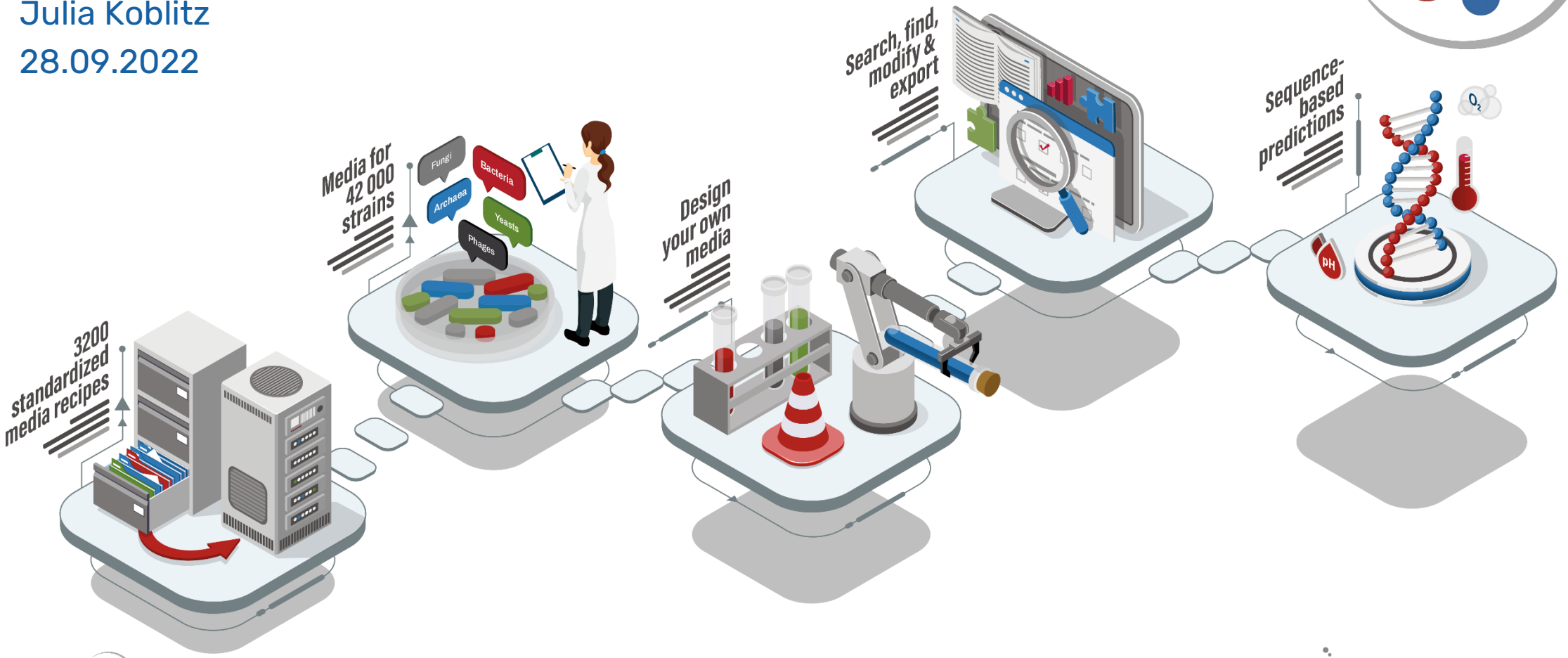
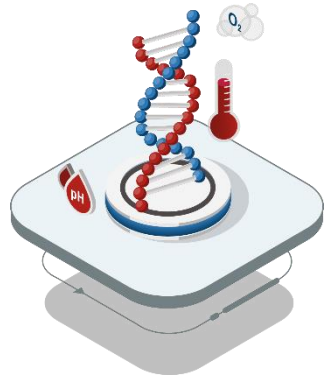
Media*Dive*: the expert-curated cultivation media database

Julia Koblitz

28.09.2022



Why did we build this database?



- **The aim:** Predict cultivation conditions and media for uncultivable prokaryotes
- Based on genome sequences
- With use of artificial intelligence

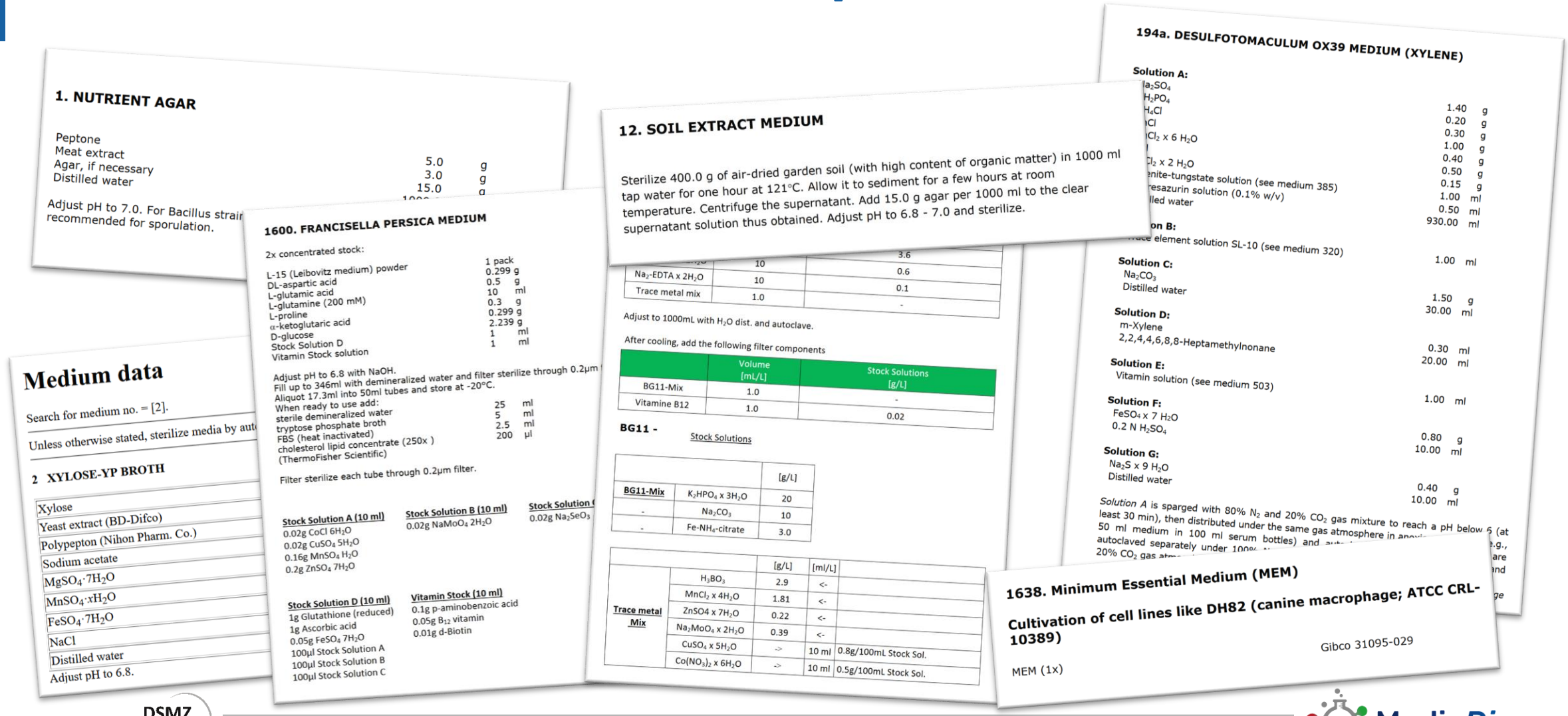
- Part of the DiASPora project

DiASPora

Digital Approaches for the Synthesis of Poorly accessible Biodiversity Information

diaspora-project.de

The cultivation media landscape



1. NUTRIENT AGAR

Peptone	5.0	g
Meat extract	3.0	g
Agar, if necessary	15.0	g
Distilled water		

Adjust pH to 7.0. For Bacillus strai
recommended for sporulation.

1600. FRANCISELLA PERSICA MEDIUM

2x concentrated stock:

L-15 (Leibovitz medium) powder	1 pack
DL-aspartic acid	0.299 g
L-glutamic acid	0.5 g
L-glutamine (200 mM)	10 ml
L-proline	0.3 g
α-ketoglutaric acid	0.299 g
D-glucose	2.239 g
Stock Solution D	1 ml
Vitamin Stock solution	1 ml

Adjust pH to 6.8 with NaOH.
Fill up to 346ml with demineralized water and filter sterilize through 0.2µm
Aliquot 17.3ml into 50ml tubes and store at -20°C.
When ready to use add:

sterile demineralized water	25	ml
tryptose phosphate broth	5	ml
FBS (heat inactivated)	2.5	ml
cholesterol lipid concentrate (250x) (ThermoFisher Scientific)	200	µl

Filter sterilize each tube through 0.2µm filter.

Stock Solution A (10 ml)
0.02g CoCl $6H_2O$
0.02g $CuSO_4$ $5H_2O$
0.16g $MnSO_4$ H_2O
0.2g $ZnSO_4$ $7H_2O$

Stock Solution B (10 ml)
0.02g $NaMoO_4$ $2H_2O$

Stock Solution
0.02g Na_2SeO_3

Stock Solution D (10 ml)
1g Glutathione (reduced)
1g Ascorbic acid
0.05g $FeSO_4$ $7H_2O$
100µl Stock Solution A
100µl Stock Solution B
100µl Stock Solution C

Vitamin Stock (10 ml)
0.1g p-aminobenzoic acid
0.05g B_{12} vitamin
0.01g d-Biotin

Medium data

Search for medium no. = [2].

Unless otherwise stated, sterilize media by aut

2 XYLOSE-YP BROTH

Xylose
Yeast extract (BD-Difco)
Polypepton (Nihon Pharm. Co.)
Sodium acetate
$MgSO_4 \cdot 7H_2O$
$MnSO_4 \cdot xH_2O$
$FeSO_4 \cdot 7H_2O$
NaCl
Distilled water
Adjust pH to 6.8.

12. SOIL EXTRACT MEDIUM

Sterilize 400.0 g of air-dried garden soil (with high content of organic matter) in 1000 ml tap water for one hour at 121°C. Allow it to sediment for a few hours at room temperature. Centrifuge the supernatant. Add 15.0 g agar per 1000 ml to the clear supernatant solution thus obtained. Adjust pH to 6.8 - 7.0 and sterilize.

	10	3.6
Na_2-EDTA x $2H_2O$	10	0.6
Trace metal mix	1.0	0.1
		-

Adjust to 1000mL with H_2O dist. and autoclave.

After cooling, add the following filter components

	Volume [mL/L]	Stock Solutions [g/L]
BG11-Mix	1.0	-
Vitamine B12	1.0	0.02

BG11 - Stock Solutions

		[g/L]
BG11-Mix	K_2HPO_4 x $3H_2O$	20
-	Na_2CO_3	10
-	Fe-NH_4-citrate	3.0

		[g/L]	[ml/L]	
Trace metal Mix	H_3BO_3	2.9	<-	
	$MnCl_2$ x $4H_2O$	1.81	<-	
	$ZnSO4$ x $7H_2O$	0.22	<-	
	Na_2MoO_4 x $2H_2O$	0.39	<-	
	$CuSO_4$ x $5H_2O$	->	10 ml	0.8g/100mL Stock Sol.
	$Co(NO_3)_2$ x $6H_2O$	->	10 ml	0.5g/100mL Stock Sol.

194a. DESULFOTOMACULUM OX39 MEDIUM (XYLENE)

Solution A:

a_2SO_4	1.40	g
H_2PO_4	0.20	g
H_4Cl	0.30	g
Cl	1.00	g
Cl_2 x $6H_2O$	0.40	g
	0.50	g
Cl_2 x $2H_2O$	0.15	g
enite-tungstate solution (see medium 385)	1.00	ml
resazurin solution (0.1% w/v)	0.50	ml
lled water	930.00	ml

on B:
ace element solution SL-10 (see medium 320) 1.00 ml

Solution C:
Na_2CO_3 1.50 g
Distilled water 30.00 ml

Solution D:
m-Xylene 0.30 ml
2,2,4,4,6,8,8-Heptamethylnonane 20.00 ml

Solution E:
Vitamin solution (see medium 503) 1.00 ml

Solution F:
$FeSO_4$ x $7H_2O$ 0.80 g
0.2 N H_2SO_4 10.00 ml

Solution G:
Na_2S x $9H_2O$ 0.40 g
Distilled water 10.00 ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in an
50 ml medium in 100 ml serum bottles) and
autoclaved separately under 100%
20% CO_2 gas atm

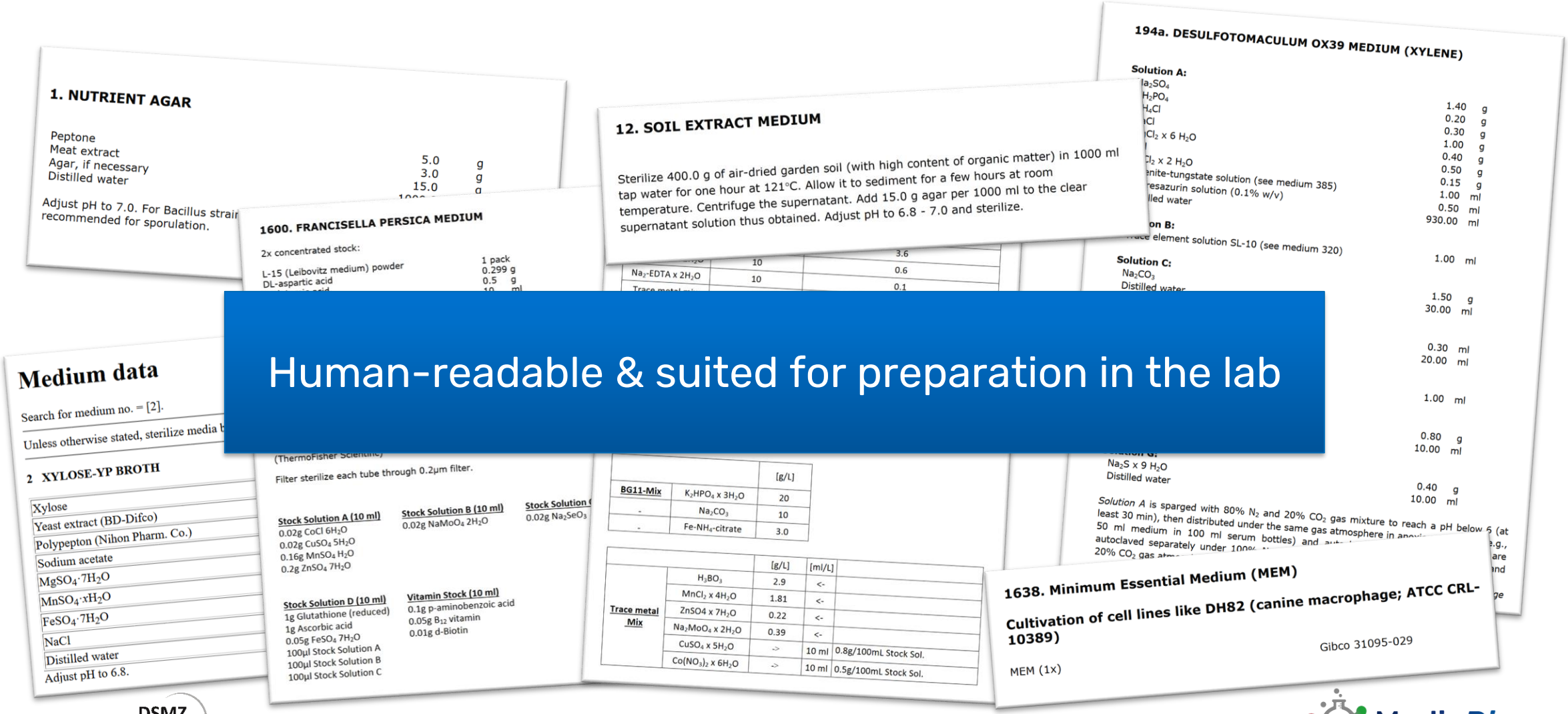
1638. Minimum Essential Medium (MEM)

Cultivation of cell lines like DH82 (canine macrophage; ATCC CRL-10389)

Gibco 31095-029

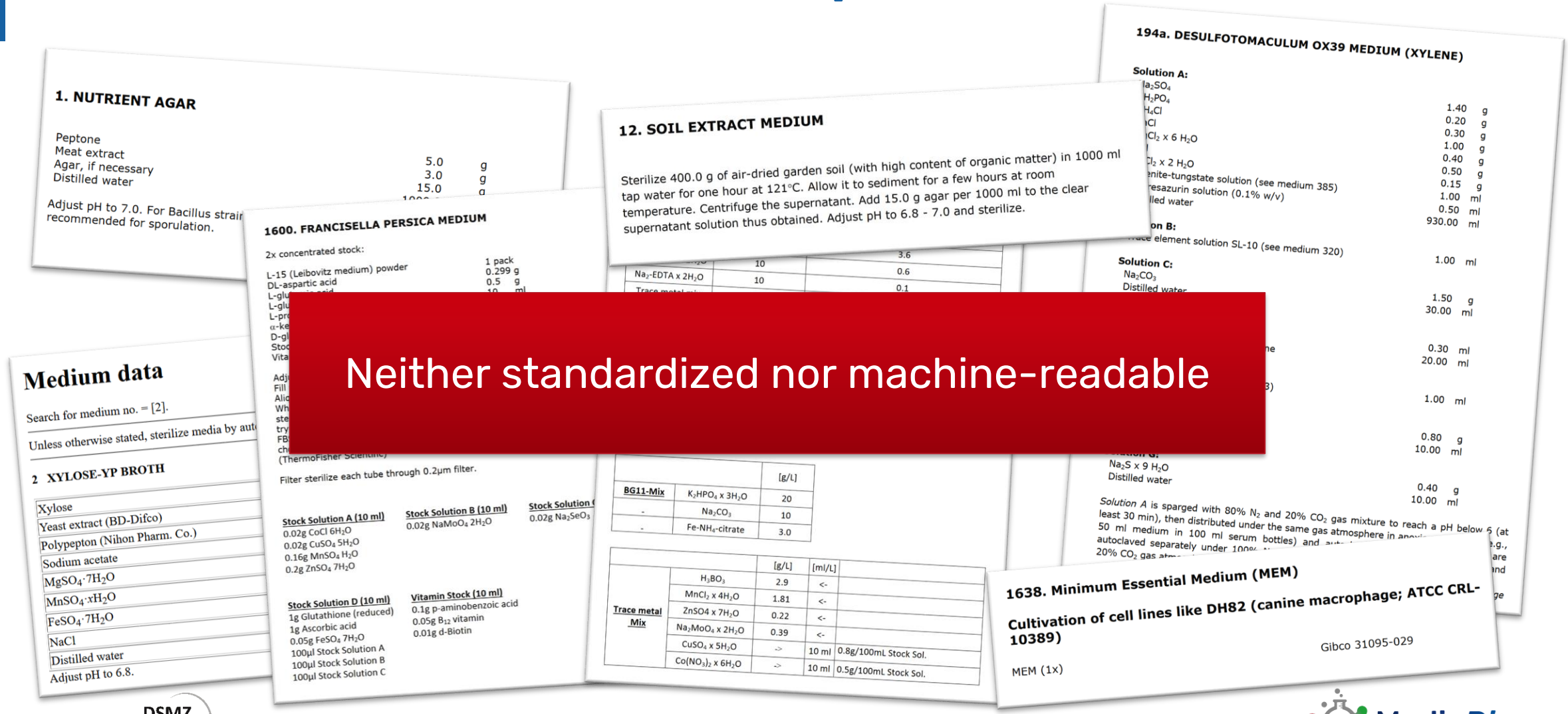
MEM (1x)

The cultivation media landscape



Human-readable & suited for preparation in the lab

The cultivation media landscape



Neither standardized nor machine-readable

What we did to change this

Japan Collection of Microorganisms

DSMZ Leibniz Institute
DSMZ-German Collection of Microorganisms and Cell Cultures GmbH

1. NUTRIENT AGAR

Peptone	5.0	g
Meat extract	3.0	g
Agar, if necessary	15.0	g
Distilled water	1000.0	ml

Adjust pH to 7.0. For Bacillus strains the addition of 10.0 mg $MnSO_4 \times H_2O$ is recommended for sporulation.

> 3,000 Media recipes

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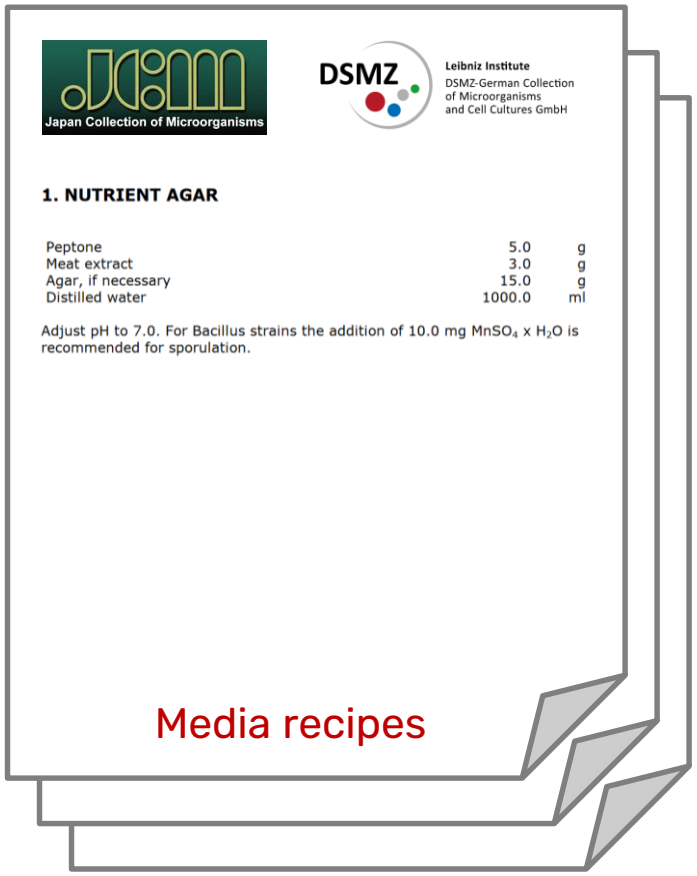

Extracted text

```
1. NUTRIENT AGAR

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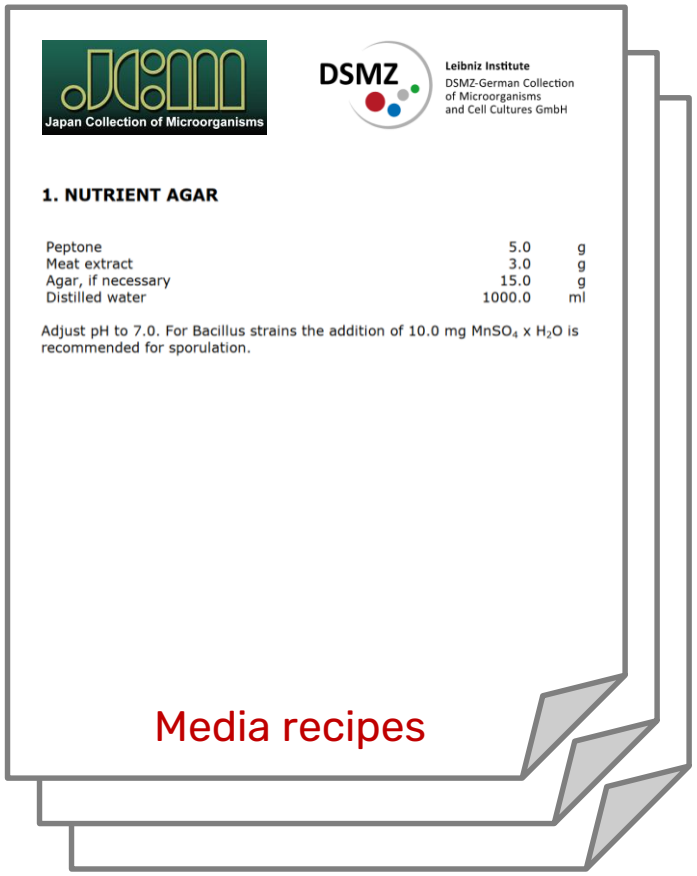
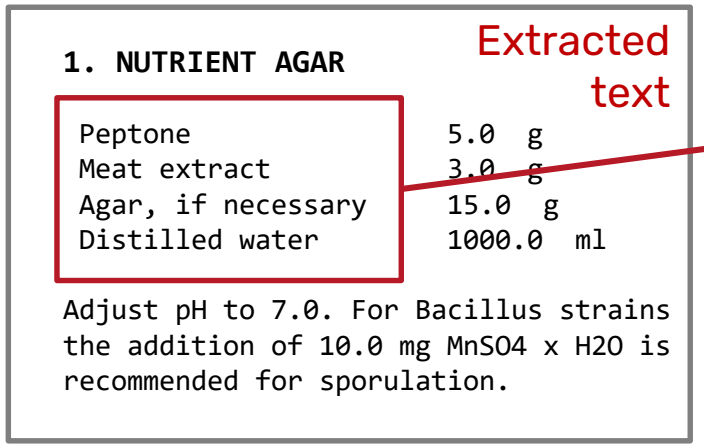


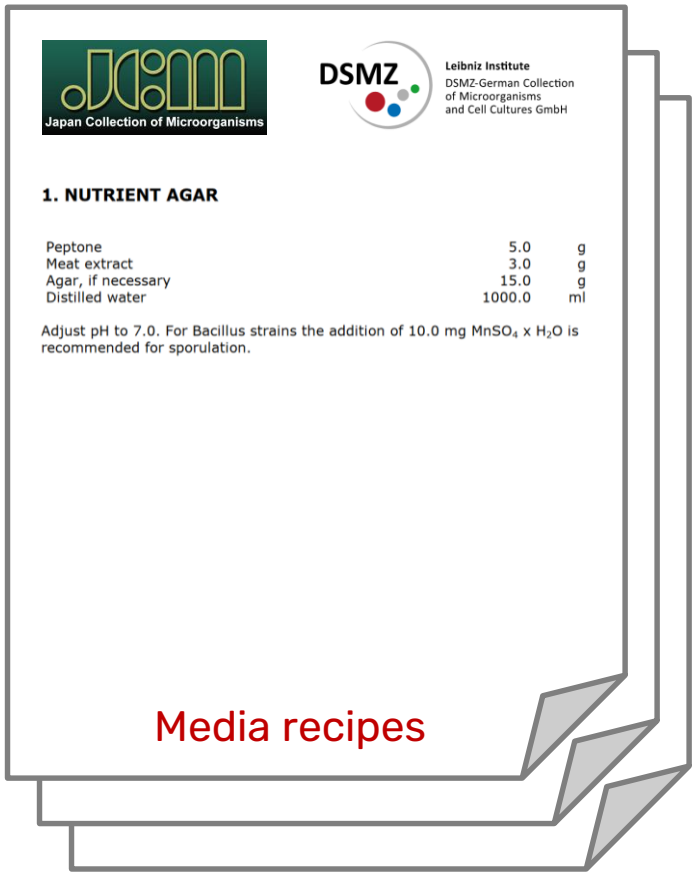


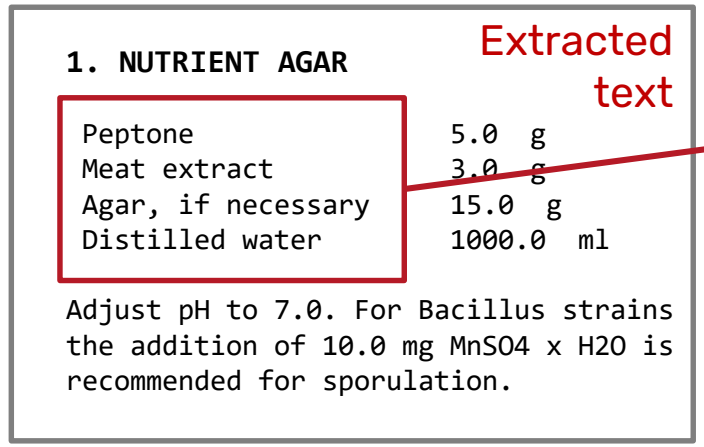
Standardization:

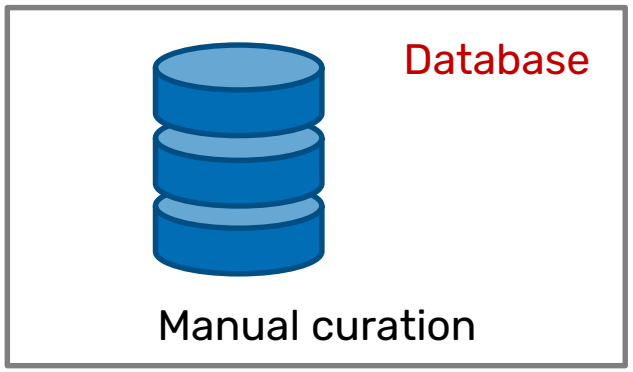
- Synonyms
- Variant forms of spelling
- Spelling mistakes
- Attributes & conditions

Mapping to other databases

What we did to change this







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Media recipes

Extracted text

```
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Meat extract            3.0   g
Agar, if necessary      15.0  g
Distilled water         1000.0  ml

Adjust pH to 7.0. For Bacillus strains
the addition of 10.0 mg MnSO4 x H2O is
recommended for sporulation.
```

Database

Manual curation

Website

1. NUTRIENT AGAR

Main sol. 1 # 1000 ml

Solution occurs in 20 other media (first defined in Medium 1).

Compound	Amount	Unit	Conc. [g/L]	Conc. [mM]
Peptone	5	g	5	-
Meat extract	3	g	3	-
Agar , for solid medium	15	g	15	-
Distilled water	1000	ml	-	-

1. Adjust pH to 7.0.
2. For Bacillus strains the addition of 10.0 mg $MnSO_4$ x H_2O is recommended for sporulation.

FAIR & continously extended

- All data is available online according to FAIR data principles
- Expert-curated and continously extended

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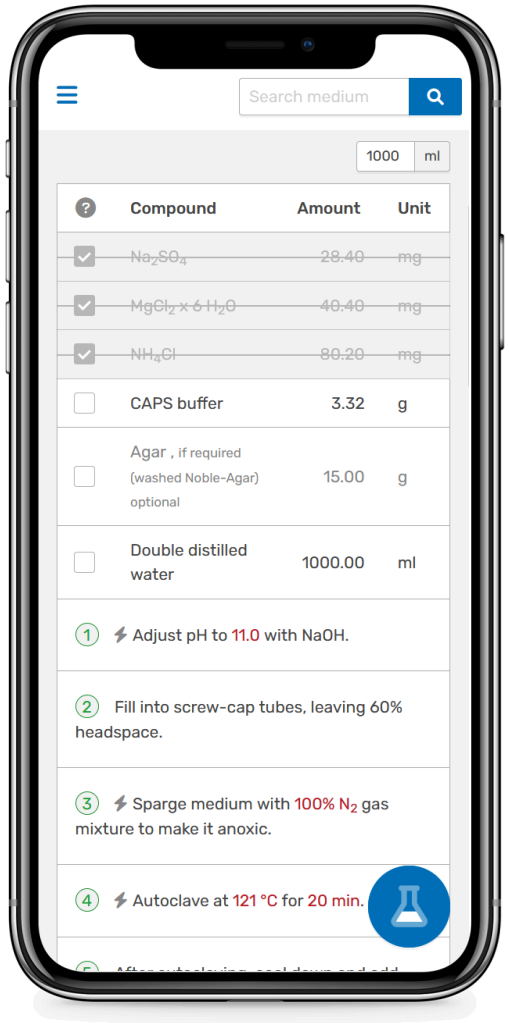
- Mobile friendly web page
- QR-code generation for easy switch to mobile
- Medium cooking guide on mobile devices



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- Mobile friendly web page
- QR-code generation for easy switch to mobile
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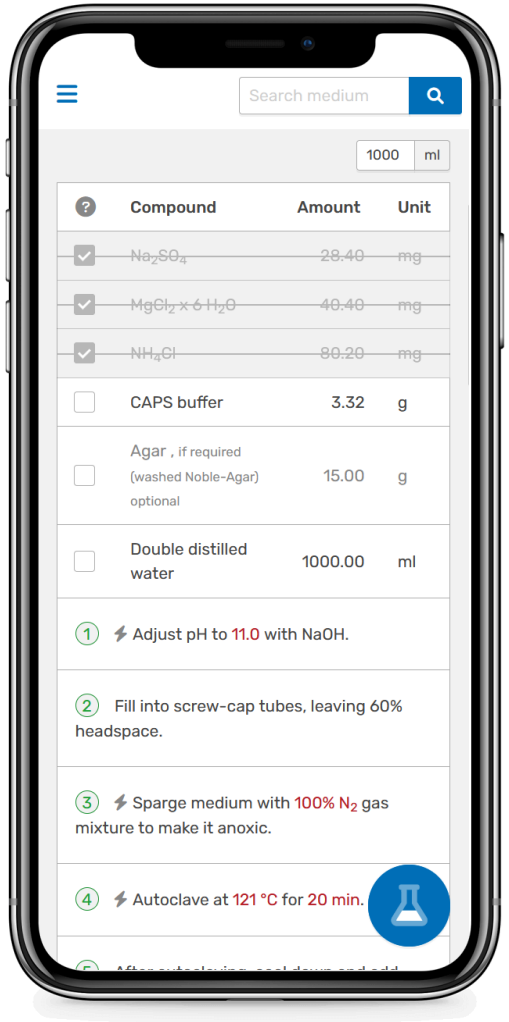
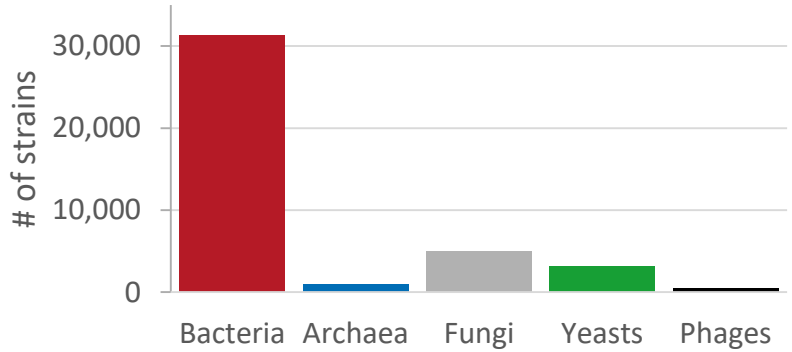
- Website available in English and German
- Accessibility features, e.g. for people with dyslexia



Standardization leads to new possibilities

- Search & find cultivation media
  - by medium name and number
  - by ingredients & concentrations
  - by strains



Standardization leads to new possibilities

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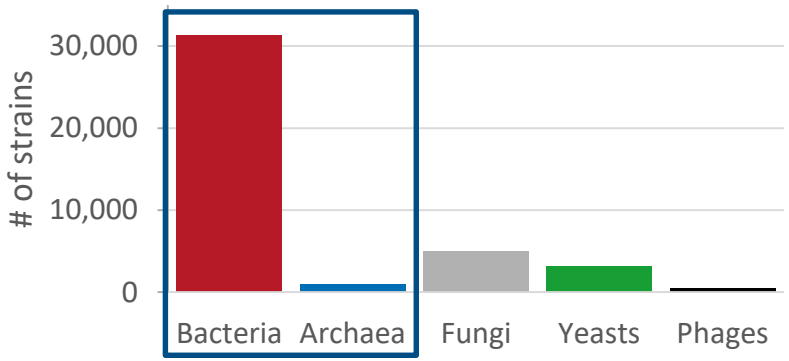

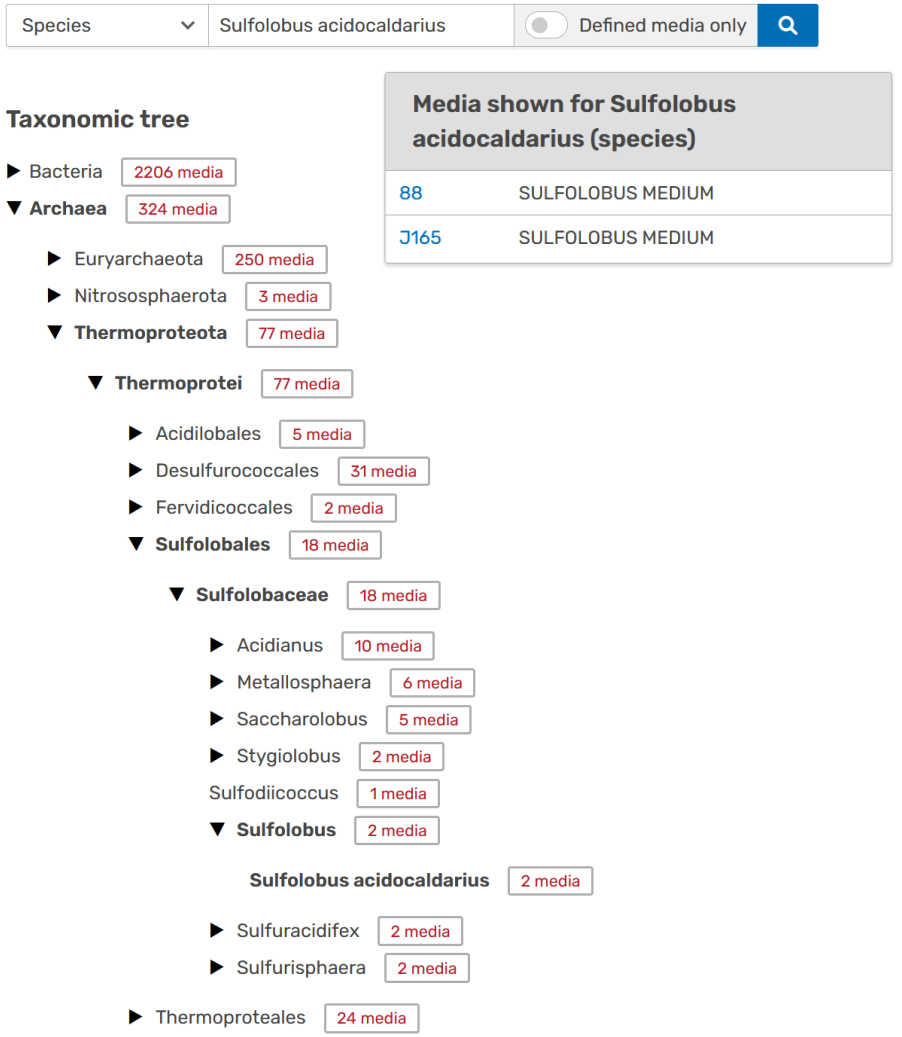

Standardization leads to new possibilities

- Search & find cultivation media
  - by medium name and number
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  - by strains & taxonomy
- Modify
  - Volume
  - Strain-specific media

*** Modified for *Thiofractor thiocaminus* DSM 22050**

Supplement medium with 1.00 g/l $NaNO_3$ and omit pyruvate, lactate and yeast extract. Upon autoclaving add 3.00 g/l sterile sulfur powder (sterilized by steaming for 3 hours on each of 3 successive days) and adjust pH to 6.0 - 6.5.

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Main sol. 1011c # — 1020 ml

Equipment needed: Filter, Autoclave, Hungate tubes, Gassing station

Compound	Amount	Unit	Conc. [g/L]	Conc. [mM]
NaCl	30.00	g	29.412	503.281
...	...	...	...	...
$CaCl_2$ x 2 H_2O	0.14	g	0.137	0.934
~~Na- pyruvate*~~	~~0.50~~	~~g~~	~~0.49~~	~~4.455~~
~~Na- lactate*~~	~~0.50~~	~~g~~	~~0.49~~	~~4.374~~
~~Yeast extract*~~	~~0.10~~	~~g~~	~~0.098~~	
$Na_2S_2O_3$ x 5 H_2O	1.50	g	1.471	5.925
Wolin's vitamin solution	10.00	ml	-	-
Sulfur powder*	**3.00**	**g**	**-**	**-**
$NaNO_3$*	**1.00**	**g**	**-**	**-**
Distilled water	1000.00	ml	-	-

Standardization leads to new possibilities

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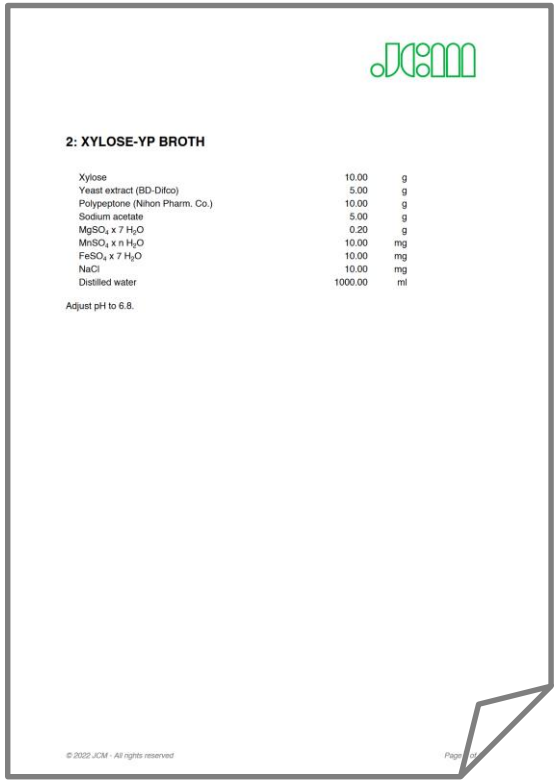
- Export
  - Download tables as CSV
  - Download media and solutions as PDF recipes

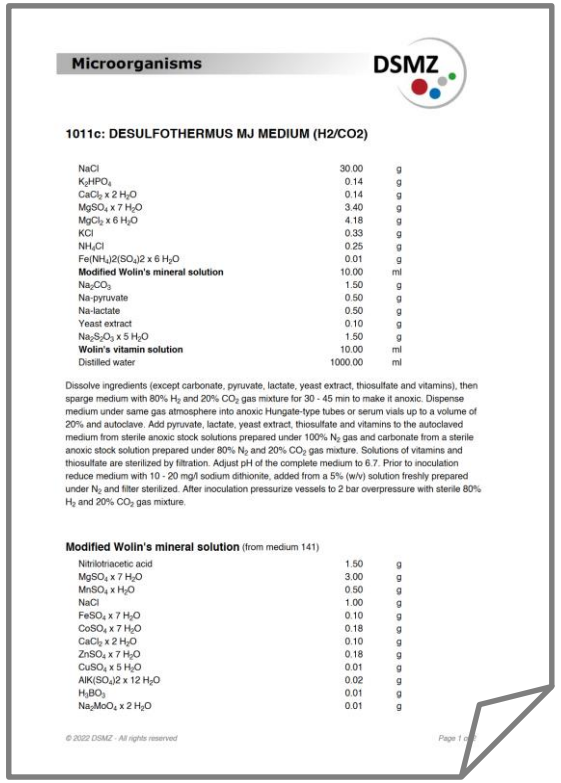


JCM

2: XYLOSE-YP BROTH

Xylose	10.00	g
Yeast extract (BD-Difco)	5.00	g
Polypeptone (Nihon Pharm. Co.)	10.00	g
Sodium acetate	5.00	g
$MgSO_4 \times 7\ H_2O$	0.20	g
$MnSO_4 \times n\ H_2O$	10.00	mg
$FeSO_4 \times 7\ H_2O$	10.00	mg
NaCl	10.00	mg
Distilled water	1000.00	ml

Adjust pH to 6.8.



Microorganisms DSMZ

1011c: DESULFOTHERMUS MJ MEDIUM (H2/CO2)

NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2 \times 2\ H_2O$	0.14	g
$MgSO_4 \times 7\ H_2O$	3.40	g
$MgCl_2 \times 6\ H_2O$	4.18	g
KCl	0.33	g
NH_4Cl	0.25	g
$Fe(NH_4)2(SO_4)2 \times 6\ H_2O$	0.01	g
Modified Wolin's mineral solution	10.00	ml
Na_2CO_3	1.50	g
Na-pyruvate	0.50	g
Na-lactate	0.50	g
Yeast extract	0.10	g
$Na_2S_2O_3 \times 5\ H_2O$	1.50	g
Wolin's vitamin solution	10.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, pyruvate, lactate, yeast extract, thiosulfate and vitamins), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add pyruvate, lactate, yeast extract, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of vitamins and thiosulfate are sterilized by filtration. Adjust pH of the complete medium to 6.7. Prior to inoculation reduce medium with 10 - 20 mg/l sodium dithionite, added from a 5% (w/v) solution freshly prepared under N_2 and filter sterilized. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnSO_4 \times 7\ H_2O$	0.18	g
$CuSO_4 \times 5\ H_2O$	0.01	g
$AlK(SO_4)2 \times 12\ H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.01	g

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- Modify
  - Volume
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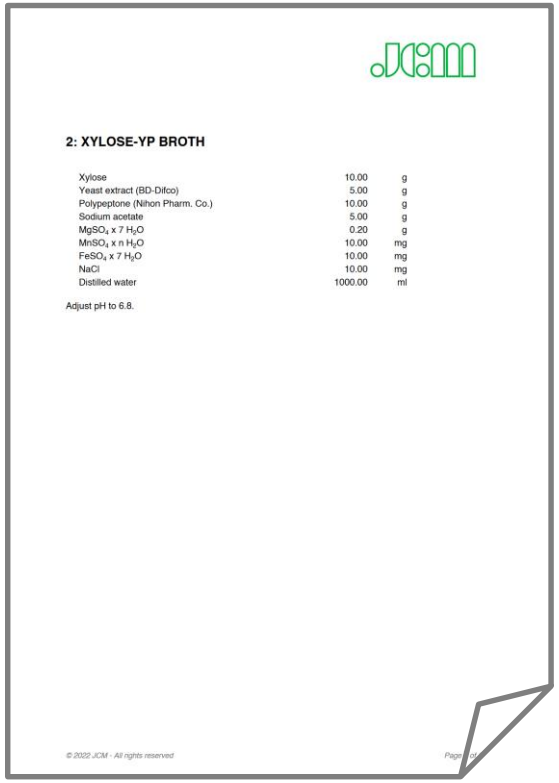
- Export
  - Download tables as CSV
  - Download media and solutions as PDF recipes
- Connect
  - Strains and ingredients are linked to other resources



JCM

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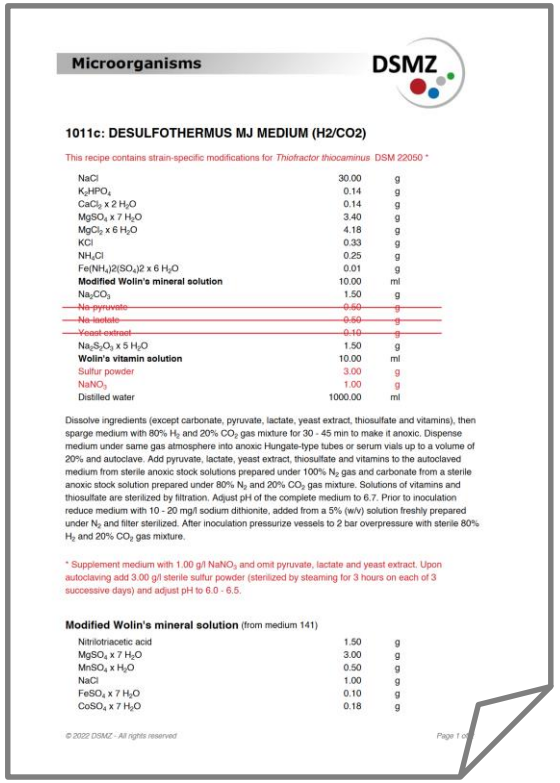
Xylose	10.00	g
Yeast extract (BD-Difco)	5.00	g
Polypeptone (Nihon Pharm. Co.)	10.00	g
Sodium acetate	5.00	g
$MgSO_4 \times 7\ H_2O$	0.20	g
$MnSO_4 \times n\ H_2O$	10.00	mg
$FeSO_4 \times 7\ H_2O$	10.00	mg
NaCl	10.00	mg
Distilled water	1000.00	ml

Adjust pH to 6.8.



Microorganisms DSMZ

1011c: DESULFOTHERMUS MJ MEDIUM (H2/CO2)

This recipe contains strain-specific modifications for *Thiofractor thiocaminus* DSM 22050 *

NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2 \times 2\ H_2O$	0.14	g
$MgSO_4 \times 7\ H_2O$	3.40	g
$MgCl_2 \times 6\ H_2O$	4.18	g
KCl	0.33	g
NH_4Cl	0.25	g
$Fe(NH_4)2(SO_4)2 \times 6\ H_2O$	0.01	g
Modified Wolin's mineral solution	10.00	ml
Na_2CO_3	1.50	g
~~Na-pyruvate~~	~~0.50~~	~~g~~
~~Na-lactate~~	~~0.50~~	~~g~~
~~Yeast extract~~	~~0.10~~	~~g~~
$Na_2S_2O_3 \times 5\ H_2O$	1.50	g
Wolin's vitamin solution	10.00	ml
Sulfur powder	3.00	g
$NaNO_3$	1.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, pyruvate, lactate, yeast extract, thiosulfate and vitamins), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add pyruvate, lactate, yeast extract, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of vitamins and thiosulfate are sterilized by filtration. Adjust pH of the complete medium to 6.7. Prior to inoculation reduce medium with 10 - 20 mg/l sodium dithionite, added from a 5% (w/v) solution freshly prepared under N_2 and filter sterilized. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

* Supplement medium with 1.00 g/l $NaNO_3$ and omit pyruvate, lactate and yeast extract. Upon autoclaving add 3.00 g/l sterile sulfur powder (sterilized by steaming for 3 hours on each of 3 successive days) and adjust pH to 6.0 - 6.5.

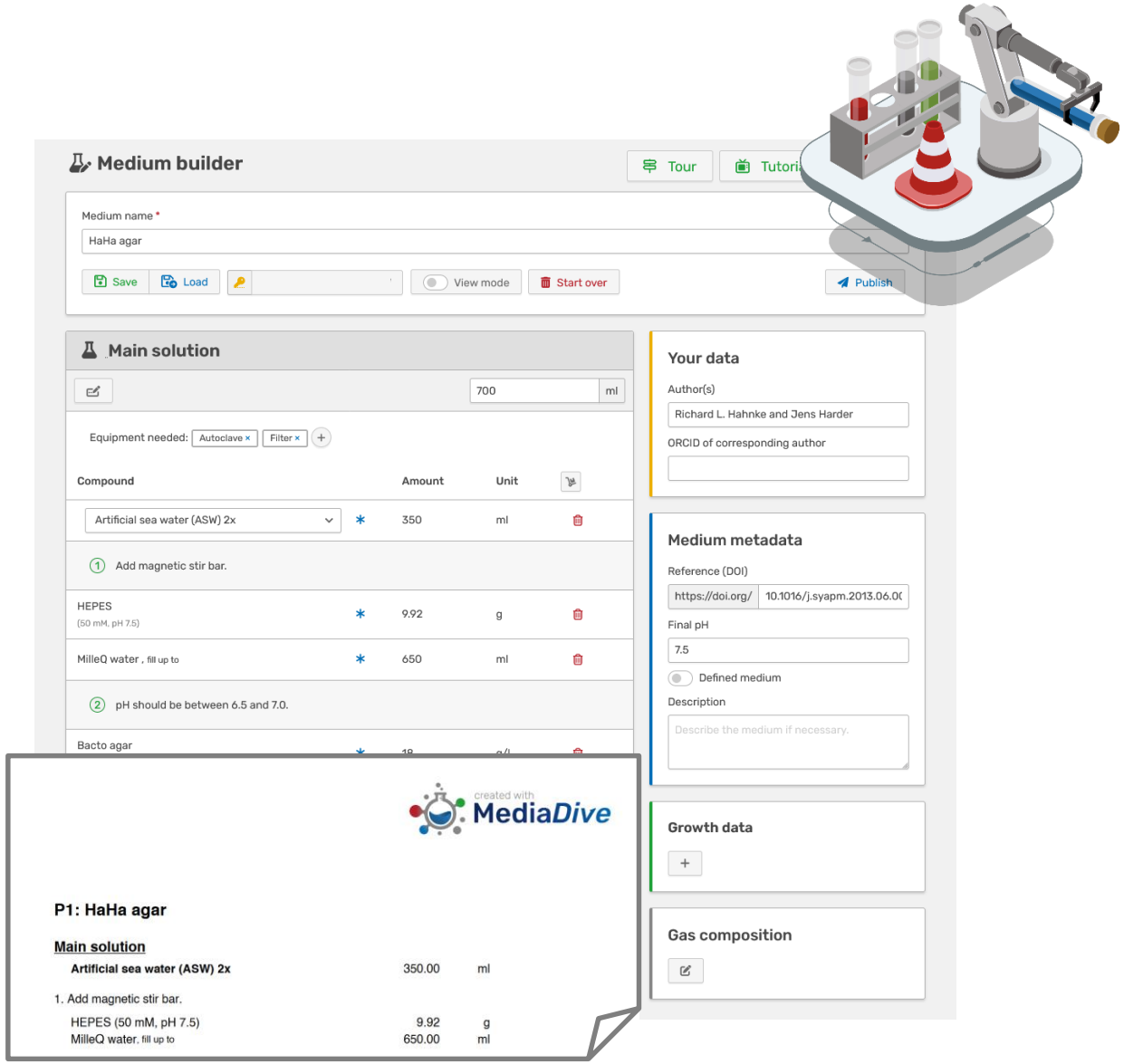
Modified Wolin's mineral solution (from medium 141)

Nitriotriacetic acid	1.50	g
$MgSO_4 \times 7\ H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7\ H_2O$	0.10	g
$CoSO_4 \times 7\ H_2O$	0.18	g

Community engagement

Medium builder

- Design your own medium
- Add metadata, references and strains
- Publish under FAIR principles
- Get a unique & persistent medium number



Community engagement

Medium builder

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- Add metadata, references and strains
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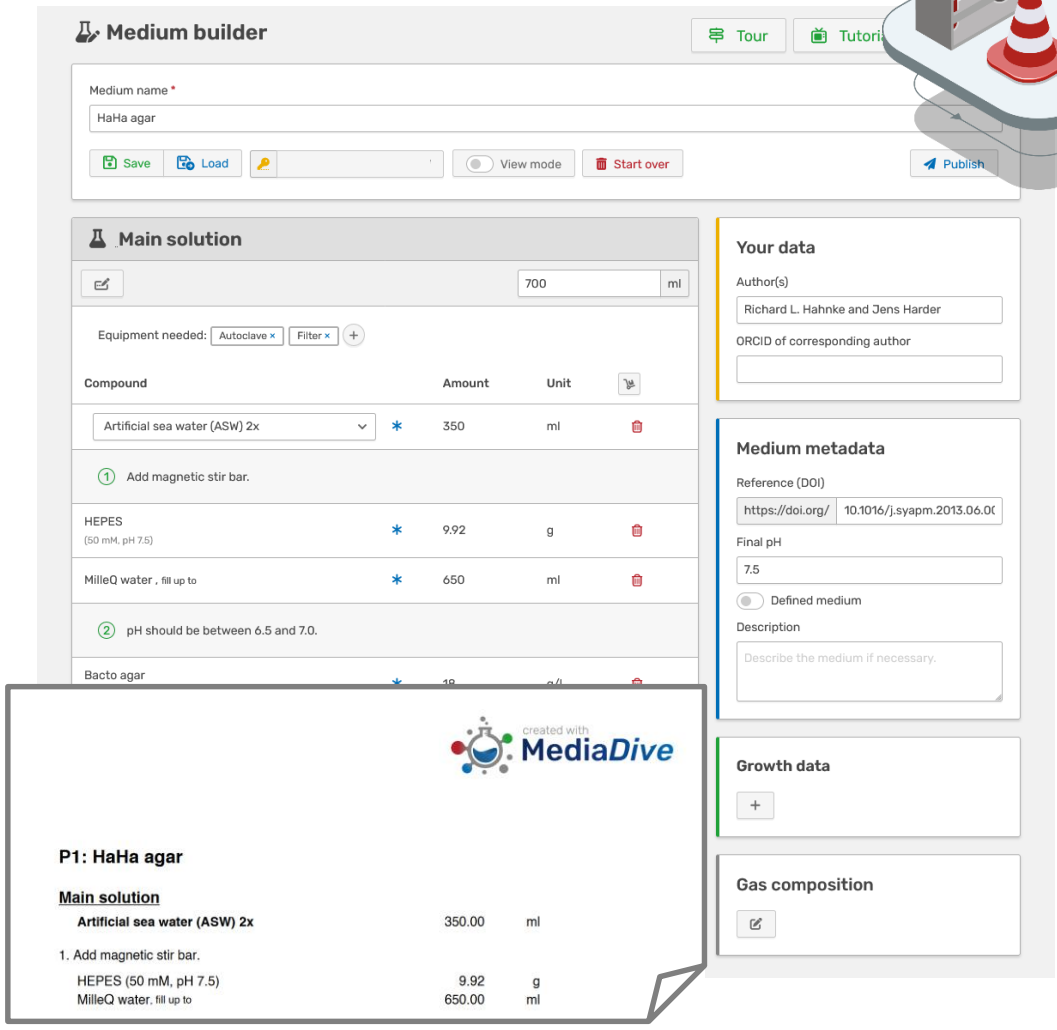
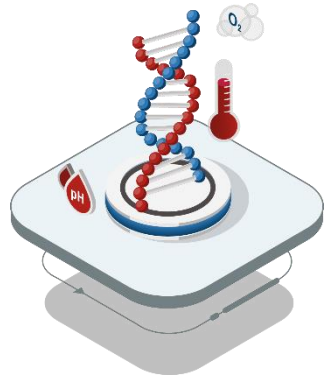
Get in touch

- Tailored transformation process for a large number of cultivation media

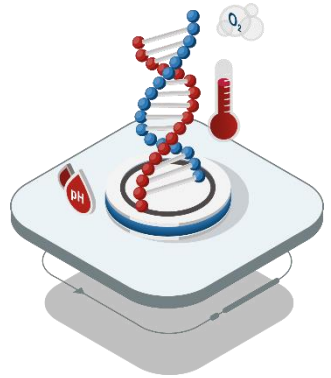


Cultivation media prediction



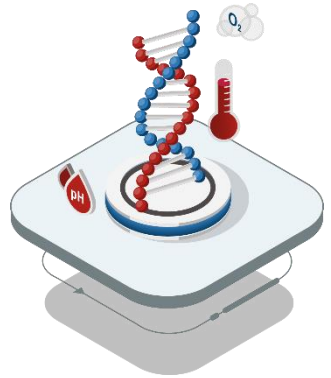
- Prototype currently in alpha test phase

Cultivation media prediction

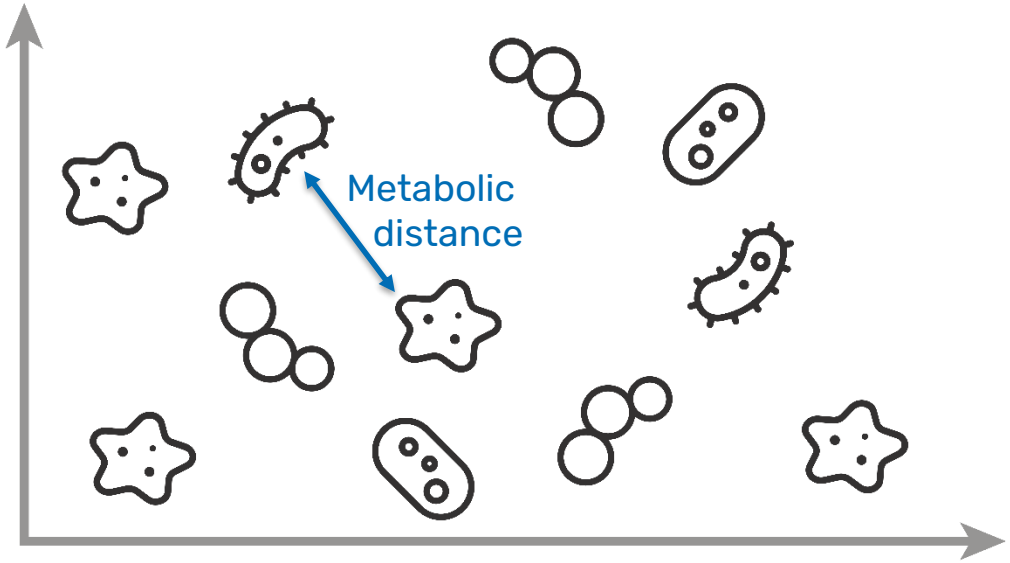


- Prototype currently in alpha test phase
- Find metabolic neighbors with *k*-nearest neighbor (KNN) and *Pfam* profiles

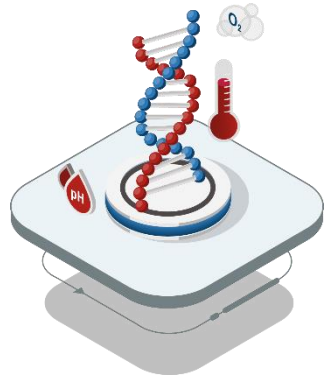
Cultivation media prediction



- Strains are arranged using their metabolic distance
- Find metabolic neighbors with *k*-nearest neighbor (KNN) and *Pfam* profiles



Cultivation media prediction



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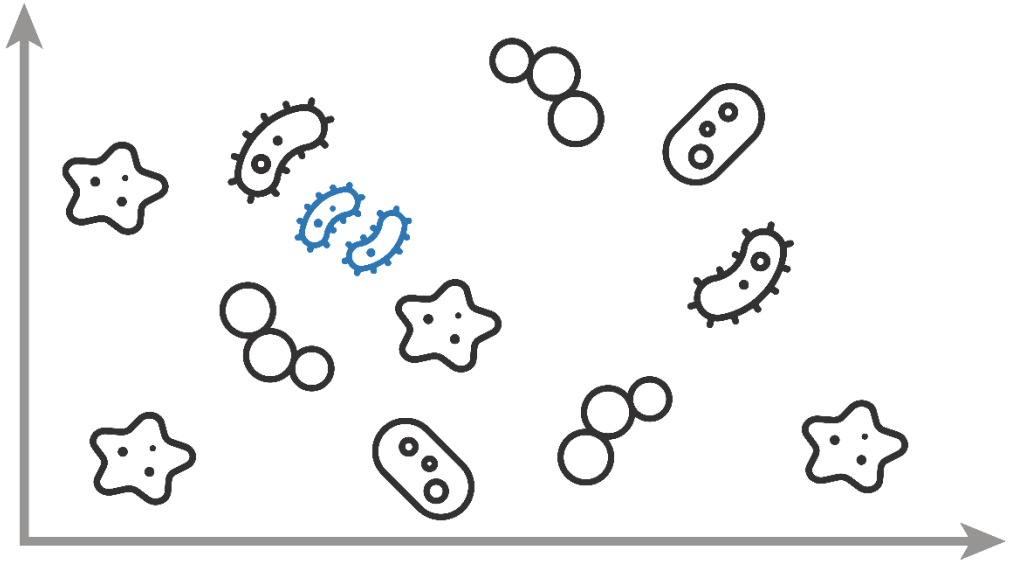
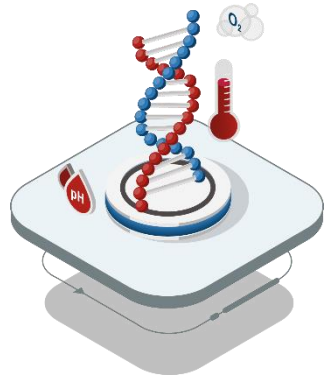
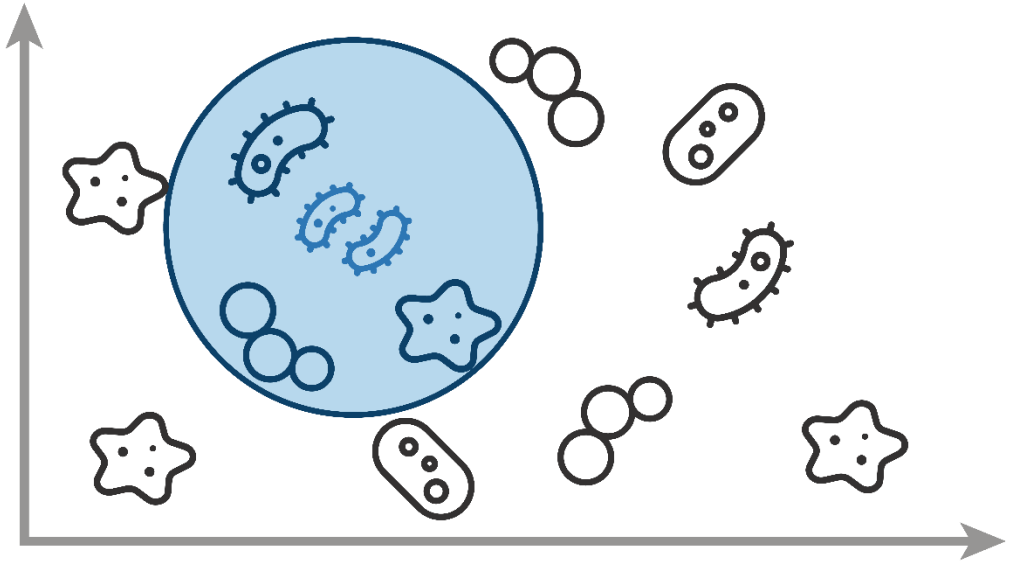


Cultivation media prediction

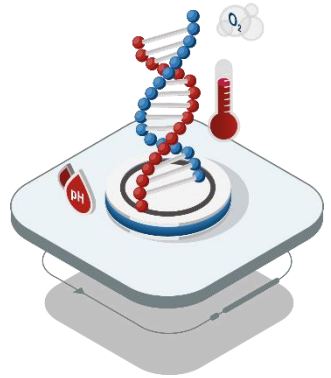


- *k*-Nearest-Neighbor

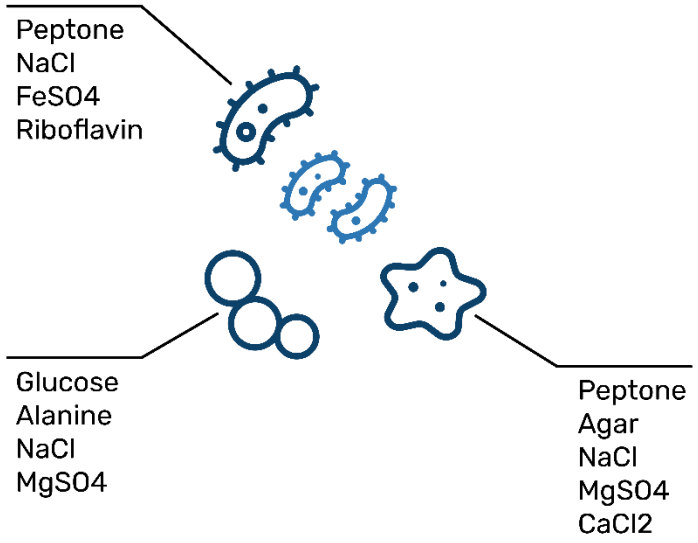
- Find *Metabolic neighbors* and calculate metabolic distances



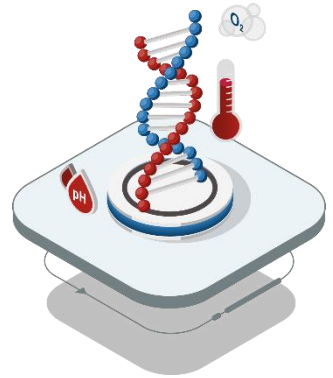
Cultivation media prediction



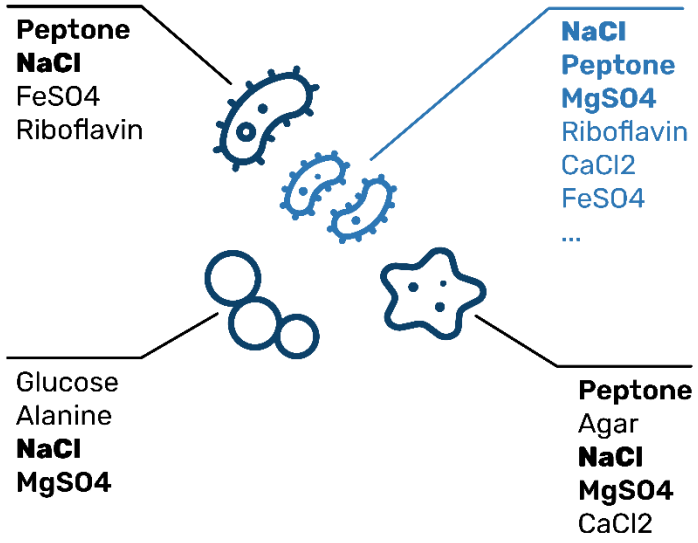
- Medium composition of metabolic neighbors



Cultivation media prediction



- Propose new medium composition
- Find matching media



Best match media

Medium	Score
Medium 87a (used by 2 neighbors) for Pyruvic acid sodium salt use one of these mods: 1386	578
Medium J319	385
Medium 462	284
Medium J303 (used by 1 neighbors)	264
Medium 771	223
Medium 1063	213

Plans for the near future

- Integrate the media prediction tool

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- Expand the taxonomy search for fungi
- Include cultivation media for cell cultures

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- Add a „source search“ based on the Bac*Dive* isolation source ontology

Isolation Source: Soil from a rice paddy in Deok-So, by enrichment with corn oil as the sole carbon

#Condition	#Anoxic (anaerobic)	
#Host	#Plants	#Herbaceous plants (Grass,Crops)
#Environmental	#Terrestrial	#Soil
#Engineered	#Agriculture	#Field

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Media*Dive*: the expert-curated cultivation media database

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