MEDIA RECIPES FOR PHENOTYPIC ASSAYS

Created/updated by: Laura Rossi Date: February 18, 2015
Surette Lab, McMaster University
Hamilton, ON, Canada
www.surettelab.ca

BACKGROUND

- The following recipes allow for the characterization of bacteria with regard to its ability to produce the secreted proteins that are specified for each media type. References are given per media type where applicable.

EQUIPMENT

- Scale, stir plate
- Autoclave

BLOOD PLATES – assay the ability to break down blood cells – Hemolysis.

- Make CBA plates with 5% sheep blood.
- CBA agar is Columbia Blood Agar base, autoclave, cool and add 5% sheep blood.

SKIM MILK PLATES – assay the ability to break down proteins – Proteinases.

- Make BHI agar with 1-1.5% final of skim milk. You can do this in any ratio you want but autoclave the two, BHI and skim milk, separately, then mix after autoclaving.
- Usually make 500mL of BHI agar and 20% skim milk. Autoclave, cool. Add 25mL of 20% skim milk to 500mL BHI for final of 1%.

DNA PLATES – assay the ability to break down DNA – DNases.

- Make BHI agar, add 2g of DNA (Sigma#D1626) to 1L dH_2O for a final of 2mg/mL DNA. Autoclave.
- After growth, for visualizing the zones, the plate needs to be flooded with 1N HCl, the zones will be visible within minutes.

PRUSSIAN BLUE PLATES – assay the ability to produce H_2O_2 (Saito et al, 2007).

- Dissolve 1g $FeCl_3 6H_2O$ in 50mL H_2O and 1g potassium ferricyanide in separate 50mL H_2O. Mix the two solutions to give a reddish brown colour.
- Dissolve BHI agar for 1L into less than 900mL H_2O.
- Add the first mixed solution, 100mL, to BHI solution to form a green precipitate. Top up to 1L.
- Autoclave. Mix well before pouring plates.
- Zones will be bright blue where H_2O_2 is being produced.

HYALURONATE PLATES – assay the ability to produce hyaluronidases. (Rodney, Smith 1968)

- 3.7g BHI broth, 1g agar, 60mL dH_2O. Autoclave. Cool to 45°C-55°C, add 20mL of 5% Bovine Serum Albumin and 20mL of 2mg/mL umbilical cord hyaluronic acid. Pour plates. Cool. Refrigerate before use.
- For analysis flood plates with 2N acetic acid to visualize the zones.