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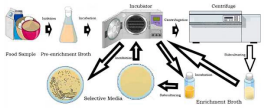
Concentration technique for Viable but Non-Culturable organisms



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Fonseca, F., Cenard, S., & Passot, S. (2015). Freeze-drying of lactic acid bacteria. *Cryopreservation and freeze-drying protocols*, 477–488.
Oxoid (2024) *CM0862 Listeria Enrichment Broth*. (n.d.). Oxoid. Retrieved May 17, 2024, from http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0862&c=UK&lang=EN

Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is based on existing protocols, such as those for resuscitating microbes, freeze-drying, and selective isolation. It merges the parts of the three protocols to create a new protocol for isolating microbes via resuscitation, centrifugation, and selective isolation. The first part involves resuscitating the microbes; the second part subjects the medium to centrifugation (concentration of the microbes); the third and final part involves the selective growth in a selective broth and subsequent growth on selective agar.

Image Attribution

Image Attribution:
The flowchart represents the process of bacterial isolation and cultivation from a food sample for laboratory analysis. The steps involved include resuscitation, centrifugation, and selective isolation. The first part involves resuscitating the microbes; the second part subjects the medium to centrifugation (concentration of the microbes); the third and final part involves the selective growth in a selective broth and subsequent growth on selective agar.

Guidelines

Laboratory protocols must be followed, and the environment must be kept as sterile as possible.

Materials

Petri-dish, Centrifuge Machine, Falcon tubes, Wire-loop, bunsen burner, spatula, vortex shaker, incubator

Safety warnings

Aseptic techniques should strictly be followed

Ethics statement

No animals are harmed/used in this experimental setup

Before start

Wear necessary safety gear before commencing the work

Resuscitation of Microorganism

1 To the resuscitation broth media (e.g., peptone water, tryptic soy broth, Luria-Bertani broth, etc.), add the sample, making a 10% solution, i.e., 10 mg in 90 mL .

2 Incubate the broth culture at 37^{o}C for 24-48 hours 1d

Centrifugation of the resuscitation media 30m

3 The grown broth culture is then centrifuged 5000 rpm, 4°C, 00:30:00 30m

4 Then, concentrate the cell suspension and decant the culture supernatant. 5m

Selective medium growth of desired organism 2d

5 Mix cell pellets with the desired selective medium broth. Suspend cells thoroughly by using a vortex mix 100 rpm, 00:05:00 until complete cell resuspension is achieved aseptically.

6 Then, incubate at the desired temperature 24:00:00 , which can be extended up to 168:00:00 . 1w 1d

7 From the above selective broth, subculture onto a selective agar for the desired organism.

8 incubate the selective agar plate at the required temperature for 24–48 hours. then observe for the desired growth and colony morphologies 1d

9 Store the isolate for further tests (Biochemical, Molecular, etc.)

Protocol references

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