Isolation and identification of listeria species

- Alesha Coleman-McCallum

Title:

Isolation and Identification of Listeria species from chicken sample using Palcam broth (pre-enrichment), UVM 11 broth (selective enrichment), Palcam and Oxford agars (selective plating) also confirmation using biochemical tests.

Objectives:

- To isolate Listeria species from chicken sample
- To observe the reaction of listeria on selective medium
- To confirm the Listeria species using biochemical tests

Introduction

Listeria is a genus of aerobic parasitic, gram positive rod-shaped bacterium (Define, n. d). This genus has more than 10 species with the commonly encountered being: Listeria monocytogenes, Listeria innocua, Listeria ivanovii, Listeria welshimeri, Listeria seeligeri, Listeria grayi, Listeria murrayi. Members of this genus are extensively spread in the environment and maybe found in soil, plants, gastrointestinal tract of animal and humans. Listeria monocytogenes species is of great concern because it is pathogenic to humans and causes Listeriosis. Listeriosis is a foodborne illness (Hardy Diagnostics, 1996).

Listeria monocytogenes is different from most bacteria since it can grow in the cold, salt, acid and air-tight conditions. The increased demand of ready to eat foods especially in first world countries has the potential of listeriosis more eminent. Pregnant women, older adults, young children and

immunocompromised persons are more susceptible to Listeriosis infections. Therefore cooking, pasteurization/applying heating steps to food, avoiding raw meat and milk/ moist or processed food and washing hands regularly may reduce the risk of infections. (FDA, 2004). According to Food Quality & Safety USA [4] a dry and clean work environment is crucial in avoiding listeria outbreaks in the food industry.

Methods for Identification

Methods used in BI208 lab for identification of Listeria were Palcam broth (pre-enrichment) then UVM 11 broth for selective enrichment step, Palcam (selective agent) and Oxford agars (selective and differential agent). For confirmation of species: organism was plated on a blood agar to check for hemolysis. Biochemical test included SIM tube for motility, mannitol, D Xylose and L Rhamnose reactions obtained. Other methods that could be used to identify listeria species are: Polymerase Chain Reaction (PCR), Rapid Identification Kits and Serological tests (MFHPB-07, 2012).

Results

Table showing results obtained from Listeria media reaction

Media	Observations	Reaction
Palcam Agar	Shiny, smooth, circular, convex	No fermentation

	colonies.	No Hydrolsis
Oxford Agar	Black colour around colonies	Esculin Reduction
Blood	Cream, hilly, glistening colonies	No Beta-hemolysis
Mannitol	Transparent colonies	Negative
D Xylose	Transparent colonies	Negative
L Rhamnose	Light yellow fermentation around colonies	Positive
SIM Tube	Umbrella shaped	Positive

growth

Gram Stain – gram positive rods

Assuming Listeria monocytogenes control was used:

Media	Expected Results
Palcam Broth	Cloudy
UVM 11	Cloudy
Palcam Agar	No fermentation Positive Hydrolysis
Oxford Agar	Esculin Reduction
Blood	Beta-hemolysis
Mannitol	Negative
D Xylose	Negative
L Rhamnose	Positive

SIM Tube	Positive

Discussion

Listeria innocua was isolated from the chicken sample. Listeria innocua is not usually implicated in food born-illnesses however an isolated death in an elderly patient was reported (Perrin, Bemer and Delamare, 2003).

Portions of chicken sample were first placed in pre-enrichment broth in order for stressed cells to become viable and all other bacterial cells to multiply. Enrichment stage is to partially suppress unwanted organisms and allow listeria to thrive. Oxford agar (OXA) is both selective and differential. The selective properties of OXA agar will not allow gram negative organisms to grow while suppressing most gram positive organisms (Oxoid, n. d). The different property of OXA agar will allow some species of Listeria to be totally inhibited, growth with or without blackened colonies (MFHPB-07, 2012). Palcam agar utilizes two indicator systems: esculin and mannitol. Listeria monocytogenes changes esculin to a black complex around colonies but does not ferment mannitol. Therefore some gram positive organisms such as enterococci and staphylococci will ferment mannitol hence can be ruled out as contaminants (Oxoid, n. d). Blood agar was used to determine if the organism is beta-hemolytic. Other Biochemical tests were mannitol, D Xylose, L Rhamnose and SIM Tube to differentiate and confirm the species. Since Listeria monocytogenes is pathogenic to humans a 2 Class Sampling Plan would be used to asses if the chicken is fit for consumption. Different species of an organism may exhibit variable characteristics and maybe mistaken. Listeria species were found and should be deemed unacceptable

for consumption. Further testing such as PCR which is very specific could be used to conclusively identify the species present.

Conclusions

Based on the objectives previously outlined, isolation and Identification of Listeria species from chicken sample using Palcam broth method was very effective.

References

1. Define, n. d. Listeria Genus. Retrieved February 22, 2017 from http://www. dictionary. com/browse/listeria
2. Hardy Diagnostics, 1996. Food Borne-illneses Retrieved February 22, 2017 fromhttps://catalog. hardydiagnostics. com/cp_prod/Content/hugo/Listeria. htm
3. FDA, 2004. Food-Borne Pathogenic Microorganisms and Natural Toxins Handbook: The “ Bad Bug Book” U. S. FDA/CFSAN. Center for Food Safety and Applied Nutrition, Food and Drug Administration, College park, MD.
4. Food Quality & Safety (formerly Food Quality), 2007. Retrieved February 20, 2017 from http://www. foodqualityandsafety. com/article/prevent-and-control-listeria
5. Jay, J. M., Loessner, M. J. & Golden, D. A. (2005). Modern Food Microbiology: Indicator of Food Microbial Quality and Safety, 476-477. United States of America: Springer Science+Business Media LLC .
6. MFHPB-07 Method, November, 2012. The isolation of Listeria monocytogenes and other listeria species from food and environment samples using Palcam broth. Retrieved February 20, 2017

fromhttp://www. hc-sc. gc. ca/fn-an/res-rech/analy-meth/microbio/volume2-eng. php

7. Monique Perrin, Michel Bemer and Catherine Delamare, 2003. Fatal Case of Listeria innocua Bacteremia: Journal of Clinical Microbiology
8. Oxoid, n. d. Enrichment Media for Listeria. Retrieved February 20, 2017 fromhttp://www. oxoid. com/uk/blue/prod_detail/prod_detail. asp? pr= CM0856vg= 91&c= uk‹= EN
9. US Government, n. d. Retrieved February 20, 2017 from https://www. foodsafety. gov/poisoning/causes/bacteriaviruses/listeria/
10. Zimbro, M. J., Power D. A., Miller, M. M., Wilson, G. E., Johnson, J. A., (Revised 2009). Difco & BBL Manual: Palcam and Oxford Agar, Culture Media for Specific Groups of Microorganisms. United States of America: Becton, Dickinson and Company.