ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(2): 340-343
doi: 10.25083/rbl/24.2/340.343

Received for publication, April, 20, 2017
Accepted, May 23, 2017

Original paper

Research Note: A comparison of media for the recovery of Campylobacter spp. from long term storage at -80° C

CLARE M. MADDEN[1], ROBERT H. MADDEN[2]

[1]School of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK
[2]Food Microbiology Branch, Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, Northern Ireland

Abstract

Pure cultures of Campylobacter spp. can be stored at -80°C for extended periods, however they eventually lose viability. To maintain cultures regular resuscitation and sub-culturing of strains needs to be undertaken but this requires that staff and media are available for this purpose. Financial pressures on many institutes have had the consequence that regular sub-culturing has become a financial burden.

Accordingly this study was undertaken to compare the ability of inexpensive media to recover campylobacters from storage of up to 12 years at -80°C. Brain heart infusion agar and modified charcoal cefoperazone deoxycholate agar base were compared with blood agar. Overall, blood agar was found to be the best medium for this purpose and is to be recommended.

Keywords Campylobacter, recovery -80°C, media, blood

To cite this article: MADDEN CM, MADDEN RH. Research Note: A comparison of media for the recovery of Campylobacter spp. from long term storage at -80° C. *Rom Biotechnol Lett*. 2019; 24(2): 340-343. DOI: 10.25083/rbl/24.2/340.343

*Corresponding author: ROBERT H. MADDEN, Food Microbiology Branch, Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, Northern Ireland
E-mail: bob_madden@hotmail.com

Introduction

Campylobacter are the principal causes of gastro-enteritis world-wide (JOHNSON & al. [1]) and groups working on this organism frequently store pure cultures of isolates for future study (CORCIONIVOSCHI & al. [2]). Campylobacters are fragile organisms and can lose viability during storage (PORTNER & al. [3]; CODY & al. [4]; PEIREN & al. [5]). Normally, such problems can be avoided by regular resuscitation and re-culturing of the isolates prior to returning them to cryogenic storage. However, reductions in research budgets can lead to resources required for such routine procedures no longer being available. Hence cultures may spend an extended period in storage.

Since frozen storage at -80°C is simpler than freeze drying, and, historically, was more likely to result in viable cultures (PEIREN & al. [5]) it was widely adopted by laboratories. In the Food Microbiology Branch of the Agri-Food and Biosciences Institute, Belfast, studies into campylobacters have been ongoing for over thirty years, with cultures stored from several studies (MADDEN & al. [6]; [7];[8] SCATES & al. [9]; SOULTOS & MADDEN [10]), but regular sub-culturing had not been possible due to a lack of resources. In order to protect the resource represented by the stored isolates a study was undertaken with the aim of discovering the most inexpensive medium for the optimal recovery using isolates which had been stored at -80°C for up to thirteen years.

Materials and Methods

All media were used were from Oxoid, (Basingstoke, UK) unless otherwise stated.

Solid media used were brain-heart infusion agar (BHI, CM1136), *Campylobacter* blood-free selective agar base (CM0739) and blood agar prepared using nutrient agar number 2 (CM0003) supplemented with 5% (v/V) defribrinated horse blood (Oxoid SR048).

The two broths studied were: BHI broth (CM1135), and BHI broth supplemented with 0.2% $FeSO_4$ 7H20, 0.025% sodium metabisulfite, and 0.05% sodium ferrous sulfate (FBP).All w/v. All incubations were microaerobic (85% N_2, 10% CO_2 and 5% O_2, all v/v), and performed in a Don Whitley MACS 500 workstation (Don Whitley Scientific, Shipley, UK). All cultures were initially incubated at 37°C and 42°C.

Isolates were removed from storage at -80°C, thawed, and a loopful streaked onto solid media, or inoculated into broth. After incubation (48h) growth was assessed using a technique based on ecometric principles (RICHARDSON & al. [11]). Sample material was streaked onto solid media in a standard pattern, then a fresh loop used to streak three further areas of the plate. Growth was assessed based on the number of quadrants on which significant growth was seen, i.e. 0 to 4. Where broths had been inoculated, after incubation for 48 h these were streaked onto blood free base, and incubated for 48 h (37°C) as above.

Statistical analysis

All results were analyzed by non parametric statistics using GenStat for Windows Release 14.1. For all analyses, a value of $p < 0.05$ was considered significant.

Results and discussion

Foodborne The study was conducted in two phases. In the first phase 50 isolates which had been stored for between three and five years were selected for study. Campylobacters are fastidious organisms and pure cultures are known to yield more growth following incubation on solid media than in broths (KIEHLBAUCH & al. [12]; KIEHLBAUCH & al. [13]. However, recovery in broth culture was assessed using brain heart infusion broth (BHI) which is a relatively rich medium in regular use in our laboratory. To further assist recovery of injured cells BHI plus FBP was also used, as the supplement can protect injured campylobacters (GEORGE & al. [14]). All 50 of the isolates grew on all of the solid media, following direct plating, Figure 1, and statistical analysis of the results showed that blood agar, frequently used for the preparation of *Campylobacter* cultures (MILITARU et al. [15]), gave the most growth of campylobacters ($p < 0.05$). Considering the broth cultures, 30% of isolates did not grow following recovery in BHI, and 8% did not grow in BHI+FBP. Accordingly work with broths was discontinued. Statistical analysis also showed that none of the solid media showed a significant difference in growth between the two temperatures.

The second phase of the study compared the growth of 50 isolates which had been stored for between ten and thirteen years on the solid media. Once again blood agar

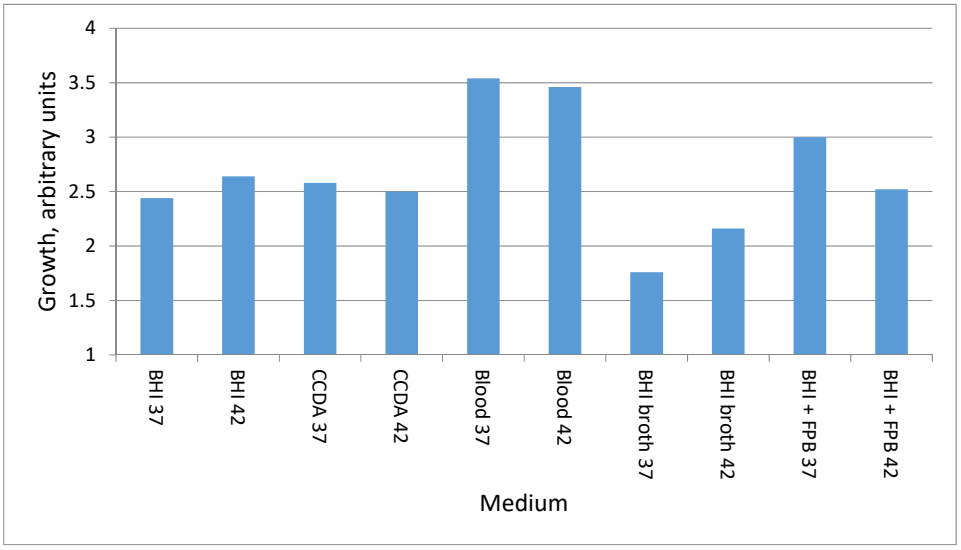

Figure 1. Comparison of growth of 50 *Campylobacter* spp. taken from long term storage at -80°C on five media, at two temperatures.

showed the highest mean growth level, $p < 0.05$, but in this case it also recovered 92% of the isolates at both 37°C and 42°C whereas the respective figures for blood free base were 72% and 64%, and for BHI 82% and 72%.

Overall, this project sought to determine if two inexpensive media, their cost being approximately half of that of blood agar, could be used to successfully recover campylobacters which had been in long term storage at -80°C. It was found that blood agar was significantly superior to brain-heart infusion agar (CM1136) and blood-free selective agar base (CM0739), and is therefore to be recommended for this task.

Conclusion

Blood agar was found to be the most effective medium for the recovery of *Campylobacter* spp. which had been in long term storage at -80°C.

Acknowledgements

This project was funded by the Society for Applied Microbiology, under the auspices of the Students and Graduates into Work Programme.

References

1. JOHNSON, T.J., SHANK, J.M. AND JOHNSON, J.G. Current and potential treatments for reducing *Campylobacter* colonization in animal hosts and disease in humans. *Frontiers in Microbiology*, 8:487 (2017).
2. CORCIONIVOSCHI, N, TELEA, A., PACALA, N. AND ALMEKA, A. A new strategy for gene deletion in *Campylobacter jejuni*. *Romanian Biotechnological Letters*, 14 (3): 4381-4389 (2008).
3. PORTNER, D.C., LEUSCHNER, R.G.K. AND MURRAY, B.S. Optimising the viability during storage of freeze-dried cell preparations of *Campylobacter jejuni*. *Cryobiology*, 54: 265-270 (2007).
4. CODY, W.L., WILSON, J.W., HENDRIXSON, D.R., MCIVER, K.S., HAGMAN, K.E., OTT, C.M., NICKERSON, C.A. AND SCHURR, M.J. Skim milk enhances the preservation of thawed-80 degrees C bacterial stocks. *Journal of Microbiological Methods*, 75: 135-138 (2008).
5. PEIREN, J., BUYSE, J., DE VOS, P., LANG, E., CLERMONT, D., HAMON, S., BEGAUD, E., BIZET, C., PASCUAL, J., RUVIRA, M.A.,

MACIAN, M.C. AND ARAHAL, D.R. Improving survival and storage stability of bacteria recalcitrant to freeze-drying: a coordinated study by European culture collections. *Applied Microbiology and Biotechnology*, 99: 3559-3571 (2015).

6. MADDEN, R.H., MORAN, L. AND SCATES, P. Sub-typing of animal and human *Campylobacter* spp. using RAPD. *Letters in Applied Microbiology*, 23: 167-170 (1996).
7. MADDEN, R.H., MORAN, L. AND SCATES, P. Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent subtyping using polymerase chain reaction restriction fragment length polymorphism and the random amplified polymorphic DNA method. *Journal of Applied Microbiology*, 84: 703-708 (1998).
8. MADDEN, R.H., MORAN, L. AND SCATES, P. Optimising recovery of *Campylobacter* spp. from the lower porcine gastrointestinal tract. *Journal of Microbiological Methods*, 42: 115-119 (2000).
9. SCATES, P., MORAN, L. AND MADDEN, R.H. Effect of incubation temperature on isolation of *Campylobacter jejuni* genotypes from foodstuffs enriched in Preston broth. Applied and *Environmental Microbiology,* 69: 4658-4661 (2003).
10. SOULTOS, N. AND MADDEN, R.H. A genotyping investigation of the colonization of piglets by *Campylobacter coli* in the first 10 weeks of life. *Journal of Applied Microbiology*, 102: 916-920 (2007).
11. RICHARDSON, L.J., COX, N.A., BAILEY, J.S., BERRANG, M.E., COX, J.M., BUHR, R.J., FEDORKA-CRAY, P.J. AND HARRISON, M.A. Evaluation of TECRA broth, Bolton broth, and direct plating for recovery of *Campylobacter* spp. from broiler carcass rinsates from commercial processing plants. *Journal of Food Protection*, 72: 972-977 (2009).
12. KIEHLBAUCH, J.A., BRENNER, D.J., NICHOLSON, M.A., BAKER, C.N., PATTON, C.M., STEIGERWALT, A.G. AND WACHSMUTH, I.K. *Campylobacter butzleri* sp-nov isolated from humans and animals with diarrheal illness. *Journal of Clinical Microbiology*, 29: 376-385 (1991a).
13. KIEHLBAUCH, J.A., PLIKAYTIS, B.D., SWAMINATHAN, B., CAMERON, D.N. AND WACHSMUTH, I.K. Restriction-fragment-length-polymorphisms in the ribosomal genes for species identification and subtyping of aerotolerant *Campylobacter* species. *Journal of Clinical Microbiology*, 29: 1670-1676 (1991b).
14. GEORGE, H.A., HOFFMAN, P.S., SMIBERT, R.M. AND KRIEG, N.R. Improved media for growth and aerotolerance of *Campylobacter fetus*. *Journal of Clinical Microbiology*, 8: 36-41 (1978).
15. MILITARU, D., POPA, V., BOTUS, D., STIRBU, B. AND CAPLAN, E.M. In vitro evaluation of the potential antibacterial effect of artemisinin on *Campylobacter jejuni. Rom Biotechnol Lett*, 20 (2), 10221-10227 (2015).