Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (10): 792-795, 2007
ISSN 1816-4935

Comparison of Three Enrichment Broths for the Isolation of Thermotolerant Acetic Acid Bacteria from Flowers and Fruits

Supawadee Sudsakda, Warinda Srichareon and Wasu Pathom-Aree
Microbiology Section, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract: The effectiveness of three enrichment broths, namely potato medium, seed culture medium and sterile distilled water for the isolation of thermotolerant acetic acid bacteria from flowers and fruits was investigated. The numbers of successful isolation obtained from each medium were nearly the same. Seed culture medium was found to give the highest numbers of successful isolation from flower samples whereas potato medium and sterile distilled water gave higher numbers for the isolation from fruits. However, there were no statistically significant differences between the media tested (α>0.05).

Key words: Acetic acid bacteria, enrichment medium, thermotolerant, isolation

INTRODUCTION

Acetic acid bacteria are obligate aerobic Gram negative bacteria which are capable of oxidize ethanol to acetic acid (Matsushita *et al*., 1994). They are classified in the family Acetobacteraceae and widely distributed in natural habitats (Holt *et al*., 1994). Members of this family are useful in industrial applications including production of vinegar and L-ascorbic acid (Deppenmeier *et al*., 2002). The global warming crisis in the past few years is a serious challenge to fermentation industries since a large cooling system is required for maintaining the optimum temperature. Recently, there has been interest in thermotolerant bacteria due to their economic profits (Saeki *et al*., 1997a). Isolation, identification and characterization of acetic acid bacteria were set in progress to develop new microbial resources for oxidative fermentation (Moonmangmee *et al*., 2000; Adachi *et al*., 2003).

The standard procedure for isolation of acetic acid bacteria from samples of natural origin usually involve the use of enrichment culture technique since acetic acid bacteria are not present as dominant specie (Yamada *et al*., 2000; Lisdiyanti *et al*., 2002; Jojima *et al*., 2004). However, to date, no studies attempting to evaluate the efficiency of enrichment media for the isolation of acetic acid bacteria have been reported. Hence, it is the purpose of this study to compare the effect of three different enrichment media for the isolation of acetic acid bacteria especially thermotolerant strains from flowers and fruits.

MATERIALS AND METHODS

This research was carried out at Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand during June 2002-March 2003.

Samples

Fifteen species of flower (Table 1) commonly found in northern part of Thailand were collected from area within Chiang Mai University main campus. Fifteen fruit samples (Table 2) were purchased from local market.

Corresponding Author: Wasu Pathom-Aree, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand Tel: 66-53-941948 Fax: 66-53-892259

Selective Isolation of Thermotolerant Acetic Acid Bacteria

Acetic acid bacteria were isolated from each sample by enrichment culture technique using three enrichment broths: potato medium (0.5% glucose, 1% yeast extract, 1% peptone, 2% glycerol and 1.5% potato extract), seed culture medium (0.5% glucose, 0.5% yeast extract, 0.5% peptone and 0.5% glycerol) and sterile distilled water. Each enrichment broth was supplemented with 4% ethanol (v/v) and the initial pH was adjusted to 7.0. Approximately 5 g of flowers or fruits were added to the medium and incubated at 37°C for 3-5 days to select thermotolerant strains. Acetic acid bacteria were isolated by streaking the content of each enrichment broth onto potato agar plate to which 0.003% bromocresol purple, 4% ethanol (v/v) and 1.5% agar were added. The colonies producing yellow halo were selected and presumptively identified as acetic acid bacteria. All selected colonies were restreaked onto fresh new plate to obtain pure culture and subjected to detailed taxonomic characterization.

Taxonomic Characterization

Morphological, physiological and biochemical characteristics of pure isolates were examined according to the ninth edition of Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The ability of the isolate to oxidize acetate to CO_2 and H_2O was used to distinguish between members of the genus *Acetobacter* and *Gluconobacter*.

Statistical Analysis

The significance of differences in numbers of successful isolation was evaluated with the non-parametric Cochran Q test (Rall *et al*., 2005) using SPSS software package.

RESULTS AND DISCUSSION

The present communication compared the effect of three different enrichment media for the isolation of thermotolerant acetic acid bacteria from flowers and fruits. Acetic acid bacteria usually present in small numbers so it is necessary to increase their numbers to detectable level using enrichment culture technique. An enrichment culture usually provides nutrients and environmental conditions that favor the growth of microorganisms of interest. In this study, ethanol was used as a selective nutrient to select acetic acid bacteria. All media were success in promoting the growth of acetic acid bacteria. This result supported the use of enrichment culture as standard procedure for the isolation of acetic acid bacteria from environmental samples (Yamada *et al*., 2000; Lisdiyanti *et al*., 2002; Jojima *et al*., 2004).

Of the media tested, seed culture medium gave the highest numbers of successful isolation from flowers (Table 1) whereas potato medium and sterile distilled water gave the best result for fruit

Table 1: Isolation of thermotolerant acetic acid bacteria from 15 flowers using three different enrichment media

Flowers	Potato medium	Seed culture medium	Sterile distilled water
Acacia auriculaeformis	+	+	+
Aster cordifolius	+	+	-
Bixa orellana	+	-	-
Brunfelsia hopeana	-	+	-
Caesalpinia pulcherrima	-	+	-
Conna indica	-	+	+
Gardenia collinsae	-	+	-
Hibiscus hybrid	-	-	+
Ixora congesta	+	-	-
Lantana trifolia	-	+	+
Mirabilis jalapa	+	+	+
Murraya paniculata	+	+	+
Portulaca grandiflora	-	-	+
Pyrostegia venusta	-	-	+
Rosa hybrid	+	+	+
Total	7	10	9

+ : Successful isolation; - : Unsuccessful isolation

Table 2: Isolation of thermotolerant acetic acid bacteria from 15 fruits using three different enrichment media

Fruits	Potato medium	Seed culture medium	Sterile distilled water
Banana	-	+	+
Cantaloup	+	+	+
Guava	+	+	+
Jack fruit	+	+	+
Java apple	+	+	+
Orange	-	-	-
Orange (Freemont)	+	-	+
Papaya	-	+	-
Pineapple	+	-	+
Rose apple	+	+	+
Strawberry	+	+	-
Sweet orange	+	+	+
Tomato	+	+	-
Water melon (red)	-	-	+
Water melon (yellow)	+	-	-
Total	12	11	12

+: Successful isolation; - : Unsuccessful isolation

samples (Table 2). However, statistical analysis revealed that there were no significant differences between the media tested. This result suggested that these media could be used interchangeably. Comparison of these results with those earlier studies was difficult as so little relevant research has been done and many studies on the isolation of acetic acid bacteria were limited to only the mesophilic group (Yamada *et al*., 2000; Lisdiyanti *et al*., 2002; Hanmoungjai *et al*., 2007).

All isolates were identified as *Gluconobacter* sp. due to their inability to oxidize acetate. Most acetic acid bacteria are known to be mesophilic with an optimum temperature for growth around 30°C. A slight increase in temperature resulted in a dramatic decrease in growth of these organisms (Saeki *et al*., 1997b). All isolates grew well at 37°C, the character which suggested that these isolates may be thermotolerant strains (Saeki *et al*., 1997a; Moonmangmee *et al*., 2000; Adachi *et al*., 2003). The observation that all isolates were member of the genus *Gluconobacter* indicated that flowers may be good sources for isolation of acetic acid bacteria. This is in agreement with previous report that natural habitats of *Gluconobacter* strains are flowers and fruits (Gupta *et al*., 2001). Several thermotolerant *Gluconobacter* strains had also been isolated from flowers, fruits and vegetables collected in Chiang Mai (Jaiinphon *et al*., 2002). Most of these isolates showed ability to utilize sorbitol, an indication of sorbitol dehydrogenase activity. This enzyme is responsible for the oxidation of D-sorbitol to L-sorbose, a precursor in fructose and ascorbic acid production (Matsushita *et al*., 1994).

CONCLUSIONS

The obtained results indicated that the three media tested are equally effective in enrichment purpose for the isolation of thermotolerant acetic acid bacteria present in flowers and fruits. However, the use of sterile distilled water supplemented with 4% ethanol (v/v) was recommended as the enrichment medium which led to the reduction in both time and cost in media preparation. These results also added to a list of references support the usefulness of enrichment culture technique for isolation of acetic acid bacteria from environmental samples.

ACKNOWLEDGMENTS

The authors are grateful to Department of Biology, Faculty of Science, Chiang Mai University for all facilities and Laboratory provided. Thanks also go to Associate Professor Dr. Araya Jatisatien for statistical analysis and CMU Herbarium for identification of all flower samples.

REFERENCES

Adachi, O., D. Moonmangmee, H. Toyama, M. Yamada, E. Shinagawa and K. Matsushita, 2003. New developments in oxidative fermentation. Applied Microbiol. Biotechnol., 60: 643-653.

Deppenmeier, U., M. Hoffmeister and C. Prust, 2002. Biochemistry and biotechnological applications of *Gluconobacter* strains. Applied Microbiol. Biotechnol., 60: 233-242.

Gupta, A., V.K. Singh, G.N. Qazi and A. Kumar, 2001. *Gluconobacter oxydans*: Its biotechnological applications. J. Mol. Microbiol. Biotechnol., 3: 445-456.

Hanmoungjai, W., E. Chukeatirote, W. Pathom-aree, Y. Yamada and S. Lumyong, 2007. Identification of acidotolerant acetic acid bacteria isolated from Thailand sources. Res. J. Microbiol., 2: 194-197.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Stanley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore.

Jaiinphon, J., K. Thonglem and W. Pathom-aree, 2002. Selective isolation of sorbitol utilizing acetic acid bacteria. Chiang Mai J. Sci., 29: 189-192.

Jojima, Y., Y. Mihira, S. Suzuki, K. Yokozeki, S. Yamanaka and R. Fudou, 2004. *Saccharibacter floricola* gen. nov., sp. nov., a novel osmophilic acetic acid bacterium isolated from pollen. Int. J. Syst. Evol. Microbiol., 54: 2263-2267.

Lisdiyanti, P., H. Kawasaki, Y. Widyastuti, S. Saona, T. Seki, Y. Yamada, T. Uchimura and K. Komagata, 2002. *Kozakia baliensis* gen. nov., sp. nov., a novel acetic acid bacterium in the α-*Proteobacteria*. Int. J. Syst. Evol. Microbiol., 52: 813-818.

Matsushita, K., H. Toyama and O. Adachi, 1994. Respiratory chain and bioenergetics of acetic acid bacteria. Adv. Microb. Physiol., 36: 247-301.

Moonmangmee, D., O. Adachi, A. Yoshitaka, E. Shinagawa, H. Toyama, G. Theeragool, N. Lotong and K. Matsushita, 2000. Isolation and characterization of thermotolerant *Gluconobacter* strains catalyzing oxidative fermentation at high temperature. Biosci. Biotechnol. Biochem., 64: 2306-2315.

Rall, V.L.M., R. Rall, L.C. Aragon and M.G. de Silva, 2005. Evaluation of three enrichment broths and five plating media for *Salmonella* detection in poultry. Brazillian J. Microbiol., 36: 147-150.

Saeki, A., G. Theeragool, K. Matsushita, H. Toyama, N. Lotong and O. Adachi, 1997a. Development of thermotolerant acetic acid bacteria useful for vinegar fermentation at higher temperatures. Biosci. Biotechnol. Biochem., 61: 138-145.

Saeki, A., M. Taniguchi, K. Matsushita, H. Toyama, G. Theeragool, N. Lotong and O. Adachi, 1997b. Microbiological aspects of acetate oxidation by acetic acid bacteria, unfavourable phenomena in vinegar fermentation. Biosci. Biotechnol. Biochem., 61: 317-323.

Yamada, Y., K. Katsura, H. Kawasaki, Y. Widyastuti, S. Saono, T. Seki, T. Uchimura and K. Komagata, 2000. *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α-*Proteobacteria*. Int. J. Syst. Evol. Microbiol., 50: 823-829.