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Evaluation the spoilage and biogenic amines formation potential of marine Gram-positive bacteria

[1*]Al Bulushi, I. M., [2]Poole, S., [3]Deeth, H.,C. and [4]Dykes, G., A.

[1]Department Food Science and Nutrition, College of Agricultural and Marine Science, Sultan Qaboos University, P. O. Box 34, Al Khod 123, Sultanate of Oman
[2]Queensland Department of Primary Industries and Fisheries, Hamilton, QLD 4007, Australia
[3]School of Land, Crop and Food Sciences, University of Queensland, Brisbane, QLD 4072, Australia
[4]Faculty of Health Sciences, School of Public Health, Bentley Campus, Curtin University, GPO Box U1987, Perth Western Australia 6845, Australia

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Abstract

The ability of Gram-positive bacteria to form biogenic amines from different sources has been well documented; however, this ability and the spoilage potential of Gram-positive bacteria from marine sources have not been investigated. Therefore, this study aimed to evaluate the spoilage potential and the potential to form biogenic amines of 228 Gram-positive bacteria isolated from sub-tropical marine fish through their abilities to utilize organic and inorganic sulphur-containing sources, reduce trimethylamine oxide (TMAO) to trimethylamine (TMAO) and decarboxylate histidine, lysine and ornithine. Strains of *Brevibacillus borstelensis* (two), *Streptococcus uberis* (one), *Vagococcus fluvialis* (two) utilized sodium thiosulphate, cysteine and methionine. However, strains varied in sulphur source utilization. *Exiguobacterium acetylicum* (one), *Exiguobacterium* spp. (one), *Carnobacterium* spp. (one), *Brev. borstelensis* (two), *Streptococcus uberis* (two) and *Vagococcus fluvialis* (two) reduced TMAO. Histidine was not decarboxylated by any Gram-positive bacteria. Lysine and ornithine were decarboxylated mainly by strains of *Staphylococcus warneri* (eight), *Staphylococcus epidermidis* (seven) and *Micrococcus luteus* (two). This study found that Gram-positive bacteria of marine source were weak spoilers, however they had good potential to produce some biogenic amines and their potential was strain-dependent.

Introduction

A few studies have studied the potential of marine Gram-positive bacteria such as *Micrococcus, Staphylococcus* and *Bacillus* species to form TMA, cadaverine, putrescine, and histamine (Chandrasekeran *et al*., 1987; Ananthalakshmy *et al.,* 1990; Lakshmanan *et al.,* 2002a) but none of these studies characterized these abilities in these genera in detail. Moreover, the production of typical spoilage products such as hydrogen sulphide by the common marine Gram-positive bacteria such as *Micrococcus, Staphylococcus* and *Bacillus* has not been reported. Lactic acid bacteria from non-marine sources, on the other hand, have been found to produce biogenic amines and sulphide compounds. For example, *Lactobacillus* spp. were found to produce H_2S, *Leuconostoc* spp. and *Lactobacillus plantarum* to form tyramine and histamine, and *Leuconostoc mesenteroides* spp. mesenteroides to form cadaverine and putrescine (Borch and Agerhem 1992; Moreno-Arribas 2003; Arena *et al*., 2007; Anita *et al*., 2007).

In some storage studies of tropical marine fish, a few genera of Gram-positive bacteria, such as *Micrococcus* spp. and *Bacillus* spp., were found to dominate at the time the product was rejected (Chinivasagam *et al.,* 1985; Barile *et al*., 1985). However, the roles of these genera in spoilage and formation of biogenic amines have not been elucidated. This study therefore aimed to investigate the spoilage and biogenic amine formation potential of some Gram-positive bacterial species isolated from three species of sub-tropical marine fish.

Materials and Methods

Bacterial isolates

Bacterial isolates (Table 1) were isolated from fresh and ambient-temperature-stored *Pseudocaranx dentex, Pagrus auratus* and *Mugil cephalus* and identified to the genus and species levels as described previously (Al Bulushi *et al*., 2008; Al Bulushi *et*

*Corresponding author.
Email: *isab@squ.edu.om, ismailalbulushi2014@gmail.com*

Table 1. Gram-positive bacteria isolated from sub-tropical marine fish and assayed for production of sulphide compounds, reduction of TMAO and decarboxylation of amino acids

Species	No. Strains	Species	No. Strains
Staphylococcus warneri	16	*B. fusiformis*	1
Staph. epidermidis	10	*B. mycoides*	2
Staph. auricularis	8	*B. cereus/B. thuringiensis*	1
Staph. capitis	3	*Brevibacillus borstelensis*	11
Staph. sciuri	4	*Corynebacterium xerosis*	3
Staph. xylosus	2	*Streptococcus uberis*	40
Staph. haemolyticus	1	*Strep. equinus*	12
Staph. cohni	1	*Strep. salivarius*	2
Staph. simulans	3	*Strep. constellatus*	4
Staphylococcus spp.	9	*Enterococcus faecium*	35
Micrococcus luteus	21	*Carnobacterium* spp.	1
M. lylae	7	*Exiguobacterium acetylicum*	1
Virgibacillus pantothenticus	7	*Exiguobacterium* spp.	1
Bacillus megaterium	5	*Vagococcus fluvialis*	2
B. sphaericus	15	Total	228

al., 2010). Initially, the isolates were prepared by subculturing in tryptone soya broth (TSB, Oxoid, UK) and incubating at 32°C for 36-48 h.

Control bacterial strains

Shewanella putrefaciens ACM 4733, (ATCC 49138) and *Morganella morganii* ssp. *morganii* ACM 2471 (ATCC 25830, JCM 1672T) were obtained from the Australian Collection of Microorganisms, Department of Microbiology, University of Queensland, Australia. *Shewanella putrefaciens* was used as a control to produce sulphidy compounds in sulphide, endole and motile agar (SIM) and in iron agar (IA) and reduce TMAO in TMAO-medium. *Morganella morganii* ssp. *Morganii* was used as a control to decarboxylate histidine in histidine-decarboxylase medium (HD-medium). *Shewanella putrefaciens* was found to produce sulphidy compounds and reduce TMAO (Poole, 2008), whereas, *Morganella morganii* ssp. morganii was found to produce histamine (Takahashi *et al*., 2003). Both strains were sub-cultured in TSB before use as the bacterial isolates.

Production of sulphide compounds and reduction of trimethylamine oxide

Fish isolates were tested for production of sulphide compounds from sodium thiosulphate (inorganic source) and from cysteine and methionine (organic sources). For the inorganic sulphur source, 100 µl of 36-48 hour-old isolate was inoculated in sulphide indole motile agar (Oxoid, UK) and incubated at 32°C for 48 h. Production of sulphide compounds was assessed by turning the medium to black colour. For assessing production of sulphide compounds from cysteine and methionine, iron agar base (Gram *et al*., 1987) was used. The ability of isolates to reduce TMAO was determined using TMAO-medium (Gram *et al.*, 1987).

Decarboxylation of amino acids

Lysine, histidine and ornithine were selected to evaluate the potential of the isolates to produce cadaverine, histamine and putrescine respectively, as these biogenic amines were associated with potentiation of histamine toxicity, scombroid poisoning and nitrosamine formation (Hwang *et al.,* 1995; Hwang *et al*., 1999; Al Bulushi *et al.,* 2009). The ability of isolates to decarboxylate histidine, lysine and ornithine was assessed using HD-medium developed by Yamani and Untermann (1985) with a slight modification in that pyridoxine hydrochloride, the cofactor that was used by Frank *et al.* (1985) to demonstrate lysine and ornithine decarboxylase activity, was added to HD-medium to study the decarboxylation of lysine and ornithine.

Results and Discussion

Production of sulphide compounds and reduction of trimethylamine oxide

No isolates of *Staphylococcus, Micrococcus, Bacillus* and *Corynebacterium* species were positive for production of sulphide compounds or TMAO reduction. Some strains of *Brev. borstelensis, Strep. uberis* and *Vag. fluvialis* produced sulphide compounds from sodium thiosulphate, cysteine and methionine (Table 2). Variation in the ability to utilize sulphur sources was found among bacterial species (Table 2). For instance, *Brev. borstelensis* 291 utilized organic-sulphur-containing cysteine and methionine but did not use inorganic sodium thiosulphate. In contrast, *Brev. borstelensis* 73 used only cysteine.

Nine species of *Exiguobacterium acetylicum, Exiguobacterium* spp., *Carnobacterium* spp., *Brev. borstelensis, Strep. uberis* and *Vag. fluvialis* reduced TMAO (Table 2) however, TMAO reduction was found to be strain dependent. *Brevibacillus borstelensis* 73, *Brev. borstelensis* 291, *Strep. uberis* 339, *Vag. fluvialis* 31 and *Vag. fluvialis* 132 were found to produce sulphide compounds and reduce TMAO. Although, TMAO has not been found to be

Table 2. Potential of Gram-positive bacterial strains to produce sulphide compounds from different sulphide sources and to reduce trimethylamine oxide

Species	Strain with spoilage potential	Production of VSC			Reduction of TMAO
		Sodium thiosulphate	Cysteine	Methionine	
Brev. borstelensis (11)	*Brev. borstelensis* 73	–	+	–	+
	Brev. borstelensis 291	–	+	+	+
Strep. uberis (40)	*Strep. uberis* 30	–	–	–	+
	Strep. uberis 339	+	–	–	+
Carnobacterium spp. (1)	*Carnobacterium* spp. 338	–	–	–	+
Ex. acetylicum (1)	*Ex. acetylicum* 5	–	–	–	+
Exiguobacterium spp. (1)	*Exiguobacterium* spp. 191	–	–	–	+
Vag. fluvialis (2)	*Vag. fluvialis* 31	+	–	–	+
	Vag. fluvialis 132	+	+	+	+
Shewanella putrefaciens (control) (1)	ACM 4733	+	+	ND	+
Total		3	3	2	9

ND : Not Determined

reduced by Gram-positive bacteria previously, the current study found initial indication of such possible potential. This potential, however, needs to be further investigated by quantitative approach.

Micrococcus luteus 790 isolated from cheese and *Staph. xylosus* 870 from a culture collection were found to produce significant levels of H_2S in brain heart infusion broth with addition of cysteine (Lozano *et al*., 2007). No strains of *Micrococcus* and *Staphylococcus* species tested in our study showed ability to produce sulphide compounds. This discrepancy could be attributed to difference in the bacterial strains and pH of the testing medium. The impacts of both factors were studied by Lozano *et al*. (2007). The effect of bacterial strain on sulphide compounds production was also apparent in our study where among 11 *Brev. borstelensis* strains with only Brev. borstelensis 73 and Brev. borstelensis 291 were found positive to produce sulphide compounds.

Although, sulphide compounds were not quantified in the current study, black colonies found on the iron agar medium showed ability to produce H_2S in a previous study (Gram *et al.*,1987). This is the first study to report a possible spoilage role of *Brev. borstelensis, Strep. uberis* and *Vag. fluvialis.* However, the significance of this role will not be clarified until a quantitative comparison with typical sulphide compounds producers such as Shewanella species is conducted.

Decarboxylation of amino acids

Amino acid decarboxylation activities were mostly found among the *Staphylococcus* species, followed by the *Micrococcus* species (Table 3). Histidine was not decarboxylated by any of the 228 bacterial isolates tested which could indicate that Gram-positive bacteria of marine source lack the potential for form histamine. Among *Staphylococcus* species, 50% of *Staph. warneri* strains decarboxylated lysine and ornithine, and 60% and 70% of *Staph. epidermidis* decarboxylated lysine and ornithine respectively. *Staphylococcus capitis* and *Staph. sciuri* showed decarboxylase activity for lysine and ornithine. Moreover, *Micrococcus luteus* decarboxylated lysine and ornithine but only a few strains had this ability.

Staphylococcus warneri accounted for 44% and 33% of total bacteria positive for decarboxylation of ornithine and lysine respectively, whereas Staph. epidermidis accounted for 33% and 29%. In many cases, the same strain decarboxylated both amino acids. No *Bacillus, Virgibacillus, Corynebacterium, Streptococcus* or *Enterococcus* species showed decarboxylase activity for lysine and ornithine; however, one isolate of *Brev. borstelensis* decarboxylated lysine (Table 3). Lysine decarboxylation was more diverse among the isolates than that which could indicate that Gram-positive bacteria had more potential to form cadaverine than putrescine in marine fish. Inability of *Bacillus* species of marine source to decarboxylate amino acids in this study conflicted with the findings of Jaw *et al.* (2012) who found *Bacillus licheniformis* (three strains), *B. amyloliquefaciens* (one strain), and *B. subtilis* (one strain) isolated from fish meal were capable to produce 1.31–6.21 ppm of histamine. This discrepancy may attribute to the effect of bacterial source besides the strain and medium as it was explained earlier.

The selection of HD-medium over other decarboxylation media in this study was based initially on the development of this medium to specifically study histidine decarboxylation and its ability to demonstrate the decarboxylation of lysine and ornithine (Yamani and Untermann 1985). In addition, the indicator combination in HD-medium clearly showed the color change when the pH increased from 5.3 to 5.6, at the same time as histamine formation was noticed quantitatively (Yamani and Untermann 1985). Other media such as that used by Moller (1955) as cited by Yamani and Untermann (1985) cannot be used for detection of

Table 3. Number of Gram-positive bacterial strains positive for decarboxylating lysine, ornithine and histidine

Species	LDC	ODC	HDC
Staph. warneri (16)	8	8	–
Staph. epidermidis (10)	7	6	–
Staph. capitis (3)	1	1	–
Staph. sciuri (4)	1	2	–
Staph. xylosus (2)	1	–	–
Staph. simulans (3)	2	–	–
Staphylococcus spp. (9)	1	–	–
M. luteus (21)	2	1	–
Brev. borstelensis (11)	1	–	–
Morganella morganii ssp. *morganii* ACM 2471 (1) (control)	ND	ND	1
Total	24	18	1

LDC : Lysine decarboxylation, ODC: Ornithine decarboxylation, HDC : Histidine decarboxylation

histidine decarboxylation (Yamani and Untermann 1985) and that of Niven *et al.* (1981) indicates false-positive histamine production for some bacteria (Kung *et al*., 2006).

As bacterial behaviour in a particular food system can only be studied in situ, rapid preliminary screening methods to study strain-dependent decarboxylation activity in vitro have become useful (Drosinos *et al*., 2007). The ability of Gram-positive bacteria to decarboxylate histidine, found in this study, did not agree with the findings of other studies where, for example, *Staphylococcus* species were found to be potent in decarboxylating histidine and producing histamine (Hernandez-Herrero *et al.*, 1999; Kung *et al.*, 2006; Tsai *et al*., 2007). It appears from the current and previous studies that histidine decarboxylation and histamine formation are influenced by bacterial strain, decarboxylation medium, NaCl and medium sensitivity.

The importance of specific strains rather than species on formation of biogenic amines was demonstrated by Garai *et al.* (2007), who indicated that *Oenococcus oeni,* isolated from Spanish ciders, was a histamine producer (Del Campo *et al*., 2000 as cited by Garai *et al*., 2007), whereas, *Oenococcus oeni* isolated from Basque country ciders did not produce histamine. Moreover, *Staph. epidermidis* and *Staph. capitis* were found to be powerful histamine formers producing more than 100 ppm of histamine in the presence of 0.5-10% NaCl , whereas > 20% NaCl inhibited histamine formation (Hernandez-Herrero *et al*., 1999; Kung *et al*., 2006). In contrast, 20% NaCl did not prevent histamine formation by the halophilic LAB, *Tetragenococcus muriaticus* (Kimura *et al*., 2001). Despite the advantages of HD-medium over other media in detection of histamine-producing bacteria, this medium can be used for strong histamine producers capable of producing at least 500 ppm (Yamani and Untermann 1985). Therefore, it is possible that Gram-positive bacteria in the current study were either weak histamine producers or did not have histidine decarboxylase activity at all.

High percentages of lysine- and ornithine-decarboxylating *Staphylococcus* species among our isolates suggested that coagulase-negative staphylococci of marine origin are cadaverine and putrescine formers. Although biogenic amine formation has not been confirmed by quantitative assessment in the current study, the efficiency of the medium used in this study has been confirmed by quantitative assessments (Yamani and Untermann 1985). Moreover, certain strains of *Staph. xylosus, Staph. simulans, Staph. warneri, Staph. epidermidis* and *M. luteus* showed lysine and ornithine decarboxylase activities (Lakshmanan *et al.*, 2002b; Martin *et al.,* 2006; Drosinos *et al*., 2007).

Conclusion

Gram-positive bacteria of fish source had a greater ability to decarboxylate lysine and ornithine than to produce sulphide compounds or reduce TMAO, and the spoilage and biogenic amines formation potential of a bacterial species was found to be a strain-dependent. Although histidine decarboxylase activity was not found in Gram-positive bacteria, lysine and ornithine decarboxylase activity which was found in many Gram-positive bacteria isolated from marine fish could potentiate histamine toxicity in marine fish.

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