Food Microbiology, 2002, **19**, 249–257 doi:10.1006/fmic.2001.0467
Available online at http://www.idealibrary.com on IDEAL®

RESEARCH NOTE

Diversity of the microflora of edible macroalga (*Palmaria palmata*)

J. E. Moore*, J. Xu and B.C. Millar

*Edible seaweed (*Palmaria palmata*) has traditionally been consumed raw after harvesting from coastal shorelines around Northern Ireland. To date, there have been no reports examining the microbiology of this material, hence it was the aim of this study to detect the diversity of the microflora found on ready-to-eat produce. Conventional microbiological analyses of the product failed to detect any gastrointestinal pathogens and gave a mean total count of* $1{\cdot}3 \times 10^5$ *cfu* g^{-1}. *16S rRNA sequencing of culturable bacteria identified the* Sanguibacter/Oerskovia/Cellulomonas *complex, the* Clavibacter/Frigoribacterium/Curtobacterium *complex,* Enterobacter agglomerans*,* Erwinia herbicola*,* Flavobacterium *spp.,* Micrococcus lylae*,* Microbacterium *spp.,* Corynebacterium *spp., and* Dietza maris*. A comparison of growth of isolated environmental organisms was performed to ascertain the most appropriate artificial culture media to employ for their culture* in vitro*. Tryptone soya broth with yeast extract (TSBYE) and brain–heart infusion broth with yeast extract (BHIYE) may be employed as suitable basal broth media for the laboratory culture of these organisms. This is the first preliminary report on the microbial diversity of edible seaweed and demonstrated the presence of several halophilic genera and species in fresh ready-to-eat edible seaweed from Northern Ireland. Although no gastrointestinal pathogens were cultured from this material, a larger study requiring examination of seasonal effects, quality of marine water and effect of drying on faecal pathogens, is required to support a functional HACCP-based approach to ensuring safety of this product.*

Introduction

Consumption of edible seaweed has been practised in Northern Ireland for at least the past 100 years and in the Republic of Ireland since before the Great Famine of 1845, traditionally accompanied by periwinkles (*Littorina littorea*). Dulse, sometimes known as sea grass (*Palmaria palmata*) is a red/purple edible macroalga, which grows abundantly in the intertidal zone in North Atlantic and North Pacific coastal waters. It is traditionally harvested directly from these zones at low tide during the spring and summer months, after which it is allowed to dry naturally on nets until the outer surface has a visible coating of crystallized sea salt. Following this, the product is packed loosely into small transparent plastic bags for the retail market and is consumed in this raw state, without any further cooking or processing. More recently, there has been a renewed interest in the consumption of this product based on anecdotal claims of it having beneficial health benefits, in particular due to its high mineral content (Morgan et al. 1980).

Unlike the classification scheme for the harvesting of shellfish from growing beds, there are no specific regulations governing the

*Corresponding author: Fax: +44 (28) 2589 2887.
E-mail: jemoore@niphl.dnet.co.uk

Received:
22 October 2001

Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast BT9 7AD, UK

0740-0020/02/2–30249+09 $35.00/0

collection of this product from coastal regions. Therefore, such produce may be collected from inshore marine waters into which human sewage effluent is discharged, as well as from agricultural run-off, containing faecal pathogens. Furthermore, there have been no reports in the literature examining the microbiology of this material, both in terms of microbial diversity and food safety. Hence it was the aim of this study to examine the diversity of the microflora found on ready-to-eat produce, employing 16S rRNA polymerase chain reaction (PCR) coupled with direct automated sequencing on culturable bacterial organisms isolated from such produce.

Materials and Methods

Collection of microalgae specimens

Specimens (100 g) of microalga were obtained within 1 week of collection from the shoreline adjacent to Cushendall, Co. Antrim (Ordinance survey grid co-ordinates 55°04′N 006°03′W).

Conventional microbiological analyses

Conventional microbiological analyses were performed for both gastrointestinal pathogens and spoilage organisms. Seaweed material was examined qualitatively for the presence of *Campylobacter* spp. and *Salmonella* spp., following selective enrichment, as previously described (Anon 1998). In addition, quantitative counts were performed for the following: total plate counts employing plate count agar (PCA; Oxoid Ltd, Basingstoke, UK) (30°C); yeasts and moulds (20°C); *Staphylococcus aureus*; *Vibrio* spp; *Listeria monocytogenes*; *Escherichia coli* O157:H7, as previously described (Anon 1998).

Extraction and PCR amplification of microbial DNA

All DNA isolation procedures were carried out in a Class II Biological Safety Cabinet in a room geographically separate from that used to set up reaction mixes and also from the 'post-PCR' room to minimize the production of false-positive results. Isolates from the total count plates were further examined to establish their taxonomic identity. These isolates were cultured for 48 h on PCA before DNA extraction. DNA was extracted from a single colony employing the Roche High Purity PCR Template kit (Roche Diagnostics Ltd, UK), in accordance with the manufacturer's instructions. All reaction mixes were set up in a PCR hood in a room separate from that used to extract DNA and the amplification and post-PCR room to minimize contamination. Reaction mixes (50 µl) were set up as follows: 10 mM Tris-HCl, pH 8·3, 50 mM KCl, 2·2 mM $MgCl_2$, 200 µM (each) dATP, dCTP, dGTP and dTTP; 1·25 U of *Taq* DNA polymerase (Amplitaq; Perkin Elmer), 0·2 µM (each) of the 16S rRNA primers P11P (forward) 5′-GAG GAA GGT GGG GAT GAC GT-3′ and P13P (reverse) 5′-AGG CCC GGG AAC GTA TTC AC-3′, as previously described (Millar et al. 2000) and 4 µl of DNA template. The reaction mixtures, following a 'hot start', were subjected to the following thermal cycling parameters in a Perkin Elmer 2400 thermocycler: 96°C for 3 min followed by 40 cycles of 96°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by a final extension at 72°C for 10 min. During each run molecular grade water was included randomly as negative controls and appropriate DNA template from *Staphylococcus aureus* was included as a positive control. Following amplification, aliquots (15 µl) were removed from each reaction mixture and examined by electrophoresis (80 V for 45 min) in gels composed of 2% (w/v) agarose (Gibco, UK) in TAE buffer (40 mM Tris, 20 mM acetic acid. 1 mM EDTA, pH 8·3), stained with ethidium bromide (5 µg/100 ml). Gels were visualized under UV illumination using a gel image analysis system (VVP) Products, UK) and all images archived as digital (*.bmp) graphic files.

Sequencing of amplicons and analysis of sequence data

Amplicons chosen for sequencing were purified using a QIAquick PCR purification kit (Qiagen Ltd, UK) eluted in Tris-HCl (10 mM, pH 8·5) prior to sequencing, particularly to remove dNTPS, polymerases, salts and primers.

Cy-5′ labelled primer, P11P, was prepared and used for sequencing in the forward direction with the ALF Express II (Amersham-Pharmacia Ltd, Buckinghamshire, UK) employing the Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech, UK; catalogue no: RPN 2438) (96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s. 60°C for 5 s, followed by a 4°C hold). The resulting sequences obtained were compared with those stored in the GenBank Data system using BLAST alignment software (http://www.blast.genome.ad.jp/). Sequence homology identity was determined in accordance with the criteria, as previously described by Goldenberger et al. (1997).

Evaluation of optimum culture media

Twelve isolates isolated were examined in this study, as detailed in Table 1. Seven commercially available non-selective basal broth media were examined in the study and included nutrient broth (NB; Oxoid CM0001, Oxoid Ltd, Basingstoke, UK), Todd Hewitt broth (TH; Oxoid CM0189), tryptone soya broth (TSB; Oxoid CM0129), tryptone soya broth supplemented with yeast extract (0·5% w/v) (TSBYE; Oxoid CM0129+Oxoid LP0021), brain–heart infusion broth supplemented with yeast extract (0·5%w.v) (BHIYE; Oxoid CM+Oxoid LP0021), salt meat broth (SMB; Oxoid CM0094) and 0·1% (w/v) peptone saline (PS; Oxoid CM0733). All media were reconstituted in accordance with the manufacturer's instructions for preparation of the media. All strains were grown at 37°C (24 h) on PCA and inocula were prepared of each isolate in PS to give approximately 10^5 cells ml^{-1}, of which 50 µl inocula was added to 250 µl prepared media in a 96-well flat-bottomed microtitre plate (Sarstedt GmbH, Germany). For each treatment series, an equal number of negative controls were established, containing uninoculated media, to check sterility and thus avoid false-positives. Plates were incubated for 60 h with sealed lids to avoid evaporation of water. Plates were examined at 0, 20, 40 and

Table 1. Identification of cultured organisms from edible seaweed based on 16S sequence analysis

Isolate number	GenBank Accession Number of isolate sequenced	Identification (Closest phylogenetic match)	Number of bases called	Percentage homology
Dulse 1	AF419302	*Sanguibacter* spp.	176	100
		Oerskovia spp.	176	100
		Cellulomonas spp.	176	100
Dulse 2	AF419303	*Clavibacter* spp.	167	99
		Frigoribacterium spp.	167	99
		Curtobacterium spp.	167	99
Dulse 3	AF419304	*Enterobacter agglomerans*	164	100
		Erwinia herbicola	164	100
Dulse 4	AF419305	*Tatumella ptyseos*	176	100
		Enterobacter spp.	176	100
		Erwinia chrysanthemi	176	100
		Leclercia adecarboxylata	176	100
Dulse 5	AF419306	*Flavobacterium* sp.	177	100
Dulse 6	AF419307	*Clavibacter* spp.	177	100
		Frigoribacterium spp.	177	100
		Curtobacterium spp.	177	100
Dulse 7	AF419308	*Micrococcus lylae*	177	100
Dulse 8	AF419309	*Microbacterium* spp.	177	100
Dulse 9	AF419310	*Flavobacterium* sp	178	100
Dulse 10	AF419311	*Clavibacter* spp.	184	99
		Frigoribacterium spp.	184	99
		Curtobacterium spp.	184	99
Dulse 11	AF421556	*Corynebacterium* sp.	182	98
Dulse 12	AF421557	*Dietzia maris*	178	99

60 h, and were shaken and read spectrophotometrically ($\lambda = 405$ nm) on an automatic microtitre plate reader (Emax, Molecular Devices Inc. Sunnyvale, California, USA) and the absorbance values noted.

Results

Conventional microbiological analyses of the product failed to detect any gastrointestinal pathogens and gave a mean total count of were $1{\cdot}3 \times 10^5$ cfu g^{-1}. Yeasts and moulds were not detected in the product (< 200 cfu g^{-1}).

16S rRNA PCR was successful in generating an amplicon of the correct size (218 bp) for each organism isolated (Fig. 1). Subsequent sequencing of each amplicon yielded an identification, described in Table 1, along with percentage homology match and number of bases called. All sequences were subsequently deposited in GenBank, as originating from edible seaweed and as detailed by their GenBank accession numbers (Table 1).

Mean spectrophotometrical values of cell density were calculated for each isolate incorporating the seven media screened (Table 2), using the equation:

$$\Delta\ \text{Absorbance}_{[405\ \text{nm}]} = \text{Absorbance}_{[t=60\ \text{h}]} - \text{Absorbance}_{[t=0\ \text{h}]}$$

Final absorbance values were taken at $t = 60$ h, when cells were in mid-stationary phase. Mean Δ absorbance results of the broth media comparison for each strain and statistical analysis of variance allowed the basal media to be ranked into the order for each organism, as shown (Table 2). Time course experiments showed that, on average, greatest cell density was achieved at 40 h (Fig. 2). Statistical analysis of the data by Student's *t*-test demonstrated that supplementation of TSB with yeast was significantly different to the unsupplemented formulation ($P = 0{\cdot}013$). However, no statistical difference was noted between TSBYE and BHIYE ($P = 0{\cdot}089$), as both were equally efficient at generating high cell densities. Although SMB was the poorest medium at increasing cell numbers, it was significantly different to PS ($P = 0{\cdot}014$).

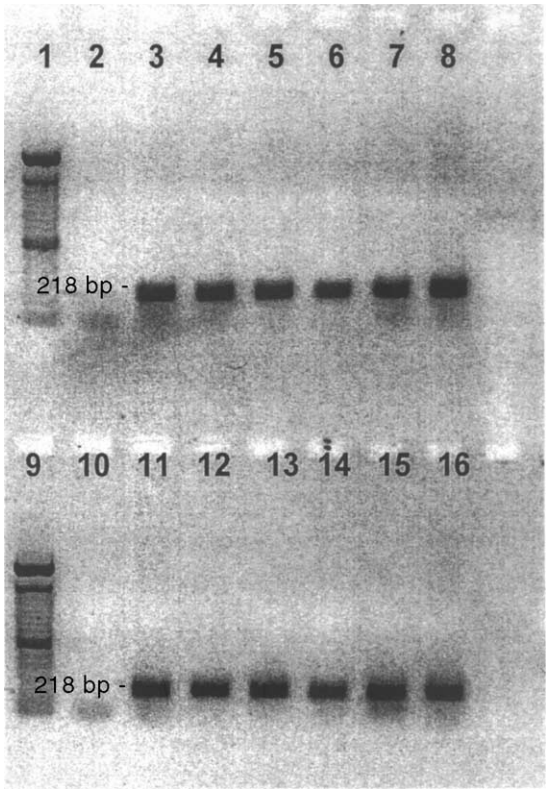

Figure 1. Amplification of a 218-bp fragment of the 16S rRNA gene in isolates cultured from edible seaweed. Lanes 1 and 9, 100-bp molecular weight marker (Gibco Life Technologies Ltd, Paisley, UK); lanes 2 and 10, negative control (molecular grade water); lane 3, *Sanguibacter*/ *Oerskovia*/*Cellulomonas* complex; lane 4, *Clavibacter*/*Frigoribacterium*/*Curtobacterium* complex; lane 5, *Enterobacter agglomerans*/*Erwinia herbicola*; lane 6, *Tatumella ptyseos*/ *Enterobacter* spp./ *Erwinia chrysanthemi*/*Leclercia adecarboxylata*; lane 7, *Flavobacterium* spp.; lane 8, *Clavibacter*/*Frigoribacterium*/*Curtobacterium* complex; lane 11, *Micrococcus lylae*; lane 12, *Microbacterium* spp.; lane 13, *Flavobacterium* spp.; lane 14, Clavibacter/*Frigoribacterium*/*Curtobacterium* complex; lane 15, *Corynebacterium* spp.; lane 16, *Dietzia maris*.

Discussion

With the consumption of any raw ready-to-eat product originating from an environment which may contain faecal and other human clinical pathogens, it is necessary to estimate the potential hazards and associated risks. Furthermore, as there have been no reports in the literature regarding this product, it is

Table 2. Comparison of growth (Δ Absorbance) of environmental organisms isolated from edible seaweed in seven basal broth media

Isolate number	Identification (closest phylogenetic match)	Mean Δ absorbance (λ=405 nm)							Optimum recovery
		NB	TH	TSB	TSBYE	BHIYE	SMB	PS	
Dulse 1	*Sanguibacter* spp. *Oerskovia* spp. *Cellulomonas* spp.	0·381	0·562	0·49	1·277	1·127	0·067	0·001	TSBYE > BHIYE > TH > TSB > NB > SMB > PS
Dulse 3	*Enterobacter agglomerans* *Erwinia herbicola*	0·089	0·027	0·042	0·207	1·034	0·02	0·015	BHIYE > TSBYE > NB > TSB > TH > SMB > PS
Dulse 4	*Tatumella ptyseos* *Enterobacter* spp. *Erwinia chrysanthemi* *Leclercia adecarboxylata*	0·045	0·029	0·038	0·291	0·780	0·022	0·013	BHIYE > TSBYE > NB > TSB > TH > SMB > PS
Dulse 5	*Flavobacterium* sp.	0·153	0·379	0·036	0·234	0·438	0·016	0·004	BHIYE > TH > TSBYE > NB > TSB > SMB > PS
Dulse 7	*Micrococcus lylae*	0·09	0·11	0·032	0·273	0·350	0·017	0.001	BHIYE > TSBYE > TH > NB > TSB > SMB > PS
Dulse 8	*Microbacterium* spp.	0·362	0·241	0·039	0·185	0·371	0·144	0.002	BHIYE > NB > TH > TSBYE > SMB > TSB > PS
Dulse 10	*Clavibacter* spp. *Frigoribacterium* spp. *Curtobacterium* spp.	0·406	0·851	0·765	0·472	0·580	0·050	0·003	TH > TSB > BHIYE > TSBYE > NB > SMB > PS
Dulse 11	*Corynebacterium* sp.	0·466	0·544	0·563	1·180	1·154	0·068	0·028	TSBYE > BHIYE > TSB > TH > NB > SMB > PS
Dulse 12	*Dietzia maris*	0·405	0·434	0·401	0·776	0·522	0·027	0·013	TSBYE > BHIYE > TH > NB > TSB > SMB > PS

NB, Nutrient broth; TH, Todd Hewitt broth; TSB, Tryptone soya broth; TSBYE, TSB supplemented with yeast extract (0·5%w/v); BHIYE, brain–heart infusion broth supplemented with yeast extract (0·5%w/v); SMB salt meat broth; PS, peptone saline (0·1% w/v).

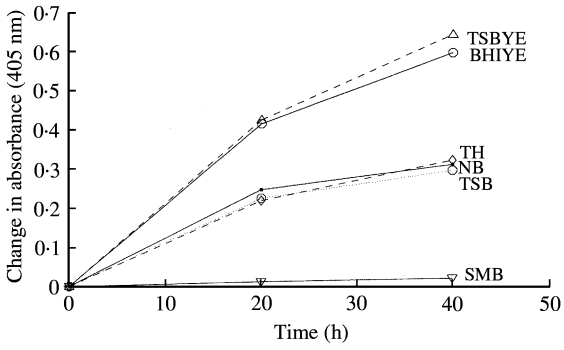

Figure 2. Time course (0–40 h) showing changes in absorbance$_{[405\ nm]}$ of *Dietza maris* isolated from edible seaweed and grown in basal broth media.
NB, Nutrient broth; TH, Todd Hewitt broth; TSB, tryptone soya broth; tryptone soya broth supplemented with yeast extract (0·5%w/v); TSBYE, BHIYE, brain–heart infusion broth supplemented with yeast extract (0·5%w/v); SMB salt meat broth.

important to examine both safety and microbio logical aspects relating to this particular product.

The aim of this study was a preliminary examination of the bacterial microflora of dulse to ascertain its potential safety, as well as diversity of culturable bacterial species present. Initially, a conventional diagnostic approach employing culture was adopted; this failed to detect *Campylobacter* spp., *Salmonella* spp., *E.coli* O157, *Vibrio* spp., *Listeria monocytogenes* and *S. aureus* following detailed laboratory analyses. One organism, *Micrococcus lylae*, which grew on the Baird–Parker selective plates for *S. aureus*, was noted to give a positive reaction with the latex agglutination assay for *S. aureus*, although this was subsequently shown not to be *S. aureus*, as it was coagulase-negative, employing the coagulase tube test on overnight incubation. Likewise, yeasts and moulds were not detected and the mean total plate count was $1{\cdot}3 \times 10^5$ cfu g^{-1}. Therefore, by categorizing edible seaweed as 'dried fruit and vegetables' and hence placing it in category 3, this product may be regarded as being 'acceptable', as interpreted using the Public Health Laboratory Service (PHLS) *Guidelines for the Microbiological Quality of Various Ready-to-eat Foods* (Gilbert et al. 2000).)

To identify the culturable flora present on the edible seaweed, each morphotype present on the non-selective PCA medium was amplified by PCR, employing 'broad-range' 16S rRNA primers, as previously described (Millar et al. 2000). The traditional basis for the identification of environmental and pathogenic organisms has been their isolation or propagation in the laboratory, where biochemical, morphological and serological tests are used to help with their identification. 16S rRNA genes are found in all bacteria and accumulate mutations at a slow, constant rate over time, hence they may be used as 'molecular clocks' (Woese 1987). Highly variable portions of the 16S rRNA sequence provide unique signatures to any bacterium and useful information about relationships between them. Alternatively, since 16S rRNA molecules have crucial structural constraints, certain conserved regions of sequence are found in all known bacteria, i.e. the eubacteria and in all known organisms. 'Broad-range' PCR primers may then be designed to recognize these conserved bacterial 16S rRNA. The 16S rRNA gene sequences and used to amplify intervening, variable or diagnostic regions. In bacteria, there are three genes which make up the rRNA functionality, i.e. 5S, 16S and 23S rRNA. The 16S rRNA gene has historically been most commonly employed; however, more recently, employment of the 16–23S rRNA intergenic spacer region has become popular, along with the 23S rRNA gene. Employment of such rRNA-based techniques has gained increased popularity as a means of identifying phenotypically difficult-to-identify organisms. Coupled with this, it was the expectation of this study that any environmental organisms detected may be difficult to identify, as the majority of identification systems used in the clinical diagnostic laboratory, e.g. API kits, would not contain phenotypic profiles of such environmental genera and species in their databases. Therefore, all isolates were identified by automated sequencing of a 218 bp hypervariable region of the 16S rRNA gene (Table 1). Unfortunately, definitive identifications were difficult to make with half of the isolates examined (isolates Dulse 1–4, Dulse 6 and Dulse 10), owing to complete homology of the isolate with between two and four of its closest phylogenetic neighbours.

Consequently, the identification of the isolate on these occassions was given from the BLAST homology search as all possibilities for the sequence obtained. Sequence analysis allowed for the definitive identification of five organisms, as described in Table 3, which have been shown to be halophilic and hence survive the high osmotic pressure associated with survival in marine environments. Previously, all of these organisms have been described as causal agents of human infection, particularly in immunocompromised patients. Although the contribution of such organisms to infection in immunocompromised patients through ingesting edible seaweed is probably very low, greater concern lies with the potential presence of gastrointestinal pathogens on this material.

To date, there has only been one isolated report in the literature regarding the safety of edible seaweed in the St Lawrence River, Canada, which described heavy metal contamination, but which failed to describe the microbiological status of the material (Phaneuf et al. 1999). Although, unlike the bivalve shellfish also grown in in-shore marine waters, the macroalga does not employ a filtering process and thus has not the ability to concentrate faecal pathogens in tissues. Therefore, contamination with faecal organisms may be a direct results of growth in polluted waters containing human or animal faecal material. Therefore, a formal approach to product safety of such material through HACCP should be carried out, with particular emphasis placed on the effect of drying on the survival of faecal pathogens, where critical points are based on moisture content in final product.

A comparison of growth of isolated environmental organisms was performed to ascertain the most appropriate artificial culture media

Table 3. Environmental habitat and previous reports of opportunistic infections associated with organisms identified from edible seaweed from this study

Organism	Halophilic/haloduric habitats in which organism is found	Evidence as pathogen in opportunistic infection	References
Flavobacterium sp.	Solar saltern, saline soil, seawater	Five cases of Gram-negative sepsis in patients undergoing chemotheraphy for leukaemia	Moriyama et al. (1982)
Micrococcus lylae	Salt used in Spanish dry-cured ham Natural flora of the Black Sea Natural flora of human skin	Six patients with sepsis yielded eight methicillin-resistant isolates most closely resembling *M. lylae*	Marples and Richardson 1980 Cordero and Zumalacarregui 2000 Dedkov et al. 1990 Kloos et al. 1976
Microbacterium sp.	Isolation from mud and saline environments and from the marine sponge, *Halichondria panicea*	Well documented opportunistic pathogen, particularly involving sepsis in immunocompromised patients as well as endophthalmitis	Alonso-Echanove et al. 2001 Wicke et al. 2000 Funke et al. 1997 Savino and Lollini 1976
Corynebacterium sp.	Marine environment	Widely documented cause of clinical infection, including its association with several cases of *otitis externa* in off-shore divers	Alcock 1977 Baross et al. 1975
Dietzia maris	Deep sea sediment	Hip prosthesis infection as well as bacteraemia in an immunocompromised patient	Pidoux et al. 2001 Bemer-Melchior et al. 1999 Colquhoun et al. 1998

Table 4. Detailed listing of components of seven basal media compared in this study

Media constituents ($g l^{-1}$)	NB	TH	TSB	TSBYE	BHIYE	SMB	PS
Lab-Lemco powder	1·0					10·0	
Infusion from 450 g fat-free minced beef		10·0					
Pancreatic digest of casein			17·0	17·0			
Brain–heart infusion solids					3·5		
Papaic digest of soybean meal			3·0	3·0			
Natural heart muscle						30·0	
Yeast extract	2·0			5·0	5·0		
Peptone	5·0				25·0	10·0	1·0
Tryptone		20·0					
Glucose		2·0	2·5	2·5	2·0		
Sodium bicarbonate		2·0					
Sodium chloride	5·0	2·0	5·0	5·0	5·0	100·0	8·5
Disodium phosphate		0·4					
Disodium hydrogen phosphate					2·5		
Dibasic potassium phosphate			2·5	2·5			
pH	7·4±0·2	7·8±0·2	7·3±0·2	7·3±0·2	7·4±0·2	7·6±0·2	7·0±0·2

For abbreviations, see Table 2.

to employ for their culture *in vitro*. Such a study was performed as there is very limited data available to allow for optimum proliferation of these organisms in any downstream characterization of other assay. Overall, TSBYE and BHIYE broth may be employed as suitable basal broth media, and this may be attributed to these media containing higher concentrations of peptones, proteins and other nutritionally rich meat infusions (Table 4). Surprisingly, SMB gave the poorest recovery of all media examined and should be considered less suitable for routine use when other nutritionally rich media are available.

In this preliminary study, 16S rRNA PCR and direct automated sequencing of the amplicons was carried out on culturable organisms isolated on the non-selective basal PCA agar. Direct PCR amplification of the 16S rRNA of a seaweed homogenate, as opposed to amplification from the PCA plate, may have given a greater diversity of genera and species present, because of the ability of the PCR assay to amplify viable but non-culturable environmental organisms which were unable to grow in artificial media under laboratory controlled conditions, Until now, separation of 16S rRNA PCR amplicons for sequence analysis of closely related genera/species has been problematical; however, several methods are not available, including denaturing grade gel electrophoresis which are suitable for the separation of mixed 16S rRNA amplicons before sequencing.

In conclusion, this is the first preliminary report on the microbial diversity of edible seaweed and demonstrated the presence of several halophilic genera and species in fresh ready-to-eat edible seaweed from Northern Ireland. Although no gastrointestinal pathogens were cultured from this material, a larger study requiring examination of seasonal effects, quality of marine water and effect of drying on faecal pathogens, is required to support a functional HACCP-based approach to ensuring safety of this product.

Acknowledgement

This work was supported financially by the Department of Health and Social Services (Northern Ireland).

References

Alcock, S. R. (1977) Acute otitis externa in divers working in the North Sea: a microbiological survey of seven saturation dives. *J. Hyg. (Lond)* **78,** 395–409.

Alonso-Echanove, J., Shah, S. S., Valenti, A. J., Dirrigl, S. N., Carson, L. A., Arduino, M. J. and Jarvis, W. R. (2001) Nosocomial outbreak of *Micro-*

bacterium species bacteremia among cancer patients. *J. Infect. Dis.* **184,** 754–760.

Anon (1998) *PHLS Methods for Food Products.* London, Public Health Laboratory Service.

Baross, J. A., Hanus, F. J. and Morita, R.Y. (1975) Survival of human enteric and other sewage microorganisms under simulated deep-sea conditions. *Appl. Microbiol.* **30,** 309–318.

Bemer-Melchior, P., Haloun, A., Riegel, P. and Drugeon, H. B. (1999) Bacteremia due to *Dietzia maris* in an immunocompromised patient. *Clin. Infect. Dis.* **29,** 1338–1340.

Colquhoun, J. A., Mexson, J., Goodfellow, M., Ward, A.C., Horikoshi, K. and Bull, A. T., (1998) Novel rhodococci and other mycolate actinomycetes from the deep sea. *Antonie Van Leeuwenhoek* **74,** 27–40.

Cordero, M. R. and Zumalacarregui, J. M. (2000) Characterization of micrococcaceae isolated from salt used for Spanish dry-cured ham. *Lett. Appl. Microbiol.* **31,** 303–306.

Dedkov, V. S., Zernov, I. P., Rechkunova, N. I. and Degtiarev, S. K. (1990). Detection of aquatic microorganisms from the Black Sea – producers of restrictions endonucleases. *Mol. Gen. Mikrobiol. Virusol.* **12,** 17–18.

Funke, G., Haase, G., Schnitzler, N., Schrage, N., and Reinert, R. R. (1997). Endophthalmitis due to *Microbacterium* species: case report and review of *Microbacterium* infections. *Clin. Infect. Dis.* **24,** 713–716.

Gilbert, R. J., de Louvois, J., Donovan, T., Little, C., Nye, K. Ribeiro, C. D., Richards, J., Roberts, D., and Bolton, F. J. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. *Commun. Dis. Public Health* **3,** 163–167.

Goldenberger, D., Kunzli, A., Vogt, P., Zbinden, R. and Altwegg, M. (1997) Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. *J. Clin. Microbiol.* **35,** 2733–2739.

Kloos, W. E., Zimmerman, R. J. and Smith, R. F. (1976) Preliminary studies on the characterization and distribution of *Staphylococcus* and *Micrococcus* species on animal skin. *Appl. Environ. Microbiol.* **31,** 53–59.

Marples, R. R. and Richardson, J. F. (1980). *Micrococcus* in the blood. *J. Med. Microbiol.* **13,** 355–362.

Millar, B. C., Jiru, X., Moore, J. E. and Earle, J. A. (2000) A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. *J. Microbiol. Methods* **42,** 139–147.

Morgan, K. C., Wright, J. L. C. and Simpson, F. J. (1980) Review of chemical constituents of the red algae Palmaria palmata (Dulse). *Econ. Bot.* **34,** 27–50.

Moriyama, Y., Urushiyama, M., Ohnishi, M., Koyama, S. Hirosawa, H., Hanano, M., Fuse, I., Takai, K., Nagayama, R., Fujiwara, M., Kishi, K., Takahashi, M., Koike, T., Takahashi, H., Sakai, C., Aoyagi, Y., Sanada, M., Hattori, A. and Shibata, A. (1982). Infections during induction chemotherapy of acute leukemia and their control V. Clinical evaluation of a large dose of amikacin injected intravenously. *Jpn. J. Antibiot.* **35,** 1579–1584.

Phaneuf, D., Cote, I., Dumas, P., Ferron, L. A. and LeBlanc, A. (1999) Evaluation of the contamination of marine algae (seaweed) from the St. Lawrence River and likely to be consumed by humans. *Environ. Res.* **80,** S175–S182.

Pidoux, O., Argenson, J. N., Jacomo, V. and Drancourt, M. (2001) Molecular identification of a *Dietzia maris* hip prosthesis infection isolate. *J. Clin. Microbiol.* **39,** 2634–2636.

Savino, A. and Lollini, M.N. (1976) Preliminary observations on the characterization of a microbial strain isolated from mud and active in saline medium with added phenanthrene. *Boll. Soc. Ital. Biol. Sper.* **52,** 749–754.

Wicke, C., Huners, M., Wray, V., Nimtz, M., Bilitewski, U. and Lang, S. (2000) Production and structure elucidation of glycoglycerolipids from a marine sponge-associated *Microbacterium* species. *J. Nat. Prod.* **63,** 621–626.

Woese, C. R. (1987) Bacterial evolution. *Mictrobiol. Rev.* **51,** 221–271.