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Article *in* Current Opinion in Biotechnology · July 2002

DOI: 10.1016/S0958-1669(02)00309-9 · Source: PubMed

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Fish spoilage bacteria – problems and solutions

Lone Gram* and Paw Dalgaard†

Microorganisms are the major cause of spoilage of most seafood products. However, only a few members of the microbial community, the specific spoilage organisms (SSOs), give rise to the offensive off-flavours associated with seafood spoilage. Combining microbial ecology, molecular techniques, analytical chemistry, sensory analysis and mathematical modelling allows us to characterise the SSOs and to develop methods to determine, predict and extend the shelf life of products.

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Current Opinion in Biotechnology 2002, 13:262–266

0958-1669/02/$ – see front matter

DOI 10.1016/S0958-1669(02)00309-9

Abbreviations

AHL	acylated homoserine lactone
LAB	lactic acid bacteria
SSO	specific spoilage organism
TMA	trimethylamine
TMAO	trimethylamine oxide

Introduction

The growth and metabolism of microorganisms is crucial for the turnover of organic and inorganic matter in all ecosystems. In foods, microbial degradation may manifest itself as spoilage, that is, changes in the sensory properties of a food product rendering it unsuitable for human consumption. Spoilage of foods can be caused by both chemical reactions and physical damage. However, the major cause of food spoilage is microbial growth and metabolism resulting in the formation of amines, sulfides, alcohols, aldehydes, ketones, and organic acids with unpleasant and unacceptable off-flavours. Microbial spoilage may also be detectable as a discolouration or slime, or might simply be identified by the appearance of colonies. The term 'unacceptable' when applied to food spoilage is product-specific — for example, ammonia odours are part of a desirable odour profile in some dried and fermented fish products, but are not acceptable in most fresh and lightly preserved seafoods.

Microbial food spoilage is an area of global concern as it has been estimated that as much as 25% of all food produced is lost post-harvest owing to microbial activity [1]. An improved science-based understanding of the growth and activity of spoilage microorganisms in seafood and other foods is crucial for the development of preservation techniques and subsequent reduction of losses due to spoilage. This review will focus on the microbial ecology of seafood spoilage and describe the specific spoilage organism concept.

Microbial ecology of seafood groups

Foods are dynamic systems in which changes occur in pH, atmosphere, nutrient composition and microflora over time. Each food product has its own unique flora, determined by the raw materials used, food processing parameters and subsequent storage conditions. Despite the variation in composition of the microflora on newly caught fish, seafood products can be categorised into groups with similar microbial ecology [2••]. During storage, the microflora changes owing to different abilities of the microorganisms to tolerate the preservation conditions. Gram-negative, fermentative bacteria (such as *Vibrionaceae*) spoil unpreserved fish, whereas psychrotolerant Gram-negative bacteria (*Pseudomonas* spp. and *Shewanella* spp) grow on chilled fish [2••]. CO_2 packing inhibits respiratory organisms and selects for *Photobacterium phosphoreum* and lactic acid bacteria (LAB) [3••]. Respiratory Gram-negative bacteria are typically inhibited in fish products preserved by the addition of low levels of NaCl, a slight acidification and chill storage in vacuum-packs (e.g. cold-smoked fish). Under these conditions, the microflora typically becomes dominated by LAB (*Lactobacillus* and *Carnobacterium*) with an association of Gram-negative fermentative bacteria such as *P. phosphoreum* and psychtrotrophic Enterobacteriaceae [4–6]. Increasing the 'preservation pressure', for example, by acidification or the addition of preservatives like sorbate and benzoate, as in the so-called semipreserved seafood (e.g. marinated herring), allows for growth of lactobacilli and yeasts. Drying or heavily dry-salting of fish eliminates bacterial growth and these products will spoil due to growth of filamentous fungi or insect infestation. Yeasts may grow in heavily wet-salted fish (e.g. barrel-salted herring). Several fish products are subjected to a mild heat treatment (equalling pasteurisation) and spore-forming bacteria (*Clostridium* or *Bacillus*) may grow in such products, particularly if unsalted (e.g. products cooked in vacuum pouches, i.e. *sous vide* products) [7].

Specific spoilage organism (SSO) concept

While early studies of seafood microbiology acknowledged that only part of the spoilage microflora participated in the spoilage process [8–10], the recent establishment of the specific spoilage organism (SSO) concept [11] has contributed significantly to our understanding of seafood spoilage. The SSOs are typically present in low numbers and constitute only a very small fraction of the microflora on newly processed seafood. Different SSOs are found in different seafoods and may be a single species (Table 1). Identification of an SSO relies on comparison of the sensory and chemical characteristics of spoiled products with those of isolates from the spoilage microflora. The qualitative ability to produce off-odours (spoilage potential) and the quantitative ability to produce spoilage metabolites (spoilage activity) are essential in the identification of an

Table 1

Examples of specific spoilage organisms of seafood products.

Product	Specific spoilage organism
Iced marine fish	*Shewanella putrefaciens*
Iced freshwater fish	Pseudomonas spp.
CO_2-packed chilled fish	*Photobacterium phosphoreum*

SSO. Quantitative comparison of the yield factor for production of trimethylamine (TMA), biogenic amines or volatile amines in products and by bacterial isolates grown in model substrates has been useful for the identification of SSOs [5,11,12]. Comparison of the chemical profiles of spoiled seafoods and of the metabolites produced by potential spoilage organisms has only been used to a limited extent in identification of SSOs. This approach, which should involve the use of multivariate statistical methods for pattern matching, deserves further study. The spoilage domain has been defined as the range of conditions (pH, temperature, water activity and atmosphere) under which an SSO can grow and produce spoilage metabolites. The identification of SSOs and their spoilage domains has substantially facilitated the development of methods to determine, predict and extend the shelf life of seafood. This concept [3••] is also applicable to other foods where spoilage is caused by microbial activity.

Microbial metabolites and seafood spoilage

Fish contains little carbohydrate but typically has a high content of free amino acids. Many fish species contain trimethylamine oxide (TMAO). The seafood SSOs produce ammonia, biogenic amines, organic acids and sulfur compounds from amino acids, hypoxantine from ATP degradation products, and acetate from lactate. TMA is produced by some bacteria capable of using TMAO in anaerobic respiration [13–18]. Many microbial metabolites produced in seafood are similar to those observed in meat and poultry products [19••,20]; however, in seafood spoilage, TMA in particular contributes to the characteristic ammonia-like and 'fishy' off-flavours. *Aeromonas* spp., psychrotolerant Enterobacteriaceae, *P. phosphoreum*, *Shewanella putrefaciens*-like organisms and *Vibrio* spp. can all reduce TMAO to TMA.

Some spoilage metabolites can be used as quality indices. Compared with microbiological methods, which are slow, chemical analyses may be significantly faster; however, for some compounds measurable concentrations are not present until close to spoilage. Classical single-compound quality index (SCQI) for seafood includes measurements of total volatile nitrogen (TVN), TMA and hypoxantine. Ratios between ATP degradation products (K values) and biogenic amines have also been used for some time as quality indices [3••]. Multiple-compound quality indices (MCQI), in which combinations of several metabolites are identified by statistical methods, have recently been introduced and correlate better with sensory properties and/or shelf life in some products [18,21,22]. This new combination of sophisticated chemical analyses, sensory assessment and multivariate statistics will be an important area in food spoilage research in the years to come.

Taxonomy and identification of spoilage bacteria

It is possible to culture the spoilage flora of fish and no examples have been reported where 'unculturable' bacteria have had any importance in seafood spoilage [23]. Comparisons of direct microscopy and culturable counts have yielded similar total numbers of cells [24]. Identification of the microflora has mostly relied on classical, simple phenotypic tests, and comparison of the phenotypic identification with that obtained by 16S rDNA gene sequencing has shown good agreement [25]. *Shewanella putrefaciens* has been named after James M Shewan who worked on fish spoilage over several decades [26]. Mesophilic, halotolerant strains are now recognized as *Shewanella algae* [27] and the psychrotolerant group has been split into *Shewanella putrefaciens* and *Shewanella baltica* [28]. Bioluminescent or non-luminous strains of *P. phosphoreum* involved in seafood spoilage can be identified by simple biochemical tests [29]. Biochemical identification of *Lactobacillus* spp. and *Carnobacterium* spp. can be problematic, but sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) of whole-cell proteins is valuable for identification of LAB at the species level [30]. Little work has been done on taxonomy of yeasts and filamentous fungi involved in fish spoilage.

Methods for detecting SSOs

It has been shown in many seafood products that numbers of SSOs can be used to predict remaining product shelf life, there is, therefore, a great interest in detection methods specifically for these bacteria. Several elegant detection methods have been designed using the spoilage reaction of a particular organism as indicative/selective criteria [31]. These include agar-media for the detection of H_2S-producing *S. putrefaciens*-like bacteria, and conductance-based incubation methods relying on TMAO reduction (e.g. by *P. phosphoreum* [32]). Furthermore, pseudomonads can be enumerated in fresh fish by impedance measurements [12]. To date, no specific agar media exists for the detection of spoilage LAB or Enterobacteriaceae.

Specific methods for the detection of SSOs should be able to detect as little as 10^2 SSO/g of products to be useful in shelf life prediction. Thus, the sensitivity of immuno-based methods (10^6–10^7 SSO/g) [33] is not sufficient to allow for practical use. Similar concerns may be raised in

relation to 16S rRNA-probe-based methods, where the limitation will be the senstivity of the fluorescence microscope. Specific probes have been developed for *S. putrefaciens* [34], but have not been tested in seafoods. A polymerase chain reaction (PCR)-based detection method has been developed for *S. putrefaciens* to differentiate between this organism and *S. algae* [35]. The PCR technique is likely to find use in the future, as polymerase-inhibiting compounds can be removed and inter-laboratory reproducibility increased. Incubation and agar-based detection methods will, owing to their sensitivity, be the most important detection methods for some time to come.

Interactions between spoilage bacteria

Spoilage bacteria are selected primarily as a result of the physical and chemical conditions in the products; however, seafood spoilage obviously involves growth of the microorganisms to high numbers ($>10^6$–10^7 cfu/g) and the interaction (antagonism or symbiosis) between different groups of microorganisms may influence their growth and metabolism. Despite the nutrient richness of fish muscle, the environment is iron-limited and siderophores are produced during bacterial growth. The high iron-binding capacity of the pseudomonad siderophores may cause this bacterial group to be positively selected for [36]. LAB inhibit growth of other bacteria due to the formation of lactic acid and bacteriocins or by competition for nutrients and this may contribute to their selection during spoilage of lightly preserved seafood products. Lactobacilli and Enterobacteriaceae might interact during spoilage of lightly preserved fish products. LAB may degrade arginine (to ornithine), which is then degraded by the Enterobacteriaceae to putrescine. This results in 10–15-fold higher levels of putrescine than produced by Enterobacteriaceae growing alone in the absence of LAB [5]. A final example of the interactive properties of food spoiling bacteria is the ability of several food spoiling Gram-negative bacteria to produce chemical communication signals, acylated homoserine lactones (AHLs) [37••]. We have recently shown that AHLs can be extracted from a range of foods (fish, meat and vegetable products) [38•], and the concentration increases as growth of Gram-negative bacteria takes place. The role of AHLs in (sea)food spoilage is currently unknown, but several phenotypes (pectinolytic, lipolytic, proteolytic and chitinolytic activities) potentially involved in spoilage of different foods have been linked to AHL regulation in several bacteria (AB Christensen *et al.*, personal communication). AHLs can be extracted from fish fillets and minced fish at point of spoilage and are produced by several important seafood spoilage bacteria. Elucidation of the role of AHLs in (sea)food spoilage will be an important area for future research.

Predicting spoilage and shelf life of seafood

An exciting area for use of the SSO concept is in the ability to use mathematical models that quantitatively describe growth of SSOs to predict the shelf life of seafood. Models for the growth of *Brochothrix thermosphacta*, *P. phosphoreum*, *Pseudomonas* spp. and *S. putrefaciens* have been successfully validated for shelf-life prediction of different aerobically stored and CO_2-packed fresh fish [12,39•,40]. In addition, stochastic models that take into account the distribution of spoilage bacteria on products and the storage temperature have been developed for shelf-life prediction of fresh aerobically stored fish [41,42]. Several, successfully validated models for the growth of SSOs have been included in application software and this has facilitated prediction of seafood shelf life under constant and dynamic temperature storage conditions [43]. A simple model to predict interactions between groups of bacteria growing on seafood was also recently suggested [44]. The construction of models to predict the development of microbial spoilage associations in new formulations of lightly preserved seafood remains an important challenge in the field of seafood microbiology.

New preservation strategies – targeted inhibition of spoilage bacteria

The spoilage of some seafoods is well understood and this understanding has enabled the development of preservation techniques targeted at the SSO. An example of this is the CO_2-packaging of fresh, marine, iced fish. This inhibits the respiratory spoilage bacteria (*Shewanella* and *Pseudomonas*) and should, in principle, result in a dramatic extension of shelf life. However, because of the presence of the CO_2-resistant, TMAO-reducing *P. phosphoreum*, the product spoils almost at the same rate as non-CO_2-packed fillets. Targeted inhibition of *P. phosphoreum* (e.g. by freezing or the addition of spices) reduces its growth and results in a significant extension of shelf life [45,46]. Non-spoilage LAB or pure bacteriocins have been used to extend the shelf life of brined shrimp which, if unpreserved, spoil due to growth of spoilage LAB [47]. The possible involvement of AHL regulation in the spoilage of some foods also opens a new field of food preservation. Although until recently preservation has relied on the elimination (killing) or growth inhibition of spoilage organisms, AHL-regulated traits can be specifically blocked (e.g. by the *Delisea pulchra* halogenated furanones [48,49]). This 'quorum-sensing interference' will not necessarily inhibit growth but will, in principle, only block the unwanted spoilage reactions; for example, the export of enzymes involved in the spoilage process.

Conclusions and future perspectives

Some aspects of seafood spoilage have been thoroughly studied and are well understood, in particular, the spoilage of products where only one SSO is involved. The spoilage of seafood products, such as lightly preserved products, where a complex microflora may interact is only partially elucidated. This and the development of predictive shelf-life models of such products are areas for future research. Furthermore, research must be undertaken to develop specific and fast detection methods for SSOs of different seafoods. The SSO concept allows the development of preservation methods targeted only at spoilage microorganisms and this concept should have parallels in other areas of food microbiology. In many situations food

microbiology has been viewed as a rather static field of research, relying heavily on 'total counts' or the presence of a particular pathogen in food commodities. When studied by combinations of microbial growth and metabolism kinetics, molecular techniques, analytical chemistry, sensory assessment and mathematical modelling, we see the dynamics in food microbiology as being as exciting and complex a research field as other areas of microbial ecology.

Acknowledgements

We thank our colleagues from the Microbiology Section at the Danish Institute for Fisheries Research (DIFRES) who were involved in many of the studies that form the basis of the present paper, and Hans Henrik Huss for critical comments.

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