Abundance and distribution of genus *Flavobacterium* in the Baltic Sea; outlook to *de novo* genomics of brackish-water *Flavobacterium*

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Abstract

The Baltic Sea is one of the largest brackish environments totalling in 377 000 km^2 with an extensive salinity gradient from east to west of ~2 to 35, while north to south gradient is less pronounced (from ~2 to 8). In addition, the water column in the Baltic Proper is stratified due to a strong pycnocline. About 60 % of the Baltic Sea surface waters are considered as brackish, with a salinity usually too low for marine organisms, while too high for freshwater organisms. Therefore, the species diversity of higher metazoan eukaryotes is low. In addition, during its short history of <11 000–12 000 years with several switches between fresh and brackish water stages, specialized brackish-water species have only had a limited time to develop. Salinity has also been suggested to be the major determinant of microbial community composition [1], exceeding the influence of other basic environmental parameters such as temperature and pH [2]. However, comprehensive studies on the effects of salinity on the bacterial communities in the transition zone between marine and freshwater systems are rare [3].

The aim of the study was to describe the biogeography of presumably abundant bacterial species within the genus *Flavobacterium* in an aquatic system with prominent salinity gradient extending in large geographical space (several thousands of kilometres). In addition, whole genome analysis of two *Flavobacterium* isolates from the Baltic Sea was carried out to comparatively investigate their functional metabolic capabilities. These two strains originate from a batch growth experiment carried out using inocula from the Bothnian Bay and Bothnian Sea transfer zone [4].

Water samples were obtained on a research cruise (MSM0803) of the RV Maria S. Merian in June/July 2008 (Fig. 1). In total, 60 stations covering the central Baltic Sea and Danish straits were sampled, including several depth layers. Conductivity, temperature, pressure, chlorophyll a fluorescence and dissolved oxygen of water samples were measured using a conductivity/temperature/depth sensor (CTD) SeaBird 911 connected to a rosette of 24 10L bottles. Concentrations of inorganic nutrients and oxygen were analysed as described by Grasshoff et al. [5].

Distribution of the total abundance of the genus *Flavobacterium* was estimated using quantitative real-time PCR (qPCR) and the values were related to salinity, temperature, chlorophyll a, dissolved oxygen, and inorganic nutrient concentrations. Additionally, the

genomes of two brackish-water isolates, *Flavobacterium* sp. GOBB3-C103-3 and *Flavobacterium* sp. GOBB3-209, were sequenced by Roche 454 GS-FLX system (Titanium) with total genome coverage of 30 fold. Short (~400 bp) Roche 454 reads were assembled with MIRA3 software, annotated and analyzed in IMG-ER [6].

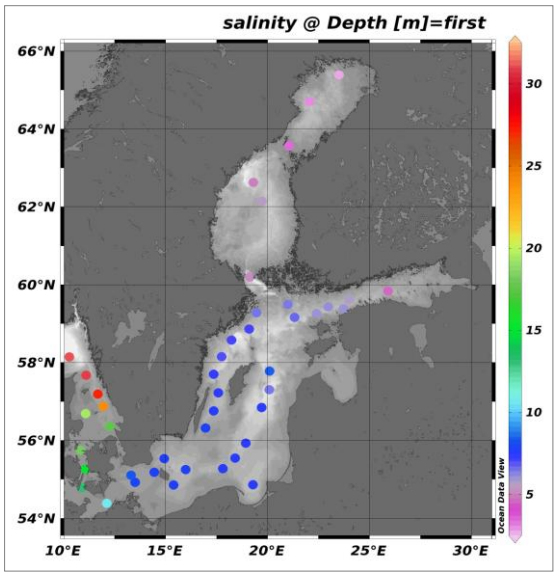

Figure 1. Sampling stations along ~2 000 km long transects. Salinity at the surface layer in sampling stations is discriminated by color coding.

Newly designed primers for 16S rRNA gene were applied to quantify cells of the genus *Flavobacterium* in qPCR assays. For this, several genus specific candidate primers were designed *in silico* using ARB [7] and PrimeGens [8], and subsequently tested for specificity using the ProbeMatch tool from Ribosomal Data Project II (RDP II). Three candidate primers were tested with *in situ* fluorescent hybridization (FISH) on test strains isolated from the Baltic Sea and its catchment area. One oligonucleotide probe was selected to be used in qPCR assays in combination with the universal 16S rRNA gene primer. Total number of bacterial 16S RNA gene copies was assessed using a pair of universal bacterial primers. The abundance of flavobacterial genes was standardized to the total small subunit ribosomal gene copy abundance.

Two geographical regions with increased abundance of *Flavobacterium* along the 2000 km transect with changing salinity from 2 to 35, from Bothnian Bay to outer Danish straits - Skagerrak, were observed (Fig. 2). These were the transfer zone from Bothnian Bay to Bothnian Sea, and the second in the area of Bornholm basin. Although the shift of salinity in these regions is moderate (few salinity units), the change of salinity is pronounced for the brackish part of the Baltic Sea. In addition, *Flavobacterium* cells were more abundant at the surface layers, in most cases deeper layers were suboxic or anoxic. This suggests that most of *Flavobacterium* species are aerobic heterotrophs in the Baltic Sea which possibly benefit from changing salinity conditions and resulting effects on terrigeneous dissolved matter as suggested earlier [9].

Draft genomes of analyzed *Flavobacterium* differed by their genome size (GOBB3-209 has 2.4 Mbps and GOBB3-C103-3 has 4.2 Mbps) and coding regions (GOBB3-209: ~1400 CDS; GOBB3-C103-3: ~2200 CDS). The results of the annotation suggest that the size of the genome is directly related to metabolic capabilities of these aquatic bacteria. The bacterium *F.* sp. GOBB3-209 is lacking several metabolic pathways and specific genes present in GOBB3-C103-3. The most prominent which were identified were proteins involved in:

– cell wall and capsule/capsular and extracellular polysaccharides synthesis;
– metabolism of carbohydrates, denitrification, ammonia and phosphorus assimilation;
– high affinity phosphate transport systems;
– resistance/efflux pumps, stress response and CRISPs.

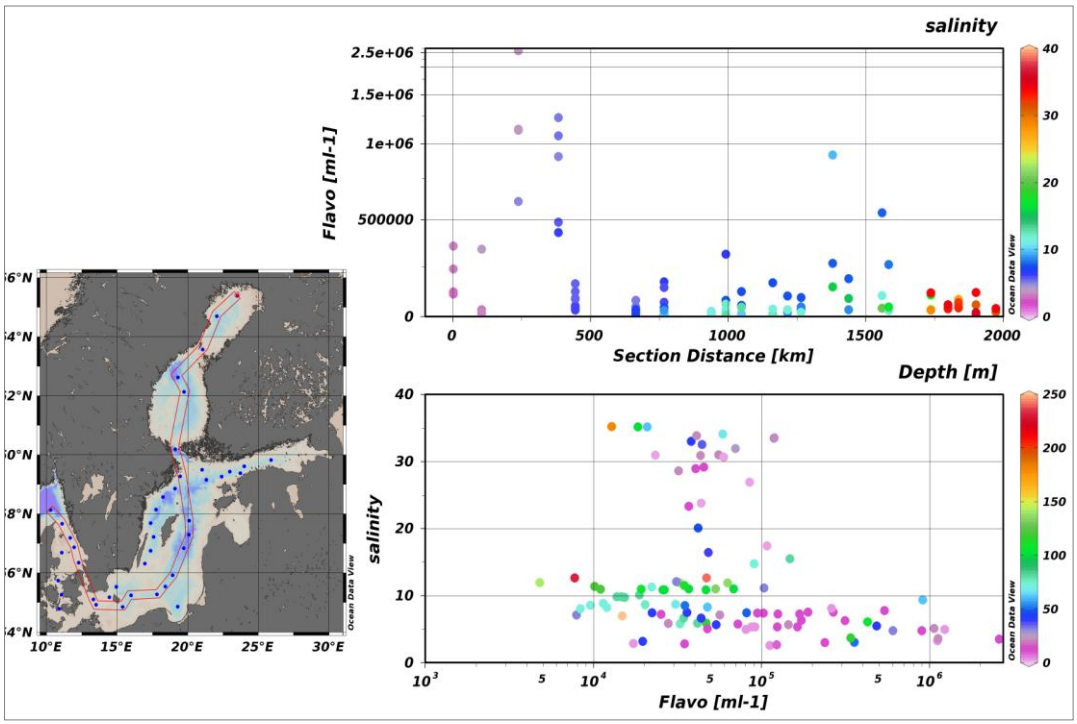

Figure 2. Abundance of the genus *Flavobacterium* bacteria along ~2 000 km transect from the Bothnian Bay to North Sea (right upper panel, salinity indicated by color coding). Samples shown originate from transect indicated on left panel map (sample used surrounded by red line). Relationship between salinity and abundance of *Flavobacterium* bacteria in the left bottom panel, color coding for depth.

The bacterium *F.* sp. GOBB3-209 contrasted from the other sequenced bacterium by possessing a bacteriorhodopsin gene which was highly similar to that found in a *Flavobacterium* sp. strain isolated earlier from the Baltic Sea.

In conclusion, genus *Flavobacterium* cells were more abundant at the surface of the water column and the elevated abundances were observed in regions where salinity conditions changed. This corroborates the suggestions from earlier studies that members of *Flavobacterium* might be largely passive immigrants from the catchment area carried into the Baltic along the rivers [10] but are adapted to conditions in certain regions of the Baltic Sea. Moreover, changing ion strength conditions due to salinity increase might provide additional substrate sources to certain bacteria, *Flavobacterium* among them. This phenomenon was observed earlier in batch culture experiments [4] and approved by observations in this study.

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