Solar Salt Works: Purifying systems from undesirable Bacteria.

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The bacterial populations of three producing units (salines) of Guérande salt works submitted to bacterial contamination of various importance are studied. Bacterial numerations show that salt works, thanks notably to the zones of cyanobacterial mats, act as depurating systems towards undesirable bacteria. In the most concentrated brines only remain and develop the extreme halophilic bacteria which favour salt precipitation and give to the salt its particular flavour.

1. INTRODUCTION

Since solar salt contains usually numbers of Extremely Halophilic Bacteria (EHB), up to 10^6 cells per g [1], the question arises whether other bacteria, especially pathogenic ones, could also be present in marketed salt. Thus, it was interesting to study bacterial populations in the sediments and brines of several salt producing units (salines) of Guérande salt works (South Brittany), submitted to various degrees of bacterial contamination.

2. MATERIALS AND METHODS

The geographical and environmental situation of Guérande salt works has been often described as well as their organisation and functioning [2]. In each studied saline, surficial sediments were sampled in winter 93 and 94 along the sequences of basins that follow the advance of water when salt is being produced. Brines were sampled 3 times during summer 1995 following the same sequences of basins.

The downstream sequence of condensers is as follows (Figure 1). The "vasière" receives marine water from tidal channels.

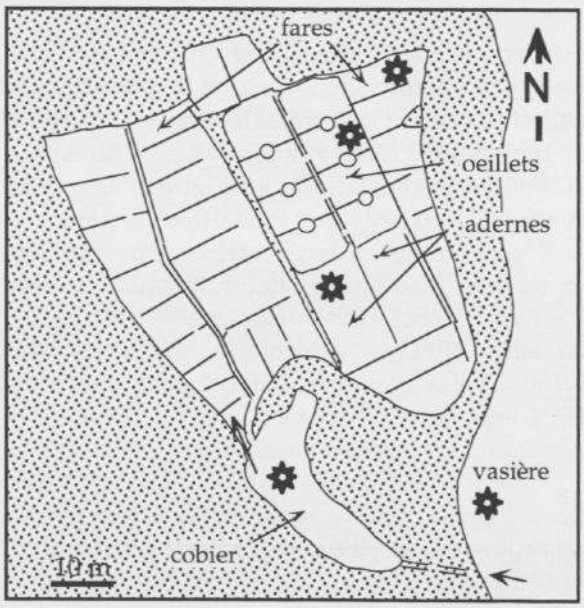

Figure 1. Typical organisation of a saline and standard location of sampling points.

It is a lagoonal type environment where organic matter production largely exceeds consumption [3,4]. The "cobier" and following nearer "fares" are covered by cyanobacterial mats, whereas the further "fares" generally bear gypsum deposits.

Table 1
Numerations of NEHAHB, vibrios and EHB in sediments (per ml).

	Pen Bron			Kervalet			Lénifun		
	NEHAHB	Vibrios	EHB	NEHAHB	Vibrios	EHB	NEHAHB	Vibrios	EHB
Winter 1993									
Vasière	$4.5\ 10^5$	0	$1.8\ 10^5$	$2.8\ 10^3$	<25	$9.8\ 10^2$	$2.0\ 10^5$	50	$1.8\ 10^2$
Cobier	$5.0\ 10^3$	775	$1.6\ 10^5$	$3.6\ 10^5$	3130	$9.4\ 10^5$	$4.0\ 10^5$	75	$2.5\ 10^3$
Fare	$1.4\ 10^5$	750	$5.0\ 10^4$	$4.0\ 10^5$	5500	$4.3\ 10^5$	$3.7\ 10^6$	300	$2.5\ 10^4$
Aderne	$1.2\ 10^6$	0	$1.1\ 10^5$	$6.0\ 10^5$	0	$4.0\ 10^5$	$1.2\ 10^6$	0	$2.8\ 10^5$
Oeillet	$2.0\ 10^5$	0	$1.3\ 10^6$	$2.2\ 10^6$	0	$2.7\ 10^6$	$4.5\ 10^5$	0	$4.8\ 10^6$
Winter 1994									
Vasière	$1.5\ 10^6$	500	<25	$3.6\ 10^5$	450	<25	$1.0\ 10^5$	450	<25
Cobier	$3.0\ 10^5$	450	$2.40\ 10^5$	$7.5\ 10^5$	150	$2.5\ 10^3$	$4.5\ 10^6$	750	$2.40\ 10^3$
Fare	$5.5\ 10^5$	5500	$1.50\ 10^6$	$3.4\ 10^5$	100	$9.1\ 10^3$	$2.0\ 10^6$	0	$7.80\ 10^5$
Aderne	$1.6\ 10^6$	0	$2.60\ 10^6$	$9.0\ 10^5$	0	$1.5\ 10^6$	$9.2\ 10^5$	0	$4.80\ 10^5$
Oeillet	$4.0\ 10^5$	0	$8.46\ 10^6$	$4.1\ 10^5$	0	$3.8\ 10^6$	$2.9\ 10^5$	0	$2.60\ 10^6$

Concentrated brines are stocked in the "adernes" before going onto the crystallisors called "oeillets" where salt, and salt flower, is harvested. During winter the salines are submitted to rainfall. In springtime, they are reworked and prepared for salt production.

Numerations were carried out on non extremely halophilic heterotrophic aerobic bacteria (NEHAHB), extremely halophilic bacteria (EHB), vibrios, in sediments and brines. Total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS), were only numbered in summer brines.

Counts (NEHAHB, EHB, vibrios) have been carried out by seeding on solid media according to the dilution methods from one ml of sediment or brine. The detection threshold is 25 bacteria per ml.

Culture solid media were the following: i. culture medium for the extreme halophiles [1] with a 2 months incubation time at 37° C, ii. Oppenheimer and Zobell medium [5], adjusted to 35 g/l of NaCl, for the culture of marine aerobic heterotrophic bacteria with an incubation at room temperature during 15 days, with perusals at 8 and 15 days, iii. TCBS medium for the culture of vibrios [6], with an incubation at 37°C during 24 hours. Counts of TC, FC and FS were carried out by culture on filtering membranes which is an easier process than the culture in lactose liquid media [7].

Variable volumes of the sample are filtered on a 0.45 µm cellulose membrane which is then deposited upright on a solid medium: eosin, methylene blue medium for the culture of the coliforms with a 24 hours incubation at 37°C for TC and 44°C for FC, and the Slanetz and Bartley medium for the culture of FS (48 hours incubation at 37°C) [8].

3. RESULTS AND DISCUSSIONS

3.1. Sediments

Except two low values, the NEHAHB strengths are generally high and relatively homogeneous in sediments of all basins, around 10^5 to 10^6 cultivable bacteria per ml (Table 1). In winter, temperature is low and bacterial activity is reduced.

Table 2
Brine concentration ratio (BCR) and cumulative mortality percentages of NEHAHB (NEH), total coliforms (TC) and faecal streptococci (FS) in summer brines. ---outcoming contamination.

	Pen Bron				Kervalet				Lénifun			
	BCR	% mortality			BCR	% mortality			BCR	% mortality		
		NEH	TC	FS		NEH	TC	FS		NEH	TC	FS
July 5-6-7												
Vasière	0	0		0	0	0	0	0	0	0	0	0
Cobier	1,19	40,1		88,2	1,00	90,0	86,7	88,8	1,00	---	86,9	94,5
Fare	2,46	83,1		100	2,85	82,5	98,2	100	3,00	89,8	99,1	99,6
Aderne	2,68	97,3		99,8	4,67	78,6	96,2	100	4,46	94,6	99,9	99,9
Oeillet	5,88	99,7		100	6,10	98,8	100	100	6,45	99,9	100	100
July 24-25-26												
Vasière	0	0			0	0	0	0	0	0	0	0
Cobier	1,42	29,5			1,36	---	80,9	84,3	1,00	---	---	---
Fare	2,73	38,0			2,91	---	94,0	100	2,43	78,9	95,1	96,5
Aderne	4,17	97,6			4,04	99,0	100	100	4,41	74,8	100	99,9
Oeillet	5,98	>99,9			6,19	>99,8	100	100	6,07	99,8	100	100
August 28-29-30												
Vasière	0	0	0		0	0		0	0	0	0	0
Cobier	1,93	89,7	45,6	---	1,36	53,9		68,8	1,00	34,4	100	100
Fare	3,29	82,4	79,1	100	3,27	69,4		100	3,30	83,5	100	100
Aderne	4,69	92,3	95,7	100	4,92	66, 7		100	4,89	89,1	100	100
Oeillet	6,40	99,9	100	100	6,66	97,8		100	6,69	99,9	100	100

As a matter of fact, the numeration technique quantifies in the same manner bacteria that could rest in the sediment as inactive spores (e.g. Bacillus) and the alive ones.

Besides, numeration reveals in no way the activity of the latter in the biotope.

Seeding on solid medium and incubation temperature certainly stimulated the activity of both kinds of cells. On another hand, for several reasons, some bacteria living in the natural environment cannot be cultivated on standard solid media [9].

Thus, presented numerations should be considered as approximate. The lowest values could be related to the cleaning and reworking of basins which are carried out during winter time but not every year.

There is no obvious relationship between NEHAHB strengths and the salinity longitudinal gradient that should exist in interstitial brines even though these are diluted by winter rainwater. Vibrios make a very small part of bacterial populations even in the first condensers. Beyond these, they totally disappear.

Table 3.
Numerations of vibrios (V), total coliforms (CT), faecal coliforms (FC), faecal streptococci (FS) in number of cultivable cells per ml, and values of salinity (*S*) in brines (‰).

	Pen Bron					Kervalet					Lénifun				
	V	TC	FC	FS	*S*	V	TC	FC	FS	*S*	V	TC	FC	FS	*S*
July 5-6-7															
Vasière	480	0	0	2,700	*67*	550	2,700	0	16,000	*53*	150	10,000	20	20,000	*50*
Cobier	0	0	0	380	*80*	200	360	0	1,800	*57*	2,100	1,780	0	1,100	*50*
Fare	0	0	0	0	*101*	0	140	0	0	*100*	200	310	0	230	*100*
Aderne	0	0	0	18	*150*	0	480	0	0	*182*	0	40	0	18	*146*
Oeillet	0	0	0	0	*290*	0	0	0	0	*260*	0	0	0	0	*290*
July 24-25-26															
Vasière	650	0	0	0	*67*	100	2,160	4	600	*36*	3,500	2,700	0	4,700	*61*
Cobier	0	0	0	0	*95*	150	560	0	128	*49*	2,700	20000	300	16,000	*60*
Fare	0	0	0	0	*125*	50	520	0	0	*76*	0	320	0	400	*86*
Aderne	0	0	0	0	*180*	0	0	0	0	*86*	0	0	0	140	*170*
Oeillet	0	0	0	0	*325*	0	0	0	0	*185*	0	0	0	0	*282*
August 28-29-30															
Vasière	500	160	0	0	*57*	50	0	0	1600	*50*	800	240	20	20,000	*55*
Cobier	0	168	0	140	*110*	0	0	0	680	*68*	380	0	0	0	*55*
Fare	0	110	0	0	*150*	0	0	0	0	*130*	0	0	0	0	*126*
Aderne	0	32	0	0	*210*	0	0	0	0	*214*	0	0	0	0	*200*
Oeillet	0	0	0	0	*360*	0	0	0	0	*372*	0	0	0	0	*360*

These results suggest that marine heterotrophic aerobic bacteria are able to withstand the wide temperature and salinity variations to which they are submitted through seasonal changes.

Numerations do not account for bacterial activity which is probably slowed down in winter due to temperature decrease and less organic matter production. In the vasières, the EHB populations are not numerous except in one point (Pen Bron, 93) where the highest salinity has been measured and reached 67‰. Below 88 gl^{-1} of NaCl, lysis of EHB cells occurs. Hence, interstitial salinity should locally overstep such values. Downstream, the EHB strengths increase and reach more than 10^6 in crystallisors. Thus, EHB, though probably inactive, are able to survive, in sediments at temperatures far below their growth optimum (35-45°C). They are present, numerous and ready to develop when the salines start functioning.

3.2. Brines

NEHAHB counts stay between 10^3 and 10^6 in condensers which contain brines ranging from 36‰ to 150 ‰. They house lagoonal biota and microbial mats which produce amounts of organic matter which should favour bacterial development. Even though

probable in the vasières this is not the case downstream since, even in such conditions, bacterial strengths globally do not increase.

Further downstream, NEHAHB numbers decrease rapidly with increasing salinity.

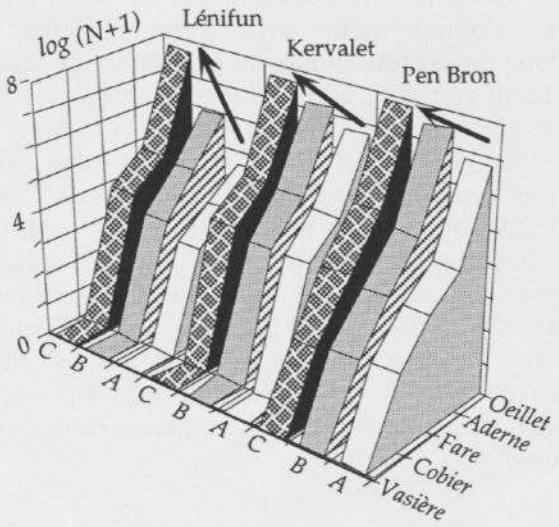

Figure 2. Variations of the strenghts of extreme halophilic bacteria in brines. Summer 1995. A: July 5/6; B: July 24/26; C: August 28/30.

In crystallisors, NEHAHB counts do not exceed 4 10^3 cultivable cells per ml. If considering that nothing happens to bacteria except mechanical concentration, the brines salt concentration ratios allow to calculate a theoretical bacterial number in each basin. It is possible to obtain cumulative mortality percentages with following formula:

$$\frac{\text{(calculated number - measured number)}}{\text{calculated number}} \times 100$$

Results are given in Table 2. Given possible contaminations by runoff from peripheral levees and by birds faeces, this means that environment conditions do not favour NEHAHB development in the most salty pans.

Studied salines are not submitted to the same degree of contamination by undesirable bacteria (Table 3). Meanwhile, in all salines the strengths of vibrios, TC and faecal bacteria, decrease rapidly, notably in the cobiers and fares. These bacteria are totally absent in crystallisors. Furthermore, apart from a few exceptions, the bacterial numbers decrease in each basin during the course of salt production season from July to August. Eventually, in salt depositing brines undesirable bacteria are totally absent.

Extreme halophilic bacteria are almost totally absent from first condensers which display salinities that do not allow EHB development. From 80‰ up to saturation, the EHB exponentially increase and reach very high values in the crystallisors, up to nearly 10^8 cultivable cells per ml. Thus, they find in their biotope high enough amounts of organic matter to allow an exponential increase of their populations.

This confirms that the NEHAHB decrease with salinity is not due to the decrease of organic substrates but to the physical and chemical conditions of environment. Organic matter is produced by planktonic blooms of the green algae Dunaliella which contributes with EHB and other bacteria (Thiorhodobacteriales), to the pink colour of concentrated brines. Organic matter is also probably originated from the upstream microbial mats as suggested by the beginning of EHB increase in the fares around 100‰ salinity.

It is worth noticing that in crystallisors of all studied salines the EHB numbers increase during the course of the salt production season (Figure 2).

Thus, high salinities favour EHB development. In crystallisors, EHB are largely dominating bacterial populations. They are ten times more numerous than in winter sediments. Additionnally, Guérande salt may contain up to 10^5 EHB per gram [1] i.e. one hundred times less than the saltiest brine which then appears as the most suitable biotope for them.

4. CONCLUSIONS

In crystallisors winter sediments, vibrios are absent but NEHAHB are numerous, mainly due to the presence of organic matter

and low salinities. But NEHAHB doubtfully resist to summer conditions. Besides, when harvesting, salt producers keep from collecting sediments. The problem is even lesser for salt flower which is collected from the brine surface.

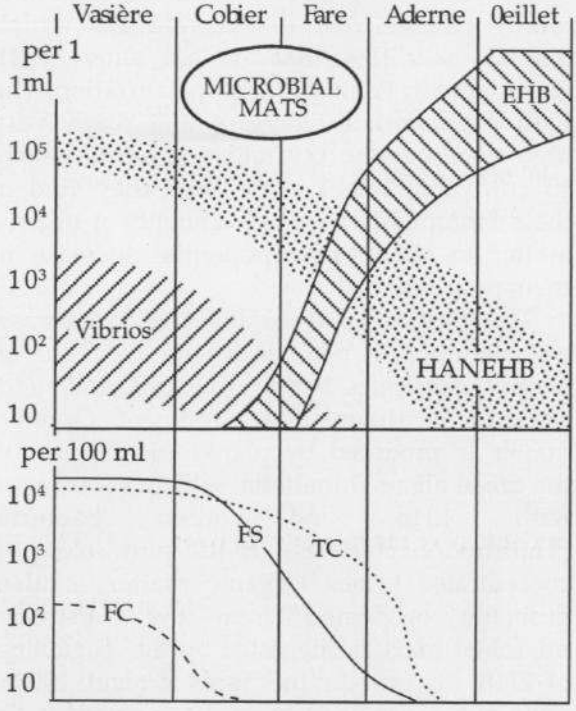

Figure. 3. Schematical evolution of bacterial planctonic populations in solar salt marshes.

In brines, NEHAHB are the most numerous in the first condensers but decrease in the following basins. Their maximal mortality occurs in the microbial mats area and, in crystallisors, the populations are reduced by 98 to 99%.

Besides, vibrios as well as faecal bacteria, which are present in the less salty basins waters, are totally absent in the most concentrated pans (Figure 3). Thus, thanks notably to the zones of cyanobacterial mats located in the cobiers and fares upstream from the crystallisors, solar salt works act as purifying systems towards undesirable bacteria. In the most concentrated brines (adernes and oeillets) only remain and develop the EHB which favour salt precipitation and give to the salt its colour and particular flavour.

When freshly harvested, early salt do be a biological product. After grinding and/or a long dessicating storage most EHB disappear. Then, salt dies and becomes a mere mineral product.

On one hand EHB contribute to salt precipitation and give early salt its inimitable taste. On another hand, notably when they develop too rapidly, they induce porosity and Mg-rich inclusions in produced salt. Thus, they would not be considered in the same manner by the gourmet or by the chemical salt producer. But all types of consumer are assured of the bacteriological quality of solar salt.

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