We study statistical properties of DNA chains of thirteen microbial complete genomes. We find that the power spectrum of several of the sequences studied flattens off in the low frequency limit. This implies the correlation length in those sequences is much smaller than the entire DNA chain. Consequently, in contradiction with previous studies, we show that the fractal behavior of DNA chains not always prevail through the entire DNA molecule.

1. INTRODUCTION

The statistics of DNA sequences is an active topic of research nowadays. There are studies on the power spectral density, random walker representation, correlation function [1], etc. Although some of the studies are in contradiction with each other, there is a consensus with respect to the reported behavior of the power spectrum of DNA sequences. For high frequencies it is roughly flat, with a sharp peak at $f = 1/3$, which has been shown to be due to nonuniform codon usage [2, 3]. For smaller frequencies, it has been reported that it presents a power-law behavior with exponent approximately equal to $-1$, that is, $1/f$ noise. Since a cutoff of the power-law exists at high frequencies, it has been called “partial power-law” [4].

The presence of “1/f” noise in a given frequency interval indicates the presence of a self-similar (fractal) structure in the corresponding range of wavelengths, whereas a flat power spectrum indicates absence of correlations (white noise).

It is an important question to know whether or not the power-law behavior of the power spectrum of a given DNA sequence is, for many sequences, much smaller than the entire length of the DNA chain. We have calculated the autocorrelation function (AF) of the nucleotides in the DNA chains of the organisms mentioned above. We have found that in some of the organisms the correlation length is of the order of a few thousand base-pairs. In others, the correlation length is very large, being not smaller than 100,000 base-pairs.

A DNA chain is represented by a sequence of four letters, corresponding to four different nucleotides: adenine (A), cytosine (C), guanine (G) and thymine (T). The calculation of the power spectrum or the autocorrelation function requires that this symbolic sequence be transformed into a numerical one. Several methods have been proposed for this [4, 7, 9]. Here we use the method introduced by Voss [3], which has been shown in [10] to be equivalent to the method used in [11]. In Voss’ method one associates 0 to the site in which a given symbol is absent and 1 to the location where it is present. So, for a given DNA sequence there will be four different numerical sequences, corresponding to the sequences associated with A, C, G and T. In his original paper, Voss calculated the PS for each one of these sequences and summed them to find the average PS. Here, we treat them distinctly, because we also want to know about the similarities and differences of the statistical features of different nucleotides in a given DNA sequence.

By artificially linking flank sequences together, Borstnik et al. [11] found a behavior for the PS as a function of the frequency that was flat, then an exponential decay, then flat again. Our studies of complete sequences show that the behavior of the PS does not
show any exponential decay in the region of intermediate frequencies. We found instead a power law. However, for low frequencies we also find a flat PS in several of the sequences studied. A flat PS at high frequencies is observed in all cases.

II. STATISTICAL ANALYSIS

A. Power Spectrum

Let us use Voss’s method and denote by \( x_j^A \) the numerical value associated with the symbol A. Then one has \( x_j^A = 1 \) if symbol A is present at location j and \( x_j^A = 0 \) otherwise. Similar transformation is made for symbols C, G and T. Consequently, the DNA can be divided into four different binary subsequences of 0’s and 1’s, associated with the symbols A, C, G, and T.

The Fourier transform of a numerical sequence \( x_k \) of length \( N \) is by definition,

\[
V(f_j) = \frac{1}{N} \sum_{k=0}^{N-1} x_k \exp(-2\pi ikf_j),
\]

where the frequency \( f_j \) is given by \( f = j/N, \) and \( j = 0, ..., N-1 \). The PS is defined as \( S(f_j) = V(f_j)V(f_j)^* = |V(f_j)|^2 \). From the definition, we can see that \( S(f_0) = <x_k>^2 \), where the brackets denote average along the chain. Consequently, this quantity carries no information about the relative positions of the nucleotides. Because of this, we usually neglect this quantity in our calculations, that is, we concentrate only on frequencies with \( j > 0 \).

Since DNA sequences have a large number of base-pairs, and the PS presents considerable fluctuation, some kind of averaging is usually done to plot this quantity as a function of the frequency. The way of averaging done so far is the following: the DNA chain of length \( N \) is divided into non-overlapping subsequences of length \( L \). Then, the power spectrum of each of these segments is computed and averaged over the \( N/L \) subsequences. In this method the smallest frequency for which the PS can be calculated is, of course, \( f = 1/L \). Consequently, the behavior of frequencies in the range \([1/N, 1/L]\) is unknown. An example of such a calculation for Ecoli is shown in Fig. 1, where the DNA chain was divided in subsequences of 8192 nucleotides. A clear power law, followed by an approximate flat region with a sharp peak at \( j = 1/3 \), is seen. To avoid overlap of the curves, we have displaced the PS of cytosine, guanine and thymine by dividing it by 10, \( 10^2 \) and \( 10^3 \), respectively. Since the power spectrum for sequences of real numbers is symmetric with respect to the axis \( f = 0.5 \), we plot only the PS for frequencies in the interval of 0 to 0.5. A similar figure for adenine is shown in Fig. 2.

In this paper we show that another way of averaging allows one to calculate the PS for smaller frequencies than

the method described above, and then verify what happens to the power-law as the frequency decreases. More specifically, we calculate the mean PS in a sliding window of \( n \) points, with adjacent windows having an overlap of \( n-1 \) points. The average PS in each window will determine the values of the smoothed resulting sequence. In mathematical terms we can express this as

\[
\overline{S}(f_j) = \frac{1}{n} \sum_{m=j-\Delta}^{j+\Delta} S(f_m)
\]

where \( \Delta = (n-1)/2, n \) is taken an odd number, and \( j \) varies from \( \Delta + 1 \) to \( N - \Delta - 1 \). Although the new sequence in this method is smoother than the original one, its length is only smaller than it by \( 2\Delta \) points. We have found that this method shows the same behavior for moderate and high frequencies as the method used in [4]. However, it much superior for studies at low frequencies.

To speed up the calculations of the PS we have used, as it is normally done, the Fast Fourier Transform algorithm [12]. This algorithm speeds up the calculation of the PS by a factor of \( N/\log_2N \), but it requires that length of the sequence analyzed be an integer power of the integer \( \alpha \), which usually is taken to be two. Since the length of DNA sequences are not generally equal to an integer power of two, we take in our computation the largest subsequence, starting from the beginning of the chain, that fulfills this requirement. More specifically, we take the first \( N' = 2^K \) nucleotides, where \( K \) is the largest power of 2 satisfying the requirement that \( N' \leq N \), with \( N \) being the total size of the DNA chain. In this way, the number of nucleotides not included in the calculation is always smaller, and in many cases much smaller, than \( N/2 \). We have also done calculations considering the entire length of the DNA and zero padding the sequence to the next integer power of 2, as described in [12]. The results remain essentially the same as the ones we show here.

Since our method shows the same behavior for the PS in the range of intermediate and large frequencies as the other averaging method, and also due to the large size of the DNA chains, we plot the PS only in the frequency range \([1/N, 0.01]\). We show in Fig. 3 the results of our calculation for \( n = 33 \) for four representative cases of the thirteen ones studied. For clarity, we have displaced the PS of C, G and T by dividing it by \( 10, 10^2 \) and \( 10^3 \), respectively. In this way, an overlap of the curves is avoided. Our results show that the low frequency PS associated with each of the nucleotides in the organisms studied fall into one of the following cases:

(a) All the four PS associated with the four different nucleotides flattens off at low frequencies. In these cases there are three regions in the PS versus frequency curve. At both low and high frequencies the PS is of white noise type and the middle regions is characterized
approximately by a power-law behavior, that is, in a log-log plot the PS satisfy $S \sim f^{-\gamma}$, with $\gamma > 0$. This is for example the case of Ecoli, shown in Fig. 2(a). When compared with Fig. 1 or with Fig. 1 of [5] we see that the averaging method of [4] does not show the true behavior of the PS at low frequencies. In this calculation we used the first $2^{22}$ nucleotides, which corresponds to 90% of the Ecoli DNA. We show another case with the same behavior in Fig. 2(b), which is the PS of *Aquíeae aeolicus*. For the PS of *Aquíeae aeolicus* we used the first $10^{20}$ sites, which corresponds to 68% of the chain length. The other organisms, among the ones studied, that show the same PS behavior are *Archaeoglobus fulgidus*, *Synechocystis PCC6803*, *Mycoplasma pneumoniae*, and *Mycobacterium tuberculosis*.

(b) The second type of behavior is the one in which the PS at small frequencies of all the nucleotides presents a power law behavior, which is approximately an extension of the PS behavior at intermediate frequencies. For these organisms, the PS presents only two regions: a flat one at high frequencies, and a power law behavior for intermediate and low frequencies. A typical case for this kind of behavior is shown in Fig. 2(c), which is the PS of *Bacillus subtilis*. In the calculation of the PS in this case we have used the first $10^{22}$ sites, which corresponds to 99% of the total length of the chain. The other organisms studied that have similar PS are: *Treponema pallidum*, *Pyro-h Pyrococcus horikoshii OT3*, and *Mycoplasma genitalium*.

(c) The third, and last, type of behavior we have seen is the one in which, for a given organism, different nucleotides present different asymptotic behavior for the PS at low frequencies. That is, the PS flattens off for some of the nucleotide sequences, and for the others it remains approximately a power-law. An example of such a behavior is shown in Fig. 2(d), which is the PS of *Haemophilus influenzae Rd*. We see that different behavior for the PS are grouped in pairs. In all the cases studied we found that the PS of A is qualitatively similar to the PS of T and the one of C is similar to the one of G. This kind pairing of the statistical features of nucleotides has been reported for yeast chromosomes in [1]. This is probably caused by the strand symmetry of DNA sequences, reported in [13]. In the calculation of the PS we ha ve used the first $10^{20}$ sites of the DNA chain, which corresponds to 57% of the total number of nucleotides. Since a large number of sites are left out of the calculation, we have also analyzed the PS of the central and final region of the chain. We verified that the results remain essentially the same as the ones shown in Fig. 2(d). The other organisms that have similar statistical features for the PS are *Chlamydia trachomatis* and *Helicobacter pylori 26695*.

### B. Autocorrelation Function

The autocorrelation function $R(l)$ of a numerical sequence is, by definition,

$$R(l) = \langle x_k x_{k+l} \rangle,$$

where the brackets denote average over the sites along the chain. For $l = 0$, Eq. (3) implies $R(0) = \langle x_k^2 \rangle$, which is a quantity carrying no information about the relative position of the nucleotides. As in the case of the power spectrum for $S(0)$, this quantity will be neglected in our calculations.

Statistical independence between sites separated a by distance $l$ implies that $\langle x_k x_{k+l} \rangle = \langle x_k \rangle^2$. The value of $l$ above which this condition is satisfied (on average) is called the correlation length. DNA molecules, depending on the organism, can form an open or a closed loop. Bacterial DNA usually forms a closed loop [14]. For circular chains, the autocorrelation function and the PS form Fourier transform pairs (this is the Wiener-Khintchine theorem) [15]. In order to consider the entire DNA sequence (without having the constrains of the Fast Fourier algorithm) we calculate the AF using its plain definition, that is, Eq. (4), and not via Fourier transforming of the PS [16]. We present results for $l$ in the interval $[1, 10^5]$. This is a much larger interval than the ones considered in previous publications [1,17], which took $l$ in $[1, 10^3]$. It is obvious that when $l \ll N$, as it occurs here, it does not matter if we consider open or closed boundary conditions. Since we find computationally easier to consider open boundary conditions, we present the results of the AF for this case. It is beyond the scope of this paper to study cross-correlation between two different kinds of nucleotides. Such a kind of study can be found for example in [18].

We show in Fig. 3 the AF versus $l$ for the sequences whose PS we displayed in Fig. 2. Since the AF presents a strong oscillation of period $3 \cdot 2^n$, we chose $n$ to be a multiple of 3 in order to smooth it out. Here we have used $n = 33$ (there was no particular reason for choosing $n$ a multiple of 3 in the calculation of the PS). In Fig. 3 the horizontal lines are the corresponding values of $\langle x_k \rangle^2$. When $R(l) \equiv \langle x_k x_{k+l} \rangle \gg \langle x_k \rangle^2$ statistical independence between the nucleotides of a given type holds. As Fig. 3 shows, when $l \lesssim 100$ the AF is roughly flat for some sequences, and for others it is approximately a power-law [18]. Then, as $l$ increases we see a regime of a power-law in all cases. For the interval of $l$ studied, we observe that the AF can get flat again as $l$ increases even more (with $R(l) \approx \langle x_k \rangle^2$), or not reach a plateau. For the sequences where the PS flattens off at low frequencies, we expect that the AF will get flat for larger $l$, with statistical independence holding. However, for most of the cases studied, this happens when $l \gg 10^5$. Only the AF of *Aquíeaeae aeolicus* seems to
reach a plateau for \( l \) in the interval \([1, 10^5]\) for all the nucleotides. This is shown in Fig. 3(c) and Fig. 3(d), where we observe that the correlation lengths for this organism appear to be between \( 10^3 \) and \( 10^4 \). For the other organisms studied, we see a wide variety of behaviors for the AF in the region of \( l \in [10^3, 10^5] \). As Fig. 3 shows, we find cases in which the AF reaches a plateau with statistical independence between the nucleotides, in others we see a slow decrease of the AF, such as the AF of A for Bacillus subtilis. We also find an abrupt change of slope in a plateau region, like the AF for A and of G in Haemophilus influenzae Rd. And most interestingly, we find the presence of anti-correlations, that is, \( < x_k x_{k+l} > \) being smaller than \( < x_k >^2 \). This implies that sites separated by a given distance tend to be occupied by different nucleotides. The case in which this appears more strongly is in the AF of C for Haemophilus influenzae Rd. We have also observed that most of the sequences present a peak in the AF at \( l \approx 100 \). The reason for this is unknown to us.

III. CONCLUSION

In summary, we have studied statistical properties of the complete DNA of thirteen microbial genomes and shown that its fractal behavior not always prevails through the entire chain. For some sequences the power spectrum gets flat at low frequencies, and for others it remains a power-law. In the study of the autocorrelation function we have found a rich variety of behaviors, including the presence of anti-correlations.

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