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Biologia molecolare. — *Base composition in* Streptomyces coelicolor *and* Streptomyces rimosus (*). Nota di PETER ANTONOV PETROV (**), ROMUALDO BENIGNI, ANGELO CARERE e ONOFRIO LOSTIA, presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — Per quanto riguarda lo studio della composizione in basi del DNA gli Streptomiceti sono, tra gli organismi viventi finora studiati, quelli con la più alta percentuale di G + C (~ 72). Si è ritenuto opportuno misurare tale percentuale anche per lo *Streptomyces coelicolor* e lo *S. rimosus*, cioè le due specie geneticamente più studiate ma non ancora caratterizzate da questo punto di vista; un ulteriore motivo d'interesse in questo studio era dato da una particolare differenza che era stata osservata nelle mappe genetiche di queste due specie e cioè la presenza, solo sul cromosoma circolare dello *S. coelicolor*, di due ampie « regioni vuote », cioè apparentemente prive di marcatori identificabili. I risultati ottenuti su DNA estratto da spore e da micelio, sia con tecniche di denaturazione termica (Tm) che di ultracentrifugazione (« Buoyant Density » in CsCl e Cs_2SO_4) non hanno mostrato differenze significative tra le due specie; i valori di G + C (%) calcolati, vanno da 68,8 a 72,2.

The base composition was estimated for numerous *Streptomycetes* [1, 2] with the exception of the two species genetically most exploited, *S. coelicolor* and *S. rimosus* whose maps are fairly comparable, but for the occurrence of two "empty" regions in *S. coelicolor* apparently missing in *S. rimosus* [3].

The studies on the base composition of DNA, either with thermal denaturation (Tm) or with density measurements by ultracentrifugation in CsCl and in Cs_2SO_4, provide us with a useful classification criterion, especially in the case of procariotes. Species very similar to each other in many characteristics have approximately the same GC percentages and even in the case of similar genuses there are no great differences in GC percentages [4, 5, 6, 7, 8].

We have determined the base composition in *S. coelicolor* and *S. rimosus* of DNA extracted both from spores and from mycelium grown in submerged culture; for this purpose we have employed sexually different strains, i.e. those with the SCP 1 plasmid (sex-factor) inserted in the circular chromosome of *S. coelicolor* (called NF strains), those with the sex-factor in the cytoplasm (called IF strains) and those without it (called UF strains) [9].

For the extraction of DNA the mycelium was lysed with the enzyme glusulase (Endo Laboratories, Inc.) while the lyophilized spores were broken mechanically in a mortar using silica powder. The purification of the DNA was achieved according to Marmur's method [10]. Thermal denaturation curves were obtained according to the method of Marmur and Doty [5] in a Beckman Acta III spectrophotometer with a temperature programmer.

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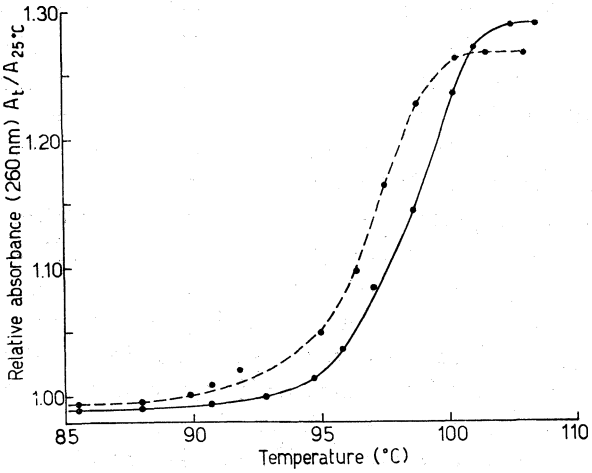

Fig. 1. – Typical curves of thermal denaturation of DNA of *S. coelicolor* performed according to Marmur and Doty [5]. Samples of DNA were about 20 μg/ml in S.S.C. The temperature was increased one degree at a time waiting for thermal stability.

– – – – – – = curve relative to DNA extracted from mycelium;

——— = curve relative to DNA extracted from spores.

The GC percentage was determined with the formula of Marmur and Doty [5] Tm = 69.3 + 0.41 (GC) in SSC (0.15 M NaCl + 0.015 M sodium citrate, pH 7).

TABLE I.

Physico-chemical parameters of DNA *in* Streptomyces

Strain and genotype	Thermal Tm (°C)	Denaturation % GC	Compositional distribution (σ)	Buoyant Density in CsCl		Buoyant Density in Cs_2SO_4
				ρ (g/cm³)	% GC	ρ (g/cm³)
S. rimosus						
Wild type . .	(*myc*) 98.2	70.5	4.7	1.730	71.4	1.436
Wild type . .	(*sp*) 98.9	72.2	4.7	1.730	71.4	1.437
S. coelicolor						
*hisA*1 NF . . .	(*myc*) 98.0	70.0	5.0	1.732	73.5	1.435
*hisA*1 NF . . .	(*sp*) 98.6	71.5	4.7	1.731	72.5	1.434
*hisA*1 UF . . .	(*myc*) 97.7	69.3	5.6	NP	NP	NP
*hisD*3 NF . . .	(*myc*) 97.9	69.7	4.5	1.732	73.5	1.429
*hisD*3 NF . . .	(*sp*) 98.3	70.7	4.6	1.730	71.4	1.431
*hisD*3*pheA*1–*StrA*1 UF . .	(*myc*) 97.5	68.8	4.5	NP	NP	1.433
*hisD*3*pheA*1–*Str* A1 IF . .	(*myc*) 97.8	69.5	2.3	NP	NP	1.431

For technical details and symbols see the text. The values reported are the averages over at least five different experiments. NP = not performed; *myc* = mycelium; *sp* = spores.

The GC % values determined using the Buoyant Density method and CsCl gradients after Schildkraut, Marmur and Doty [6] were obtained in a Spinco Model E ultracentrifuge. The DNA base compositions were calculated according to the equation $d = 1.660 + 0.098$ (GC) where d = Buoyant Density. The density values determined using Cs_2SO_4 gradients were calculated according to the method of Erikson and Szybalsky [11].

The standard deviation (σ) (expressed as GC %) of the compositional distribution of GC % values around the mean was calculated according to the modified formula of De Ley [8] $\sigma = (\Delta t - 0.6) \times 1.25$, where Δt is the temperature range between 15.9 and 84.1 % of the relative total increase in optical density. In Table I are illustrated the results obtained. These show that the GC percentages of the strains studied are in agreement with the the results obtained by the authors previously named [1, 2].

Furthemore these results do not show any significant difference between *S. coelicolor* and *S. rimosus*. The same is true also for sexually different strains. We have found some differences among the Tm values of DNA obtained from spores and from mycelia as did Enquist and Bradley [12]. We did not find any difference between the Buoyant Density values, either in CsCl, or in Cs_2SO_4, where any eventual difference would have been greatly enhanced. This leads us to presume that the observed differences are not due to variations in the GC %, but to some other factor.

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