GALACTOSE INDUCED COLONIAL DISSOCIATION IN *STREPTOMYCES AUREOFACIENS*

Sir:

Streptomyces aureofaciens SA-1 is an axenic culture derived by a series of natural selections from a soil streptomycete that was identified as *S. aureofaciens* NRRL 5758. Narasin[1], a polyether antibiotic and A36640 (unpublished data), a broad spectrum antibiotic of the enteromycin group[2], are produced by this isolate. Spore and vegetative suspensions of SA-1, plated on either a conventional complex or a chemically defined agar medium, produced many colonies with yellow (Y) aerial mycelia (AM^+) and white (W) colonies representing no greater than 5% of the population. Approximately the same number of colony forming units were obtained with a variety of media conditions. However, when SA-1 was plated on an inorganic salts medium with galactose as the sole carbon source, a classical example of colonial pleomorphism was observed. The most prominent characteristic was colonies with a non-uniform sectoring of white and yellow areas (Fig. 1); colonies from other agar media exhibited a similar effect when replica plated to galactose plates. The most unexpected observation was that galactose was the only carbon source inducing colonial pleomorphism. This inductive process was termed "galactose induced colonial dissociation." The induction was not effected with related compounds such as dulcitol (galactitol), galacturonic acid, polygalacturonic acid, galactosamine and tagatose[3]. Dissociation can be inhibited by adding a second carbon source such as glucose to galactose plates. When the second carbon source was limiting (0.01%), SA-1 initially exhibited normal colonial morphology but was transformed to a pleomorphic state when glucose was depleted.

When two well-isolated galactose grown colonies were macerated and plated on a defined or a complex medium, four distinct colony types were isolated (Table 1). One type, YAM^+ (G-22), resembled SA-1 in all aspects but was refractory

Fig. 1. SA-1 colonies on inorganic salts agar medium supplemented with (A) glucose and (B) galactose at final concentration of 1.0%. The inorganic salts medium contained K_2HPO_4 (0.01%), $MgSO_4 \cdot 7H_2O$ (0.025%), NH_4NO_3 (0.2%), $CaCO_3$ (0.25%), $FeSO_4 \cdot 7H_2O$ (0.0001%), $MnCl_2 \cdot 7H_2O$ (0.0001%), $ZnSO_4$ (0.0001%) and Noble agar (2.0%).

(A) (B)

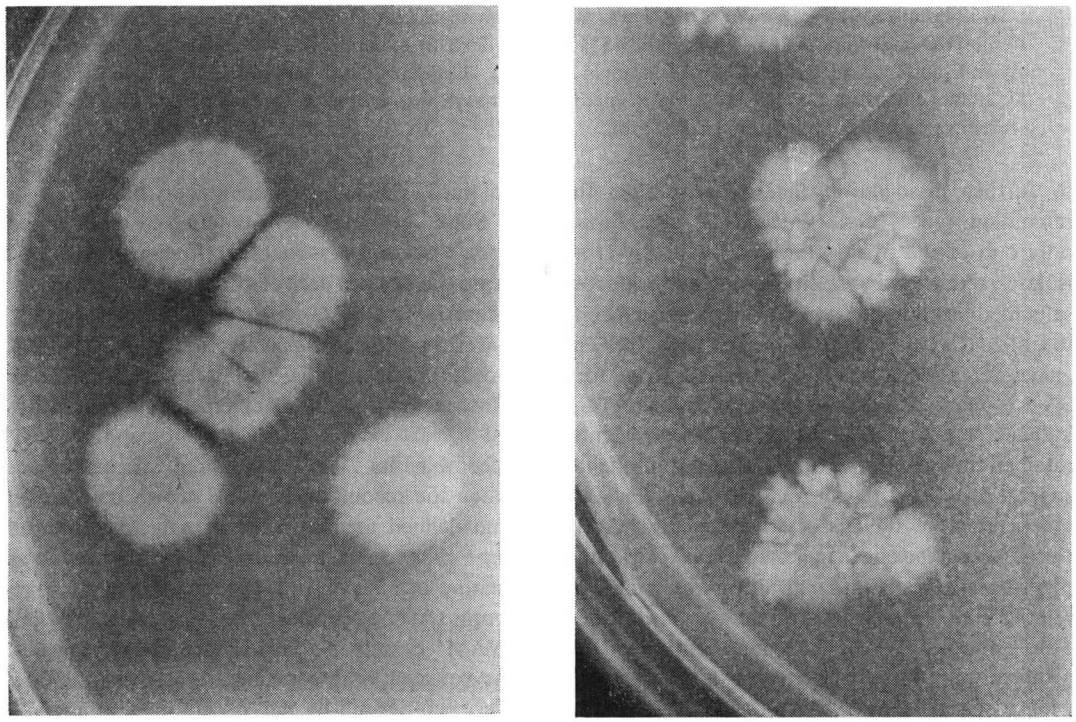


Table 1. Galactose induced isolates[a]

Culture	Colony type	Rhamnose utilization[b]	Antibiotics synthesized	Percent[c]
SA-1	YAM$^+$	+	Narasin A36640	—
G-4	YAM$^-$	—	A36640	3
G-9	WAM$^-$	—	A36640	1
G-22	YAM$^+$ (Galactose stable)	+	Narasin A36640	54
G-23	WAM$^+$	+	Narasin	41

[a] A well isolated galactose grown colony was completely removed from the agar surface, homogenized in a tissue homogenizer, diluted and plated on complex (beef extract, 0.1%; dextrine, 1.0%; yeast extract, 0.1%; NZ amine A, 0.2%, $CoCl_2$, 0.001%, agar, 2.0%. pH adjusted to 7.0), glucose and galactose salts media.

[b] +: positive utilization; —: no utilization.

[c] Average of population from two galactose grown colonies plated on glucose salts medium.

Table 2. Effect of mitomycin C on SA-1

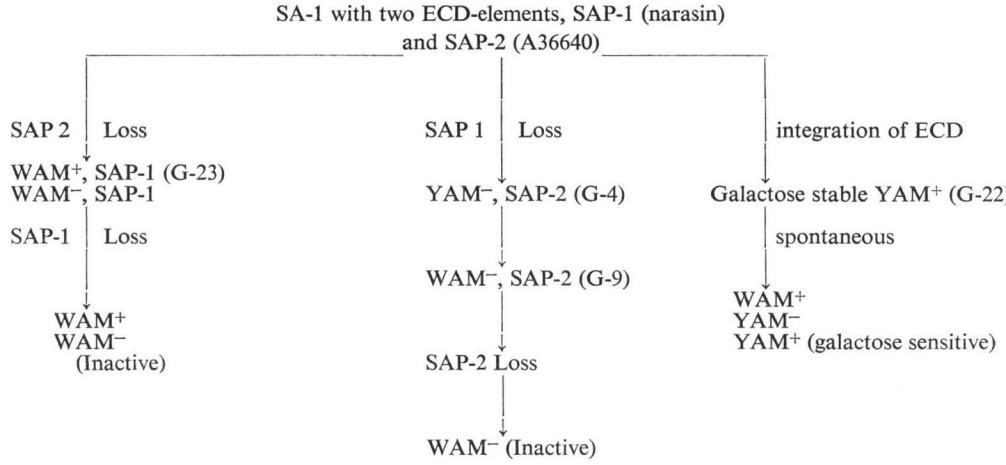
Mitomycin C mM	Growth % mycelial volume	Total No. colonies counted	No. white colonies	% white colonies
0.19	4	456	188	41
0.093	10	482	182	38
0.047	12	236	90	38
0.024	19	362	86	24
0.012	14	429	51	12
None	16	410	18	4

A 72-hour vegetative growth of SA-1 was used to inoculate (2.0%) 50 ml Erlenmeyer flasks containing 10 ml TSB-11 (trypticase soy broth plus 1.5% tapioca dextrin) medium with the indicated levels of mitomycin C and incubated at 30°C for 24 hours in the dark. Cellular growth was washed two times with TSB-11, homogenized and plated on complex medium. Growth was estimated by centrifugation in a conical centrifuge tube for 15 minutes at 1,800 rpm.

to further dissociation (galactose stable); the remaining types (G-4, G-9, G-23) synthesized only one of the two antibiotics. Like SA-1, all isolates retained the ability to grow on glucose salts medium but G-4 and G-9 lost their capacity to utilize rhamnose as a sole carbon source. The galactose induced selections derived from four SA-1 N-methyl-N′-nitro-nitrosoguanidine(NTG)-induced amino acid auxotrophs consistently retained their respective amino acid requirements, suggesting that the amino acid markers were independent of the dissociative process. Additional observations suggested that AM and pigment synthesizing capabilities were not correlated with the synthesis of the two antibiotics. For example, WAM$^-$ variants continued to produce narasin when derived from WAM$^+$; when derived from YAM$^-$, the WAM$^-$ strains retained A36640 producing capabilities.

Since dissociation was effected with galactose, it seemed reasonable to "reconstruct" SA-1 from the isolates derived through the dissociative process. Two NTG-induced auxotrophs (WAM$^+$, narasin producer, aspartate; and YAM$^-$, A36640 producer, isoleucine, valine) were mated on glucose agar plates supplemented with 250 μg ml^{-1} yeast extract to yield prototrophic YAM$^+$ recombinants. The YAM$^+$ state was maintained when the recombinants were grown on complex and defined media, including galactose, indicating a galactose-stable recombinant. An additional mating of a second NTG-induced YAM$^+$ auxotroph (A36640 producer, leucine) with the same WAM$^+$ described above yielded identical results. As expected, both narasin and A36640 antibiotics were synthesized by the YAM$^+$ recombinants.

Fig. 2. Proposed model for galactose-induced colonial dissociation



The galactose induced dissociation process suggested that galactose was mimicking a curing agent. Therefore, SA-1 was exposed to well known curing agents and the percent appearance of WAM+ was scored as an index of curing. The data summarized on Table 2 indicate that mitomycin C[4] profoundly altered the phenotypic character of SA-1. The resulting WAM+ isolates were prototrophs exclusively producing narasin and appeared to be identical to galactose-induced and spontaneous WAM+. Other curing agents such as acriflavine, acridine orange, ethidium bromide and rifampicin failed to induce the desired effect or other changes in colonial appearance.

It is clear that galactose imposes a dramatic effect on SA-1 and, to our knowledge, represents a novel observation of a simple monosaccharide inducing differentiation of a seemingly ordinary culture into four morphological types. The data appear to be most consistent with the involvement of extrachromosomal DNA (ECD) in the dissociation process. Thus far, our efforts to physically demonstrate the existence of plasmids in SA-1 have been unsuccessful. A reason for this failure was probably attributed to the presence of potent nucleases (detected on DNA agar plates) in our preparations. A model schematically presented in Fig. 2 summarizes our current views on galactose-induced colonial dissociation. We propose two ECD elements, SAP-1 and SAP-2, coding for narasin and A36640 respectively. Exposure of SA-1 to galactose appears to result in the following changes: (1) SAP-1 loss generates A36640 only producers; (2) SAP-2 loss results in the generation of narasin only producing strains; (3) Loss of both SAP-1 and SAP-2 results in inactive strains; these strains have been isolated from both narasin and A36640 only producing isolates; (4) The galactose-stable type is envisioned to be a SAP-1 and SAP-2 chromosome integrated form of SA-1 which may be the dominant form found in nature. The detection of this stable state suggests a mechanism whereby SA-1 can enter a galactose-resistant form to insure survival of the YAM+ phenotype. The spontaneous appearance of WAM+, YAM− and galactose-sensitive YAM+ isolates from the integrated state may represent a natural return to the described autonomous states.

In summary, galactose appears to possess a property similar to curing agents, profoundly altering the phenotypic character and biosynthetic capabilities of SA-1. Retention of nutritional independence in the four morphological types implies no major alterations in glucose and galactose metabolism; all attempts to detect nutritionally dependent strains, particularly galactose requiring variants, were unsuccessful. The simultaneous loss of narasin synthesis and rhamnose utilization by A36640 only producers suggest a close relationship with each other. For the present, we propose that galactose is an inducer of dissociation, possibly resulting in the loss of ECD elements. Our present efforts are

being directed towards physical proof of the proposed model and a more critical evaluation of nutritional differences in the four morphologically distinct colony types.

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