Morphological and physiological changes in *Streptomyces lividans* induced by different yeasts.

Key words: *Streptomyces*, yeast, invertase, glucose, fructose, differentiation

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Summary

Streptomyces development is a complex process that eventually finishes with the formation of individual unigenomic spores from the aerial hyphae. Intraspecific and interspecific signals must play a key role in triggering or blocking this process. Here we show that interaction between two types of microorganisms, *Streptomyces* and yeasts, leads to alteration of the *Streptomyces* developmental programme. This alteration is due to the action of invertase produced by the yeast on the sucrose present in the culture media, making glucose and fructose readly available for growth.

Introduction

Streptomycetes have a complex life cycle in which spore germination leads to the development of a branching vegetative mycelium out of which aerial hyphal branches grow. This process ends with the formation of equidistant sporulation crosswalls that finally generate unigenomic differentiated spores (Chater 1998). This program involves the exchange of several different intercellular signalling molecules, making it a function of the entire interactive population of hyphal branches and compartments.

Intercellular communication among bacteria has been widely described (Kaiser and Losick 1993; Wirth et al. 1996; Gray 1997) and several kinds of molecules have been implicated (reviewed in Shapiro 1998). The homoserine lactones are the most common in Gram-negative species, (Kaiser and Losick 1993; Wirth et al. 1996; Gray 1997) and the γ-butyrolactones in *Streptomyces* (Beppu 1995). Other types of signals include oligopeptides (Klerebezem et al. 1997) amino acids, and proteins (Kim et al. 1992; Kaplan and Plamann 1996; Shimkets and Dworkin 1997).

Due to the natural soil-dwelling habits of *Streptomyces* species, interactions with the environment and other organisms are likely to influence the specific developmental pathways of these bacteria. This is obvious even in a simplified ecosystem such as a contaminated Petri dish in the laboratory, where *Streptomyces* colonies often change their morphology as a response to the contaminant. Thus, during the comparatively long periods (up to two weeks) needed for mature colonies to form on Petri dishes, it is not uncommon to find the plates contaminated with other microorganisms, such as filamentous fungi, yeast or unicellular bacteria. In these circumstances, the *Streptomyces* colonies may overproduce pigments, antibiotics

and chemical signals and show changes in their growth patterns, even leading to obvious changes in the development of aerial mycelium.

In this work we describe the effect that several yeasts have on *Streptomyces* differentiation on solid medium, and demonstrate that the enzyme invertase, produced by different microorganisms, can trigger this change in morphology.

Materials and Methods

Microorganisms used. *Streptomyces lividans* 66 was used in most of the experiments. Other *Streptomyces* strains used are referred to in the legend of Fig. 1. Most of these strains were obtained from the John Innes Centre. The yeast strain routinely used was *Saccharomyces cerevisiae* W303 or its derivatives. Other strains were: *Candida albicans* CA14, *Hansenula polymorpha* NCYC495, *Kluyveromyces lactis* 2359/152, *Pichia anomala* ATCC 8168, *Pichia angusta* ATCC 14755, *Schizosaccharomyces pombe* 972, *Schwanniomyces occidentalis* ATCC 26077, and *Yarrowia lipolytica* W29.

Media and culture conditions. *Streptomyces* spores were obtained on R2YE or SFM agar plates (Hopwood et al. 1985; van Wezel et al. 1997). R2YE was normally used to detect the biological effect on solid medium. $CuSO_4$ (10 μM) was added to increase sporulation (Keijser et al. 2000). R2YE without sucrose was also used in some experiments.

Co-cultures of *Streptomyces* and different yeast strains were carried out on R2YE; *Streptomyces* strains were inoculated as a lawn using a spore suspension (approximately 10^7 spores/plate) and the yeasts were inoculated by stabbing with a toothpick. The plates were incubated at 28°C and observed every 24h.

The biological activity present in the supernatant of yeast cultures was evaluated in a similar way. Different yeast strains were grown in liquid YEPD (Rose et al. 1990) on a rotary shaker at 28°C and 200 rpm from 4 to 8 days. The cells were harvested by centrifugation and the supernatant was sterilised by filtration through a Millex-GP 0.22 μm filter. Different quantities of the filtered supernatant were tested in wells on

R2YE plates inoculated with a lawn of *S. lividans* 66. Manipulation of *Saccharomyces cerevisiae* utilized protocols described in Rose et al. (1990).

Liquid cultures were made up in three-baffled flasks with 1/10 volume of medium; YE (1% Yeast extract, 5 mM of $MgCl_2$, pH 7) supplemented with 10,3% sucrose (Ruiz-Arribas et al. 1995). Growth was carried out at 28°C and 200 rpm in an orbital shaker (Adolf Kühner AG, Birsfelden, Switzerland) for as long as required for each assay.

Protein purification. For effector purification,four two-litre flasks containing 500 ml of YEPD each were inoculated with *Saccharomyces cerevisiae* W303 and incubated on a rotary shaker at 28°C for 7 days. The supernatant was concentrated 100 times in an Amicon cell equipped with a YM100 membrane and diafiltered with 20 mM Tris-HCl, pH 7,9. The concentrated sample and fractions of the different columns were tested for biological activity against *S. lividans* as described above.

Chromatographic procedures were carried out with a Fast Performance Liquid Chromatography system (FPLC, Pharmacia Fine Chemicals). Absorbance at 280 nm and 205 nm was monitored with a Waters 690 multiwavelength detector coupled via a System Interface Module (SIM) to a computer running the Maxima 820 software (Waters).

The sample was loaded on a MonoQ (HR 5/5 anion exchange) column (Pharmacia Fine Chemicals) equilibrated with the same buffer. Fractions from the anionic exchange column showing biological activity were pooled, diafiltered with PBS (137 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1,5 mM KH_2PO_4 pH 7.2) and concentrated approximately 6-fold by an Amicon C-30 (YM-30), and rendered 1.7 M ammonium sulfate (final concentration) prior to loading on a Phenyl-Superose column (HR 5/5

hydrophobic interaction column; Pharmacia Fine Chemicals). The active fractions were pooled and concentrated 15-fold by an Amicon C-30. Aliquots of 200 μl were loaded onto a Superdex-200 column (HR 10/30 gel filtration column; Pharmacia Fine Chemicals) that had been equilibrated in PBS, pH 6.8. The active fractions were concentrated by Amicon C-30 and re-chromatographed in the same column under the same conditions to give homogeneous preparations of a single protein. Protein contents were estimated by spectrophotometry at 280, 260 and 205 nm. The amino-terminal end was determined in an Applied Biosystems Protein Sequenator .

SDS-PAGE was carried out as described by Laemmli (1970) and the gels were calibrated with broad range of SDS-PAGE standards (Life Technologies).

Photography. Photographs of plates were taken by direct scanning with a Snap Scan 1236 (Agfa) equipped with a transparency option. A Stemi 11 Zeis stereomicroscope was used to obtain photographs of part of the plates.

RESULTS

Streptomyces detects its neighbours

This work started with the occurrence of a pink, yeast-like colony, at the edge of an R2YE agar plate inoculated with a lawn of *S. lividans* 66. The yeast was surrounded by a zone of growth inhibition (about 1.5 cm in diameter) of *S. lividans* aerial mycelium and sporulation **(FIG. 1A)**. Blockage of aerial mycelium and sporulation did not seem to kill the substrate mycelium in the inhibition zone since samples taken from this area produced abundant normal growth and differentiated when inoculated into fresh R2YE plates. At the boundary the bald area, with the normally sporulated part of the lawn, an overgrowth of white-pink aerial mycelium was formed, sometimes decorated with droplets of liquid **(FIG. 1B)**. Isolation and microscopic examination indicated that the contamination was an undefined species of yeast. A similar effect was also produced by 100 µl of filtered supernatant from a four-day old submerged YEPD culture of this yeast deposited in a well. The substance was retained in a Centricon apparatus equipped with a 100-kDa cut-off cellulose filter (Amicon, YM-100), pointing to a high molecular mass, and was inactivated by boiling the supernatant for 15 min. indicating a proteinaceous nature (data not shown). We named this active supernatant "the effector".

Other yeasts induce a similar effect

Nine laboratory yeast strains of different species were stabbed into an R2YE plate inoculated with a lawn of *S. lividans* 66, and incubated at 28°C. Substrate mycelium grew normally all over the plate, regardless of the presence of the faster growing yeast colonies. After 6 days, halos of inhibition of aerial mycelium formation and

sporulation were observed around some, but not all, of the yeast colonies checked. *Kluyveromyces lactis* 2359/152 caused the strongest effect, while *Pichia anomala* ATCC 8168, *Saccharomyces cerevisiae* W303 and *Schizosaccharomyces pombe* 972 produced an inhibition zone similar to that caused by the original yeast contaminant. *Candida albicans* CA14, *Hansenula polymorpha* NCYC 495, *Pichia angusta* ATCC 14755*, Schwanniomyces occidentalis* ATCC 26077 and *Yarrowia lipolytica* W 29 did not cause any effect **(FIG. 1C)**.

Fractionation experiments with *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Kluyveromyces lactis* cultures grown for four days in liquid YEPD showed that the release of the effector was slightly different in these three yeasts. While in *Saccharomyces cerevisiae* most of the effector was released to the culture supernatant, in *Schizosaccharomyces pombe* most of the effector was in the cell extract, and in *Kluyveromyces lactis* the effector was more abundant in the supernatant but was also clearly present in the cell extracts (data not shown). Again, *Kluyveromyces lactis* was the most active, and hence 10 *µ*l of 4-day culture supernatant was sufficient to induce the phenotype while 100 *µ*l and 150 *µ*l were necessary for *Saccharomyces cerevisiae* and for *Schizosaccharomyces pombe* respectively.

Although *Kluyveromyces lactis* produced more effector than *Saccharomyces cerevisiae* this last microorganism was used in the following experiments because its genome sequence was available.

Other *Streptomyces* species are also sensitive to the effector

All the experiments described above were done with *S. lividans* 66 as indicator strain. Other *Streptomyces* species were also tested for their sensitivity to the yeast effector. *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Kluyveromyces lactis* were stabbed into lawns of twelve different *Streptomyces* species on R2YE plates, and incubated at 28°C. The growth and differentiation responses were monitored every 24 h and finally recorded after 8 days, to allow for all the strains to develop completely. Not all the species showed the same sensitivity to the yeast. The most sensitive strains were *S. lividans* 66 and *S. griseus* IMRU 3570. Some other species, such as *S. badius* ATCC 39117*, S. coelicolor* A3(2)*, S. flavogriseus* ATCC 33331*, S. halstedii* JM8 CECT 3310, *S. olivaceus* SC 3107*, S. parvulus* JI 2283 and *S. rochei* ATCC 10739, displayed halos of different sizes, and normally *Kluyveromyces lactis* was the most active yeast. Other species such as *S. albus* J1074*, S. vinaceus* JI 2838*,* and *S. viridochromogenes* ATCC 14920 did not show evident inhibition halos **(FIG. 1D)**. We concluded that sensitivity to yeast-induced developmental alteration is widespread among streptomycetes although its intensity is species dependent.

Time of addition of the effector.

So far, all our studies had been done with simultaneous inoculation of the yeast (or fractions) and the *Streptomyces* spores. To test whether the inhibitory effect depended on the time of addition of the effector, filter-sterilised supernatant (50 and 100 µl) of a four-day old *Saccharomyces cerevisiae* culture in YEPD broth was added at 24-h intervals (0, 24, 48, 72, and 96 h) to R2YE agar plates previously inoculated with *S. lividans* at time zero. The plates were incubated for up to ten days

at 28°C. The inhibitory effect was identical to that previously observed when the effector was added at 0 and 24 h, and decreased in intensity thereafter (particularly for the 50 μl sample). At 72 and 96 h, only the 100-μl sample caused a small halo of delayed sporulation, observed as a small white zone of aerial mycelium around the application point **(FIG. 2)**. Apart from the dose-dependent effect, this experiment indicated that the sensitivity of the *Streptomyces* cells to the effector decreased as the developmental programme progressed (the aerial mycelium formation begins about 65 h), perhaps due to the different physiological conditions at each time point or to the formation of a permeability barrier.

Invertase is the effector.

In order to purify the effector, the dialysed and concentrated supernatant of *S.cerevisiae* possessing the ability to inhibit the formation of *Streptomyces* aerial mycelium was chromatographed as indicated in Materials and Methods. After three chromatographic steps, 160 μg of a pure protein was obtained. The amino-terminal end, xMTxETSDxPLVxFT (where x is an unidentified amino acid), was determined and compared against the protein databases. The invertase from *Saccharomyces cerevisiae* presented the highest identity. Enzymatic invertase activity was detected in the purified protein while no other activities, such as glucanase, protease or chitinase, were detected (data not shown).

That invertase activity was responsible for the observed effect was also demonstrated by using two commercial purified invertases from Sigma (I-9274, from baker's yeast and I-4753, from *Candida utilis*). One microgram of both invertases deposited in a well of a R2YE plate inoculated with a lawn of *S. lividans,* caused a halo of aerial mycelium inhibition (data not shown).

Additional genetic evidence was obtained when a *Saccharomyces cerevisiae* wild-type strain (X2180-1A) and a Suc2 deletion mutant (SEY2101) (Emr et al. 1983) were stabbed into an R2YE plate inoculated with a lawn of *S. lividans.* After 7 days at 28°C, the inhibition halo was clear around X2180-1A (the wild-type strain) but was absent around the SEY2101 (Suc2 mutant) colony **(Fig. 3)**. Identical results were obtained when *Kluyveromyces lactis* JA6 (wild-type) and the invertase-negative mutant Y165 (Georis et al. 1999) were used (data not shown) .

The introduction of pRB58, a 2 *μ*m plasmid derivative overexpressing the SUC2 gene from *Saccharomyces cerevisiae* (Carlson and Botstein 1982), into strain SEY2101 restored effector production and curing of the plasmid rendered colonies unable to display any inhibitory effect on *Streptomyces* **(Fig. 3)**. These results clearly demonstrated that invertase was involved in the observed phenotype.

How does invertase produce its effect?

Since the morphological effect exerted by invertase was only observed on conventional R2YE and not when sucrose (10.3%) was excluded, we decided to test whether the products generated by this enzyme would induce the same phenotype. Forty millilitres of 10.3% sucrose were treated with 40 *μ*g commercial invertase (Sigma I-9274)/ml for 24 h at 30°C. The sample was filtered through a Centricon plus-20 cartridge equipped with a cellulose membrane with a 30 kDa cut-off. The eluate was free of residual invertase activity and no biological activity (assayed on plates inoculated with *S. lividans*) was detected, even when more than 2 ml were deposited in the well. However, when this hydrolysate was used to replace the sucrose in the R2YE, inhibition of differentiation was observed.

Considering that R2YE medium was originally designed for protoplast regeneration by the inclusion of 10.3% sucrose for osmotic stabilization (Okanishi et al. 1974) and that total enzymatic hydrolysis would generate equimolar amounts of glucose and fructose, we prepared R2YE without sucrose but containing 5.15% of glucose and/or 5.15% of fructose. *S. lividans* 66 inoculated on these plates was unable to differentiate when both sugars were present and differentiated only partially in plates containing this concentration of just one of the sugars (**Fig. 4A**). However, higher concentrations of glucose or fructose alone were also able to inhibit differentiation.

Although no morphological changes (differentiation) has been observed in this strain on liquid media, we decided to study whether the conditions described above had any effect on the normal mycelial growth of this strain. This study was accomplished using 100-ml baffled flasks containing 10 ml of YE liquid medium supplemented with 10.3% sucrose and inoculated with *S. lividans* spores (10^7). Several amounts of pure invertase were added and the cultures incubated at 28^{o}C in a shaker. A sample of each flask was observed under light microscopy every 24 h. The mycelial morphology was identical during the first 48 h but after 3 days the cultures that had 1 μg of pure invertase/ml appeared as fragmented, irregular and thicker mycelium. The change in morphology was more dramatic on ensuing days; thus, after 5-7 days these cultures had a morphology similar to unicelular bacteria **Fig. 4B**. A similar result was obtained in cultures in which the sucrose had been replaced by 5.15% glucose and 5.15% fructose and not invertase was added. When only 5.15% of one of the sugars was present, the culture appeared as small pellets containing short fragments of mycelium but no unicellular structures were observed at all **Fig. 4B**.

Again, higher concentrations of glucose, 8-10%, mimicked the effect of both sugars and resulted in mycelium with a fragmented aspect.

The main proof that invertase exerts its effect through glucose and fructose was obtained when *S. lividans* G015, a glucokinase (*glkA*) mutant, was used as the test strain. Metabolism, but not transport of glucose is blocked in this strain (Saito et al. 1998). Glucose kinase plays an essential role in the glucose repression of genes required for arabinose and glycerol transport and of genes required for the metabolism of galactose, fructose and glycerol in *S. coelicolor* (Hodgson 1982; Seno and Chater 1983). *S. lividans* G015 differentiated normally on plates of R2YE on which the sucrose had been hydrolysed with invertase or in plates of R2YE without sucrose containing 5.15% of glucose and/or 5.15% of fructose **(Fig. 4C)**. Morphology was also observed on liquid media (YES) suplemented with 1 μg invertase/ml and on YE suplemented with 5.15% glucose or/and 5.15% fructose. The effect observed was very weak when compared with the fragmentation observed in the wild type strain **(Fig. 4D)**.

Discussion

Complex morphological development such as that occurring in *Streptomyces* involves intricate pathways where intracellular and extracellular signals are perceived by regulatory systems that repress or trigger the process. Among the effectors of these pathways, sugars act not only as nutrients but also as important regulators of gene expression. Growth in poor carbon sources, such as mannitol, rescues the developmental defects in some *Streptomyces* "bald", *bld*, mutants (Chater 1984). In this paper we show that interaction between two microorganisms (*Streptomyces* and yeast) in a simple ecosystem (a plate of R2YE medium) leads to an alteration in the developmental programme of one of them: i.e., *Streptomyces*. This alteration is not the consequence of antibiosis, pheromone production, or competition for resources, but rather of an alteration in the availability of a carbon source, glucose and fructose, in this case, through the action of the invertase produced by the yeast. We have since isolated a bacterium (pressumed to be a *Bacillus sp.*) that is able to induce the same effect by the action of the same enzyme: invertase. This supports the idea that the interaction described here is quite common for *Streptomyces* and other microorganisms, and different from cases where co-habitation can actually accelerate the sporulation process. Along the same line, nutrient levels are thought to affect colony morphology in *Bacillus* (Kawasaki et al. 1997). Morphological changes and the induction of pigment production have been described in *Monascus* when co-cultivated with *Saccharomyces cerevisiae* or with *Aspergillus oryzae*. The effector in that case was a chitinase produced by *Saccharomyces cerevisiae*, that caused a partial hydrolysis of the *Monascus* cell wall (Shin et al. 1998).

In our experimental system, glucose and fructose are the final effectors, causing developmental blockage in *Streptomyces lividans*. The inhibition of *Streptomyces* differentiation by glucose has been previously described by several authors (Redshaw et al. 1976; Surowitz and Pfister 1985; Ueda et al. 1999; Umeyama et al. 1999). In *S. alboniger,* this glucose excess elicits an uncoupling between glycolysis and the TCA cycle and originates the excretion of pyruvate and acidification of the media (Surowitz and Pfister 1985) that may prevent differentiation. In *S. coelicolor* grown on unbuffered glucose based minimal media cAMP was required to avoid a developmental block caused by acid toxicity (Süsstrunk et al. 1998). We have observed that, on solid media, the inhibition area has pH5-6 and similar pH values were obtained in the fragmented submerged liquid cultures, while the pH on the controls was about 7. These results suggest that acidosis could be implicated in this process, but we have not followed this further. On other set of experiments, the glucose kinase mutant *S. lividans* G015 is resistant to the inhibitory effect, which argues again in favour of glucose and fructose or its metabolic derivatives as being responsible for blocking differentiation. Although glucose kinase mutants are able to transport glucose into the cells, they are unable to use it as a carbon source and are relieved from catabolite repression. Kelemen et al. (1995) reported that deletion of DNA lying close to, but distinct from *glkA*, induced ectopic sporulation in *S. coelicolor*, but no such a deletion has been reported to occur in the *S. lividans* G015 mutant. An alternative explanation -the osmotic effect of a high glucose concentration- could also be ruled out since, again, the glucose kinase mutant subjected to the same osmotic stress was still able to differentiate. Thus, either glucose derivatives produced through the action of GlkA, or GlkA itself due to its

suggested direct regulatory intervention in catabolite repression, must be considered as being responsible for the observed effect. Pope et al. (1996) and Viollier et al (2001) suggested that some *bld* genes play a key role in the generation or detection of signals required to evaluate the nutritional environment of the colony.

Several promoters have been described as targets for carbon regulation, most of them related to polysaccharide-hydrolase genes but, as far as we know, no development-associated promoters have been identified as direct targets for catabolite repression. It would be interesting to study which of the *bld* and *whi* genes are expressed or repressed in the presence of glucose and the phenotype of strains carrying both the *glkA* and the different *bld* and *whi* mutations.

Another possibility is that sucrose itself may have some effect on differentiation in *Streptomyces*. Most *Streptomyces* strains are unable to use sucrose as a carbon source, due to the lack of invertase or sucrase activity that would cleave it into hexoses. However, glucose-induced of sucrose uptake and utilization has been described in *S. alboniger* (Surowitz and Pfister 1985). To our knowledge, no other reports of sucrose use have been published and, so far, no putative invertase genes have appeared in the *S. coelicolor* sequencing project. It could be speculated that if sucrose itself had a positive effect on sporulation, its breakdown would prevent that positive effect from being exerted. Corroborating this, we have observed a positive effect, in *S. lividans* sporulation, when sterile filtered sucrose is used to prepare R2YE instead of the normal recipe, where sucrose is autoclaved with most of the media components and can be partialy hydrolysed during the sterilisation process. Sucrose and invertases play a key role in the differentiation of plants, where they have different functions depending on the type of invertase and its location (Sturm

1999). The sugar concentration and/or the resulting changes in osmotic values maintain a certain state of differentiation or lead to changes in the developmental programme. Whereas a high hexose-to-sucrose ratio favours cell division, a low hexose-to-sucrose ratio favours differentiation. This would indicate that the ability to switch from the undifferentiated to the differentiated state is not only an intrinsic property of the cells but also a result of metabolic signals (Weber et al. 1997; Wobus and Weber 1999; Viollier et al. 2001). A positive correlation between increased acid invertase activity and the infection of plants by certain pathogens has also been reported (Sturm and Chrispeels 1990). Although the physiological significance of this response is not yet clear, it is possible that the hexoses released might be involved in stress signalling and/or might act as gene expression regulators for the pathogen.

In *Saccharomyces cerevisiae*, several glucose transporters have been described and two of them, Snf3p and Rgt2p, act as glucose sensors (Özcan et al. 1996). We used their sequences to search for the corresponding proteins in the *S. coelicolor* sequence database. Two proteins were identified that may have similar functions in *Streptomyces* and in a forthcoming work we intend to study their involvement in glucose sensing and the control of development in this organism.

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Figure legends

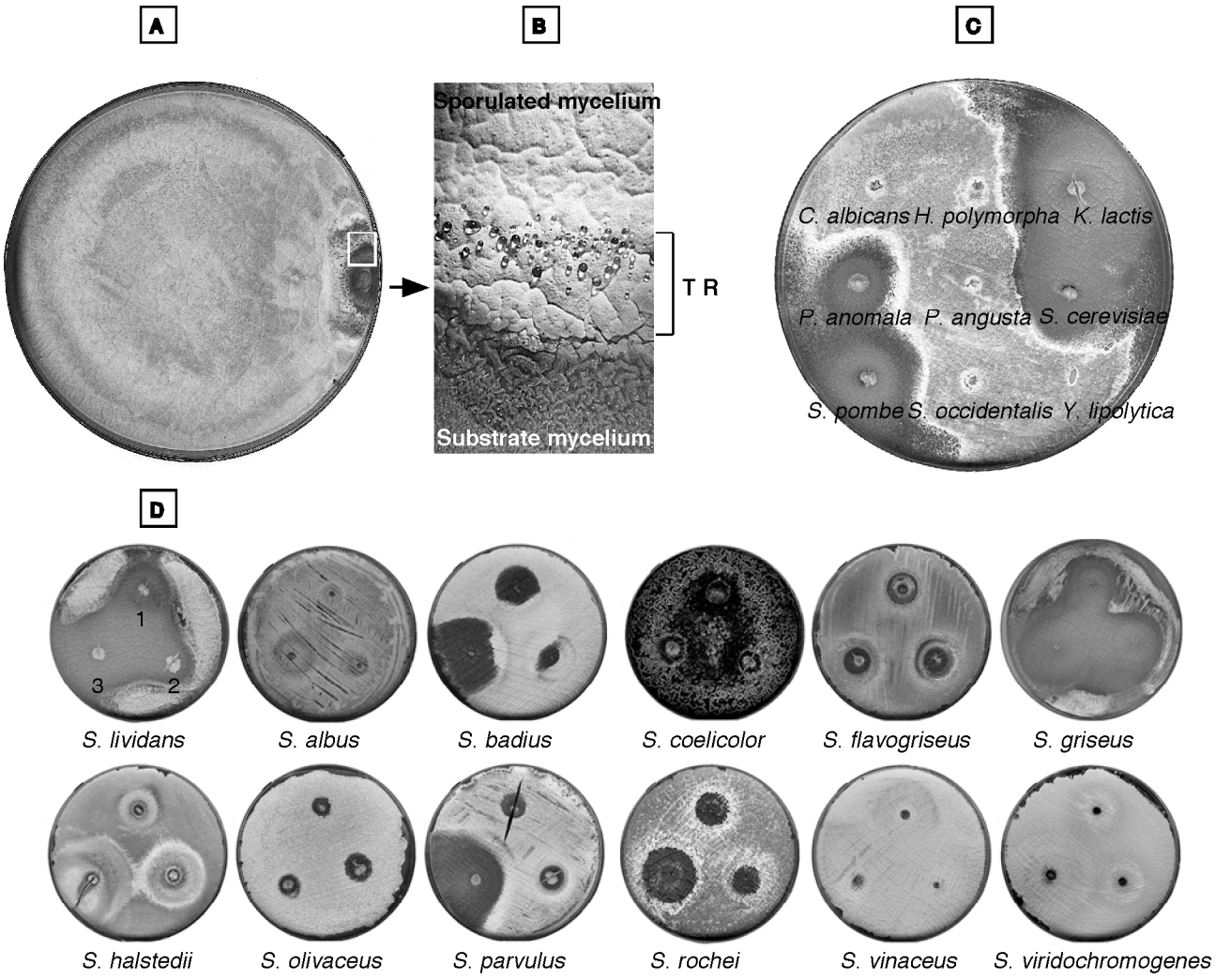
Fig. 1 A) Plate of R2YE showing the effect of yeast contamination (right side) on the differentiation of *Streptomyces lividans* 66. B) A closer view of the area indicated in Fig 1A. Droplets were sometimes visible in the transition region (TR) to the normally sporulated part. C) Plate of R2YE inoculated with a lawn of *S. lividans* 66 showing the effect of different yeasts. *Candida albicans* CA14; *H. polymorpha* NCYC 495; *Kluyveromyces lactis* 2359/152; *Pichia anomala* ATCC 8168; *Pichia angusta* ATCC 14755; *Saccharomyces cerevisiae* W303; *Schizosaccharomyces pombe* 972; *Schwanniomyces occidentalis* ATCC 26077; *Yarrowia lipolytica* W29.
D) Effect of *Saccharomyces cerevisiae* (1), *Schizosaccharomyces pombe* (2) and *Kluyveromyces lactis* (3) on the differentiation of several *Streptomyces* species. *S. lividans* 66; *S. albus* J1074; *S. badius* ATCC 39117; *S. coelicolor* A3(2); *S. flavogriseus* ATCC 33331; *S. griseus* ATCC 10137; *S. halstedii* JM8 CECT 3310; *S. olivaceus* SC3107; *S. parvulus* JI 2283; *S. rocheii* ATCC 10739; *S. vinaceus* JI 2838; *S. viridochromogenes* ATCC 14920.

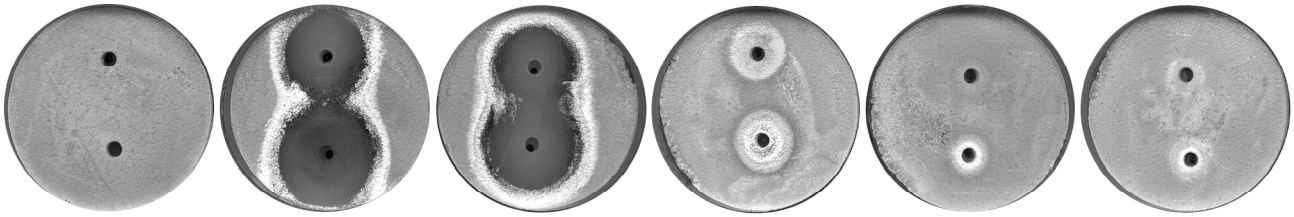
Fig. 2. Effect of the addition of 50 µl (upper part) or 100 µl (lower part) of *Saccharomyces cerevisiae* supernatant on *S. lividans* 66 at the indicated time. 50 or 100 µl of water was added in the control (0h).

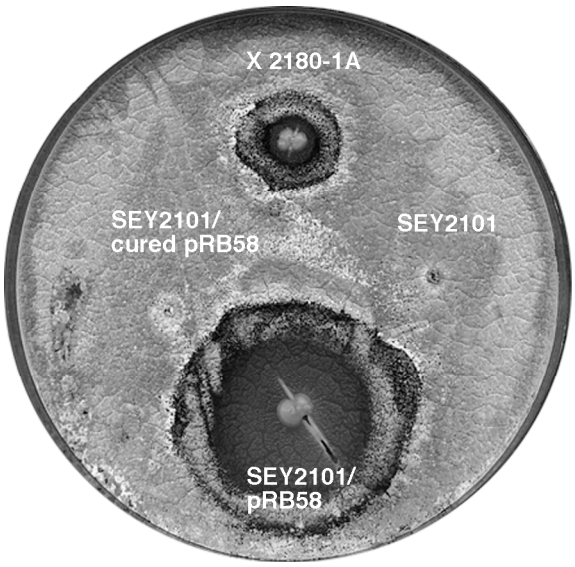
Fig. 3. Plate of R2YE inoculated with a lawn of *S. lividans* 66 showing the effect of different *Saccharomyces cerevisiae* strains. X2180-1A (wild type strain). SEY2101

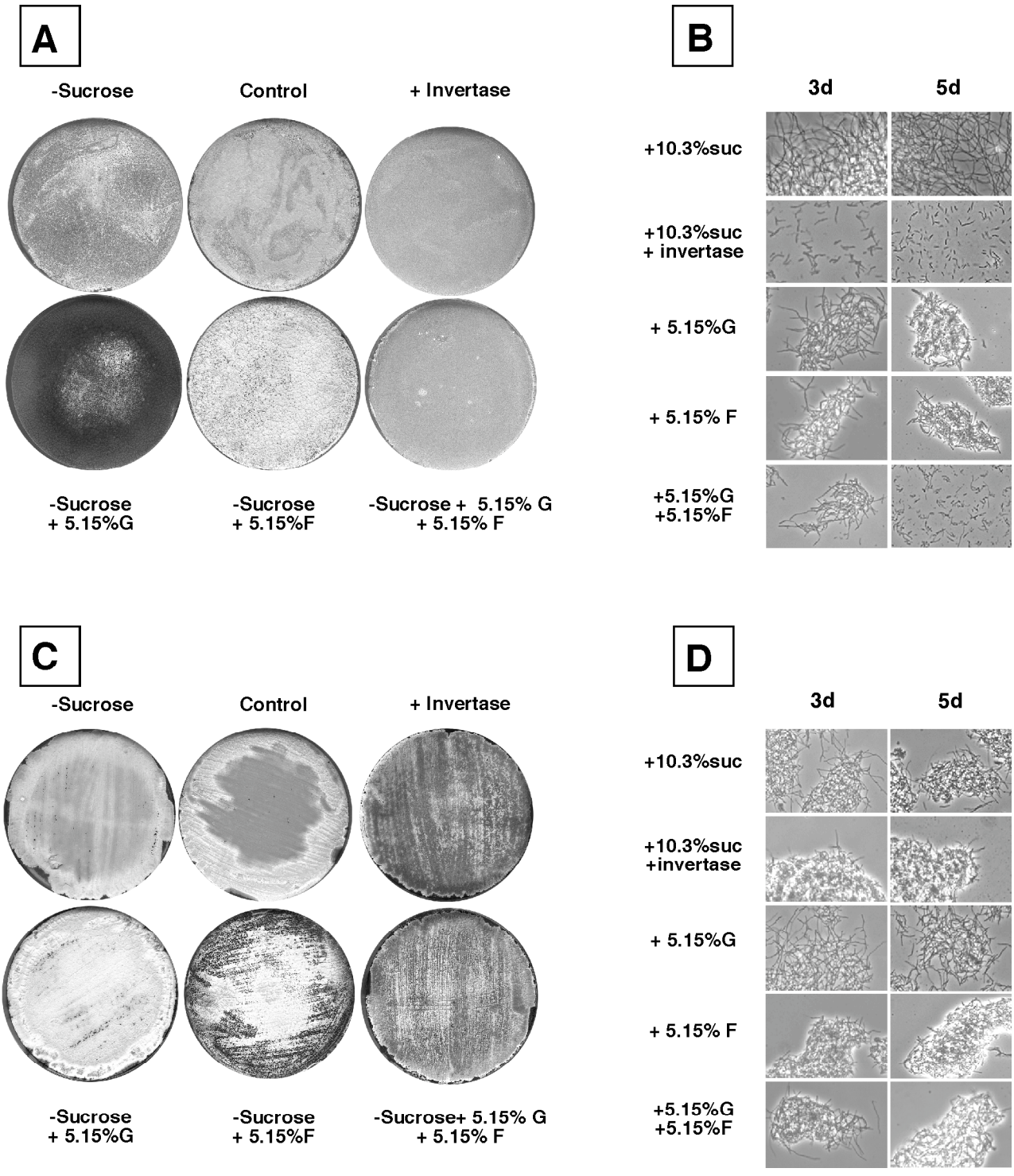
(Suc2 mutant). SEY2101/ pRB58 (plasmid carrying SUC2 in multicopy). SEY2101/ cured pRB58 (the plasmid pRB58 has been eliminated).

Fig. 4. A, C) *S. lividans* 66 (A) and *S. lividans* G015 (*glkA*) (C) inoculated on plates of R2YE with the following modifications: without sucrose (–Sucrose); Unmodified R2YE (Control); R2YE +10*µ*g invertase/ml (+ Invertase); without sucrose containing 5.15% glucose (–Sucrose + 5.15%G); without sucrose containing 5.15% fructose (–Sucrose + 5.15%F) or R2YE without sucrose containing 5.15% glucose and 5.15% fructose (–Sucrose + 5.15%G + 5.15% F). .
B and D). *S. lividans* 66 (B) and *S. lividans* G015 (*glkA*) (D) cultures on YE liquid media with different modifications: YE with 10.3% sucrose (+ 10.3% suc); Effect of 1 *µ*g/ml of invertase (+ 10.3% suc + invertase); YE with 5.15% glucose (+5.15% G); YE with 5.15% fructose (+5.15% F), or YE with 5.15% of both (+5.15% G+5.15%F). The cultures were observed after 3 (3d) and 5days (5d). B) *S. lividans* 66; D) *S. lividans* G015 (*glkA*).


A
B
C
Sporulated mycelium
Substrate mycelium
T R
C. albicans H. polymorpha K. lactis
P. anomala P. angusta S. cerevisiae
S. pombe S. occidentalis Y. lipolytica
D
1
2
3
S. lividans
S. albus
S. badius
S. coelicolor
S. flavogriseus
S. griseus
S. halstedii
S. olivaceus
S. parvulus
S. rochei
S. vinaceus
S. viridochromogenes


0h (control)
0 h
24 h
48 h
72 h
96 h


X 2180-1A
SEY2101/
cured pRB58
SEY2101
SEY2101/
pRB58


A
-Sucrose
Control
+ Invertase
-Sucrose
+ 5.15%G
-Sucrose
+ 5.15%F
-Sucrose + 5.15% G
+ 5.15% F
B
3d
5d
+10.3%suc
+10.3%suc
+ invertase
+ 5.15%G
+ 5.15% F
+5.15%G
+5.15%F
C
-Sucrose
Control
+ Invertase
-Sucrose
+ 5.15%G
-Sucrose
+ 5.15%F
-Sucrose+ 5.15% G
+ 5.15% F
D
3d
5d
+10.3%suc
+10.3%suc
+invertase
+ 5.15%G
+ 5.15% F
+5.15%G
+5.15%F