Appl Microbiol Biotechnol (2005) 69: 200–206
DOI 10.1007/s00253-005-1965-7

Silvia Guzmán · Itzel Ramos · Elizabeth Moreno · Beatriz Ruiz · Romina Rodríguez-Sanoja · Laura Escalante · Elizabeth Langley · Sergio Sanchez

Sugar uptake and sensitivity to carbon catabolite regulation in *Streptomyces peucetius* var. *caesius*

Received: 7 January 2005 / Revised: 7 March 2005 / Accepted: 12 March 2005 / Published online: 6 April 2005

Abstract *Streptomyces peucetius* var. *caesius* produces a family of secondary metabolites called anthracyclines. Production of these compounds is negatively affected in the presence of glucose, galactose, and lactose, but the greatest effect is observed under conditions of excess glucose. Other carbon sources, such as arabinose or glutamate, show either no effect or stimulate production. Among the carbon sources that negatively affect anthracycline production, glucose is consumed in greater concentrations. We determined glucose and galactose transport in *S. peucetius* var. *caesius* and in a mutant of this strain whose anthracycline production is insensitive to carbon catabolite repression (CCR). In the original strain, incorporation of glucose and galactose was stimulated when the microorganism was grown in media containing these sugars, although we also observed basal galactose incorporation. Both the induced and the basal incorporation of galactose were suppressed when the microorganism was grown in the presence of glucose. Furthermore, adding glucose directly during the transport assay also inhibited galactose incorporation. In the mutant strain, we observed a reduction in both glucose (48%) and galactose (81%) incorporation compared to the original. Galactose transport in this mutant showed reduced sensitivity to the negative effect of glucose; however, it was still sensitive to inhibition. The deficient transport of these sugars, as well as CCR sensitivity to glucose in this mutant was corrected when the mutant was transformed with the *SCO2127* region of the *Streptomyces coelicolor* genome. Our results support a role for glucose as the most easily utilized carbon source capable of exerting the greatest repression on anthracycline biosynthesis. In consequence, glucose also prevented the repressive effect of galactose by suppressing its incorporation. This suggests the participation of an integral regulatory system, which is initiated by an increase in incorporation of repressive sugars and their metabolism as a prerequisite for establishing the phenomenon of CCR in *S. peucetius* var. *caesius*.

S. Guzmán · I. Ramos · E. Moreno · B. Ruiz · R. Rodríguez-Sanoja · L. Escalante · E. Langley · S. Sanchez (✉)
Departamento de Biología Molecular y Biotecnología,
Instituto de Investigaciones Biomédicas,
Universidad Nacional Autónoma de México,
México, DF 04510, Mexico
e-mail: sersan@servidor.unam.mx

E. Langley
Unidad de Biología Molecular y Medicina Genómica,
Instituto Nacional de Ciencias Médicas y Nutrición
"Salvador Zubirán", México,
DF 14000, Mexico

Present address:
I. Ramos
Department of Bacteriology, Enzyme Institute,
The University of Wisconsin,
Madison, WI 53706, USA

Introduction

Streptomyces are Gram positive microorganisms capable of synthesizing a great number of hydrolytic enzymes that allow them to utilize a wide variety of proteins and polysaccharides as carbon source (Rose 1979; Crandall and Hamill 1986; Frost and Moss 1987). Analysis of the *Streptomyces coelicolor* genome has revealed the presence of genes encoding proteases, chitinases, amylases, and cellulases (Bertram et al. 2004). In complex culture media, these enzymes can generate primary degradation products (amino acids, fatty acids and sugars) for further utilization. Among these carbon sources, the type and concentration of sugars has played an important role in microbial fermentations of industrial interest. One of the most important systems for sugar uptake in Gram positive and Gram negative microorganisms is the phosphoenolpyruvate:sugar system (PTS) (Saier et al. 1995). This system has been described in three *Streptomyces* species (*S. coelicolor*, *S. griseofuscus* and *S. lividans*) (Titgemeyer et al. 1995). In *S. coelicolor*, this system comprises three proteins, EI, HPr and enzyme II (II^{Fru}), which are induced by *N*-acetylglucosamine and permit fructose incorporation into the cell and its phosphorylation to fructose 1-phosphate (Nothaft et al. 2003). However, this system does not seem to be

involved in the uptake of other carbon sources such as glucose and mannitol (Titgemeyer et al. 1995). Besides PTS, fructose can be phosphorylated by an ATP-dependent fructokinase (Titgemeyer et al. 1995). Similarly, it has been reported that glucose and galactose phosphorylation is carried out by their respective kinases (Hodgson 1982; Kendall et al. 1987), implying the existence of specific permeases for their transport into the cell. Recently, van Wezel et al. (2005) provided elegant evidence supporting a glucose transporter in *S. coelicolor* encoded by *glcP* and induced by D-glucose.

Streptomyces peucetius var. *caesius* produces a family of polyketide antibiotics known as anthracyclines. Among these, daunorubicin and doxorubicin are of great clinical importance due to their antineoplastic activity (Arcamone et al. 1969). This strain grows and produces anthracyclines in the presence of a number of carbon sources such as mannose, cellobiose, lactose, fructose, maltose and starch (Dekleva et al. 1985; Segura et al. 1996). Both carbon utilization and anthracycline production are susceptible to regulation by glucose in this microorganism (Escalante et al. 1999). From the original *S. peucetius* var. *caesius* strain, we have isolated mutants resistant to a non-metabolizable glucose analogue, 2-deoxyglucose (DOG). In these mutants (Dog^R), anthracycline production is less sensitive to the repressive effects of glucose (Segura et al. 1996). Additionally, the mutants present low glucose kinase (Glk) activity and decreased glucose incorporation (Escalante et al. 1999). Although anthracycline production in Dog^R mutants is resistant to glucose, supplementation of the culture medium with fructose 1,6 bis-phosphate or phosphoenolpyruvate, both glucose degradation products, exerted a negative effect on anthracycline biosynthesis (Ramos et al. 2004). Analysis of these results has suggested that glucose exerts catabolic repression on anthracycline synthesis (Escalante et al. 1999), and that this effect depends on both the microorganism's ability to incorporate glucose and its phosphorylation by Glk, culminating in the production of the metabolic signals possibly responsible for repression (Ramos et al. 2004). The role for Glk in this regulatory process has been intensively studied in *Streptomyces* (Angell et al. 1992; Imriskova et al. 2001; Kwakman and Postma 1994; Ramos et al. 2004). In this study we propose to delve into the role that transport of various repressive carbon sources plays in the process of carbon catabolite repression (CCR). Additionally, we will explore the possible relationship between these carbon sources and their manifestations in this phenomenon. With this purpose in mind, we selected carbohydrates that have a negative effect on anthracycline production. The compounds selected were analyzed in terms of their incorporation into the cell in various strains under different experimental conditions. The strains utilized were the original *S. peucetius* var. *caesius* strain, a mutant derivative insensitive to regulation by glucose (Dog^R), and the same mutant carrying a DNA fragment from *S. coelicolor* termed *SCO2127*. This fragment encodes a putative 572 bp long open reading frame (ORF) that is localized just upstream of the *glkA* gene encoding Glk (Angell et al. 1992). *SCO2127* has been shown to be necessary to completely restore CCR sensitivity in mutants (Dog^R) complemented with the *glkA* gene (Angell et al. 1994) and is suggested to be involved in glucose transport or its metabolism. Both genes are expressed as part of a polycistronic mRNA; however, the function of the putative protein produced by the *SCO2127* region is as yet undetermined.

Materials and methods

Bacterial strains and cultivation

S. peucetius var. *caesius* NRRL B-5337 was kindly provided by the ARS culture collection (United States Department of Agriculture, Peoria, Ill.). The Dog^R strain is a spontaneous mutant previously isolated in this laboratory through its resistance to growth inhibition by DOG (Segura et al. 1996). Compared to the original strain, this mutant contains only 14% Glk activity and is insensitive to CCR by glucose (Escalante et al. 1999). Both strains are maintained in a viable condition at the UNAM-48 Culture Collection (Mexico D.F.). For anthracycline production, we used 2.5 ml samples of a seed culture (Segura et al. 1996), washed and resuspended in sterile distilled water, to inoculate 50 ml YM medium with the desired carbon source concentration, contained in 250-ml baffled Erlenmeyer flasks. YM medium contains (l^{-1}): 4 g yeast extract, 10 g malt extract, pH 7.2. For uptake experiments, 50 ml seed culture (Segura et al. 1996) previously washed and resuspended in sterile distilled water were used to inoculate a 2.8-l Fernbach flask containing 500 ml uptake minimal medium (UM; Escalante et al. 1999). The UM medium was supplemented with 10 mM $(NH_4)_2SO_4$ and 100 mM of the desired carbon source (D-glucose or D-galactose). To test the effect of glucose on galactose transport, the sugar uptake was determined in cultures grown in UM medium supplemented with 100 mM galactose plus 300 mM glucose. To measure the effect of glucose on galactose incorporation, D-[1-^{14}C]galactose uptake was determined in 10 mM galactose plus 2 and 5 mM D-glucose. Cultures were grown for 36 h at 29°C in a rotary shaker at 180 rpm.

Construction of the recombinant strain

The *SCO2127* gene from *S. coelicolor* was amplified using PCR from total DNA of strain M145. Oligonucleotides were designed based on the reported gene sequences (Angell et al. 1992) covering the promoter region and the transcriptional terminator. For *SCO2127* the primers were: forward (5′-CGGAGATCTGGCCGCGGGG-3′) and reverse (5′-GGCAAGCTTACCCGAGGC-3′). The primers contain restriction sites for *Bgl* II and *Hin*dIII (underlined), respectively, for cloning into the corresponding sites within the vector pIJ486. PCR conditions for *SCO2127* amplification were an initial denaturation step at 94°C (5 min), then 94°C (1 min), 57°C (1 min), 72°C (1 min) for 20 cycles and a final extension period at 72°C (5 min). PCR products of 694 bp were di-

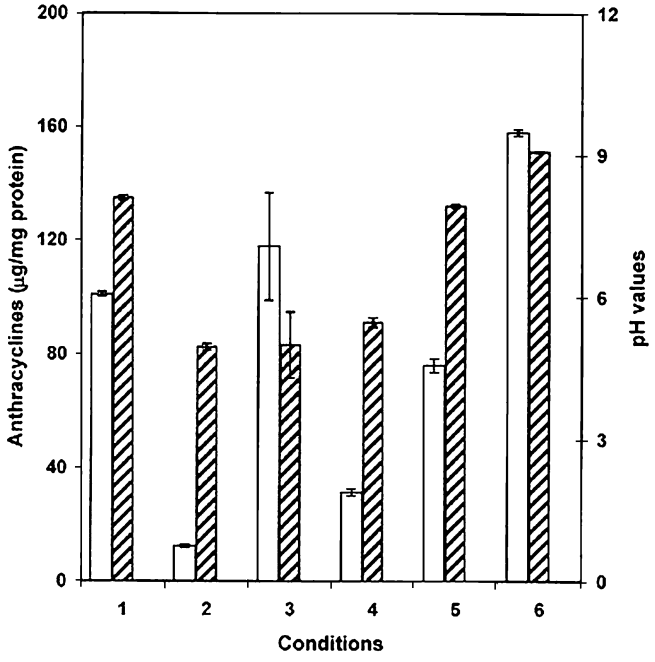

Fig. 1 Effect of various carbon sources on anthracycline production in the original *Streptomyces peucetius* var. *caesius* strain. Production (*white bars*) was determined at 120 h of fermentation. *1* Control, *2* glucose 330 mM, *3* arabinose 330 mM, *4* galactose 330 mM, *5* lactose 330 mM, *6* glutamate 40 mM. *Hatched bars* pH values for each condition at the same fermentation time point

gested, purified and cloned into pIJ486, generating pSG210 containing *SCO2127*.

These plasmids were used to transform *S. peucetius* var. *caesius* protoplasts from the Dog^R mutant using traditional methods (Kieser et al. 2000); a transformation frequency of 300 transformants/µg DNA was obtained. Transformation of the pSG210 plasmid into the mutant strain was relatively stable, showing little or no integration into the chromosome, as substantiated by the fact that after several generations we were able to recover the plasmid from our transformed strain.

Protoplast formation and regeneration

Protoplast formation was carried out from strains grown in 50 ml YEME medium complemented with 5 mM $MgCl_2 \cdot 6H_2O$ and 0.5% glycine as described by Kieser et al. (2000). Protoplast regeneration was carried out in R6 medium (Balts and Matsushima 1981) for 40 h. After regeneration, 1 ml sucrose (10.3%) and 50 µg thiostrepton were added and incubation continued for another 48 h.

Anthracycline and protein determination

Anthracyclines were extracted from harvested mycelia (120 h cultures) using acetone and 0.05 M sulfuric acid [4:1] according to Arcamone et al. (1969). Anthracyclines were quantified at 495 nm, using a molar extinction coefficient of 220. For protein determination, samples were processed as previously reported (Segura et al. 1996), and assayed by the Lowry method, using bovine serum albumin as standard (Lowry et al. 1951).

Uptake experiments

Seed culture (50 ml) was used to inoculate a 2.8-l Fernbach flask containing 500 ml UM medium and the desired carbon

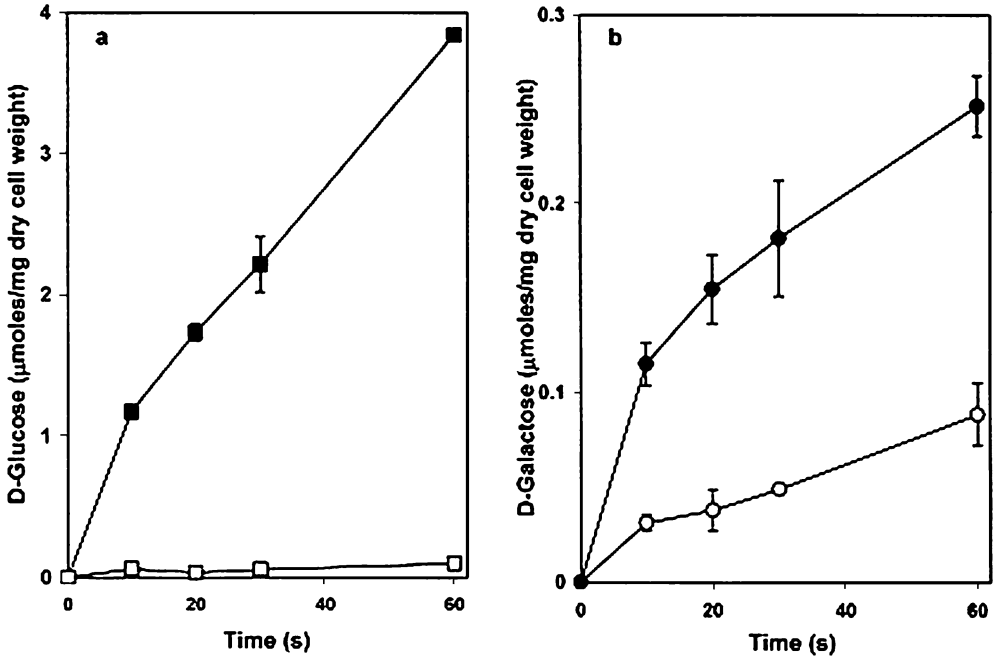

Fig. 2 Glucose (**a**) and galactose (**b**) (10 mM) transport in mycelia from the original *S. peucetius* var. *caesius* strain grown in 100 mM glucose or galactose (*filled symbols*). Controls (*open symbols*) were grown in 20 mM glutamate

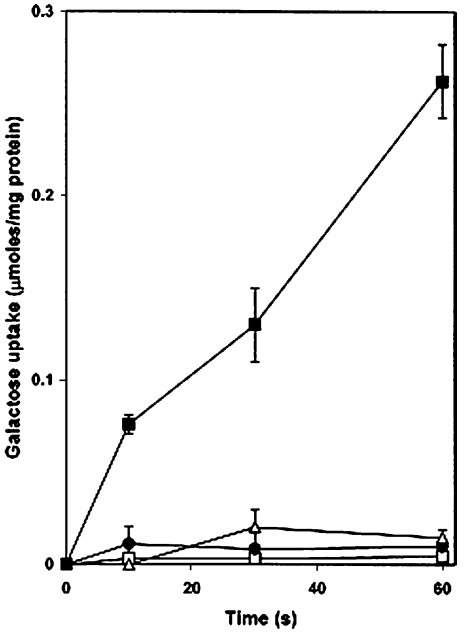

Fig. 3 Effect of glucose on galactose (10 mM) transport into cells of the original *S. peucetius* var. *caesius* strain grown in 100 mM galactose. *Filled squares* Galactose transport in the absence of glucose. Glucose concentrations tested were 2 mM (*open triangles*) and 5 mM (*filled circles*). *Open squares* Galactose transport in cells grown in 100 mM galactose plus 300 mM glucose

source. After 36 h, mycelia (250 mg wet weight) were harvested, washed with distilled water and resuspended in a vial containing 4.5 ml saline solution. The suspension was incubated under agitation and transport initiated by the addition of 25 μl of either D-[^{14}C]glucose (38.8 MBq mmol^{-1}) or D-[1-^{14}C]galactose (19.2 MBq mmol^{-1}), in 475 μl containing 10 mM non-radioactive sugar, as previously reported (Escalante et al. 1999). Cells were recovered by filtration, and radioactivity was determined by soaking the filter in vials containing 4 ml of a commercial liquid scintillation counting solution.

Results

Effect of various carbon sources on anthracycline production

Streptomyces peucetius var. *caesius* produces a family of secondary metabolites named anthracyclines. We determined the effect of a variety of carbon sources on anthracycline biosynthesis (Fig. 1) and found that, compared to a control, production was negatively affected in the presence of glucose (88%), galactose (69%) and lactose (25%). Other carbon sources, such as arabinose and glutamate, had no effect or were able to stimulate biosynthesis. Additionally, pH values varied between 5 and 9 depending on the carbon source utilized. However, we were unable to establish a correlation with the production of anthracyclines in each case since at similar pH values we found low (condition 2) and high (condition 3) anthracycline production.

Glucose and galactose transport

In order to establish a possible relationship between the negative effects exerted by glucose and galactose on an-

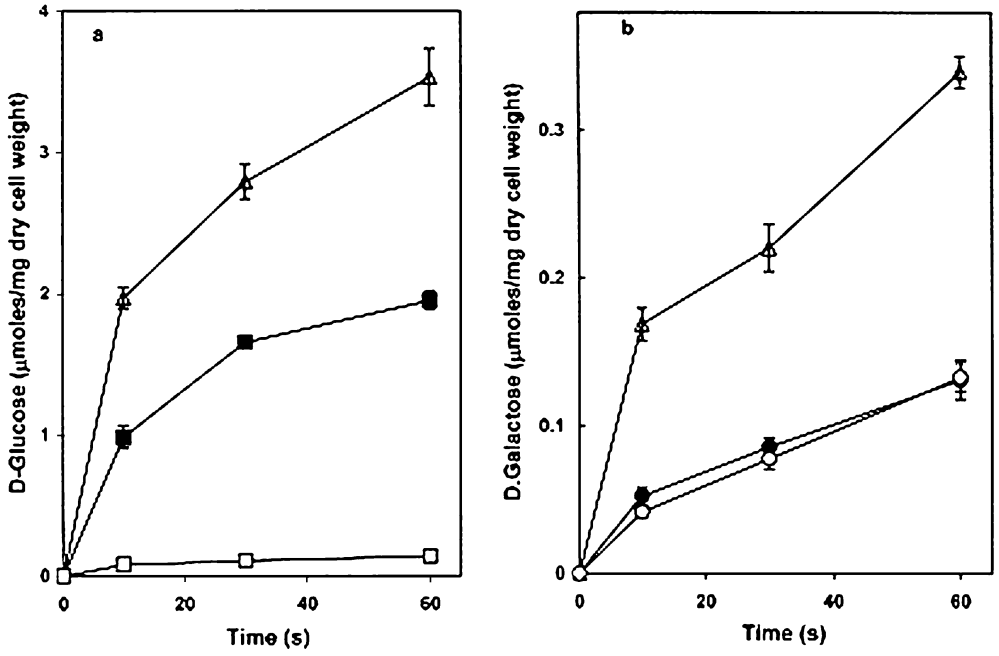

Fig. 4 Glucose (**a**) and galactose (**b**) (10 mM) transport in mycelia from the Dog^{R} mutant derived from *S. peucetius* var. *caesius* grown in 100 mM glucose or galactose (*filled squares*, *filled circles*). Glucose (*open squares*) and galactose (*open circles*) transport in cells grown in 20 mM glutamate. *Open triangles* Glucose and galactose transport in the original strain grown in 100 mM of the respective sugar as a control

Table 1 Galactose transport in the Dog^R mutant of *Streptomyces peucetius* var. *caesius*. *Gal* Galactose, *Glc* glucose

Growth	Assay[a]	Nanomoles per milligram dry cell weight[b]	Percent
Gal 100 mM	Gal 10 mM	69.90	100.00
Gal 100 mM	Gal 10 Glc 10 mM	2.63	3.76
Gal 100 Glc 330 mM	Gal 10 mM	24.80	35.40

[a]Five-milliliter system containing the cellular pellet suspended in 4.5 ml saline. Transport is initiated by addition of 25 µl D-[1-^{14}C]galactose (19.2 MBq mmol^{-1}), in 475 µl 10 mM galactose (or 10 mM galactose plus 10 mM glucose)
[b]Galactose incorporated at 30 s of incubation time

thracycline synthesis and the incorporation of these sugars into the cell, we carried out transport assays using the original *S. peucetius* var. *caesius* strain. As seen in Fig. 2, compared to a control in which the original strain was grown in the absence of sugar, cultures grown in the presence of glucose or galactose (100 mM) showed an increase in glucose and galactose incorporation of 20 and 4 times, respectively after 10 s. Additionally, galactose showed a basal incorporation of 25%. Figure 2 also shows that glucose is transported in greater concentrations (almost 15-fold higher) than galactose. It is interesting to note that although glucose and galactose (lactose degradation products) can be transported into these cells, incorporation of lactose was minimal (almost 100 times less than that of glucose) and there were no differences between cells grown in the presence or absence of lactose (not shown).

Effect of glucose on galactose transport

In order to elucidate the relationship between these two carbon sources, we determined the effect of glucose on galactose transport, and vice versa. As shown in Fig. 3, when the original strain was grown in galactose plus glucose, galactose transport was almost completely suppressed. Conversely, galactose did not significantly affect glucose transport (data not shown). Additionally, adding different concentrations of glucose directly to the galactose transport assay system inhibited galactose transport by almost 95%. This effect was observed even when glucose was added in concentrations five and two times lower than that of galactose.

Table 2 Effect of the *SCO2127* region of *S. coelicolor* on glucose transport and sensitivity to glucose repression

Strains	Glucose uptake (%)[a]	Galactose uptake (%)[a]	Sensitivity to D-glucose or D-galactose[b]
Original	100	100	S
Dog^R	51	35	R
Dog^R+*SCO2127*	108	120	S

[a]Cultures were grown in 100 mM glucose or 100 mM galactose. Values for incorporation of glucose (3.84±0.02 µmol/mg dry cell weight) and galactose (0.26±0.02 µmol/mg dry cell weight) at 60 s of incubation time were set as 100%
[b]Cultures were grown in D-glucose or D-galactose (330 mM). Sensitivity was visualized as red colored pigments (anthracyclines) formed in the presence of the sugars. Sensitive strains (S) do not produce pigments, resistant mutants (R) do

Glucose and galactose transport in a mutant resistant to CCR

To delve into the relationship between sugar transport and sensitivity to CCR, we repeated the glucose and galactose incorporation assays in a mutant strain (Dog^R) resistant to this regulatory effect. As shown in Fig. 4, when the mutant was grown in the presence of glucose or galactose, transport of these sugars was reduced 50 and 70%, respectively compared to the original *S. peucetius* var. *caesius* strain. Furthermore, glucose transport in this mutant was still inducible and basal galactose transport was conserved.

In order to understand the sensitivity of the galactose transport system to the negative effects mediated by glucose in this mutant, we measured galactose transport in cultures grown in galactose alone and galactose plus glucose. As can be seen in Table 1, the mutant exhibits lower sensitivity to glucose (65%) compared to the original strain (95%) (Fig. 3), but it remained sensitive to the inhibitory effect of glucose. The lower glucose sensitivity of galactose uptake by the mutant probably reflects its 50% reduction in glucose transport.

Glucose and galactose transport in the Dog^R mutant transformed with region *SCO2127*

In order to evaluate whether the *SCO2127* region has an effect over the transport system for glucose and galactose, we constructed a recombinant strain of the mutant Dog^R expressing this region. As can be seen in Table 2, compared to the Dog^R mutant, the recombinant strain not only regained glucose and galactose transport to an extent greater than the original *S. peucetius* var. *caesius* strain, but also regained its sensitivity to catabolic repression induced by glucose or galactose.

Discussion

Many different mechanisms for negative carbon catabolite effects exist in microorganisms (Saier 1996). In *S. peucetius* var. *caesius*, glucose, usually an excellent carbon source for growth, interferes with the formation of anthracyclines (Escalante et al. 1999). Additionally, in this microorganism glucose represses enzymes involved in the utilization of lactose and xylose (Segura et al. 1996). Glk seems to play an important role in the mechanism of glucose repression. However, its regulatory function has not yet been elu-

cidated. In the present work we found that, in addition to glucose, galactose and lactose also repressed anthracycline formation, although glucose has the highest effect. In agreement with these results, of the carbon sources negatively affecting anthracycline production, glucose is incorporated with greater efficiency by this microorganism.

In a previous paper, we reported that glucose uptake seemed to be inducible by its own substrate (Ramos et al. 2004). A similar effect was obtained in the present work, since glucose transport was stimulated 20-fold in mycelia grown in this sugar while there was practically no incorporation when other sugars were used as carbon source for growth. Recently, a glucose transport system was identified in *S. coelicolor* (van Wezel et al. 2005). Although this system is encoded by two distinct loci (*glcP1 and glcP2*), only *glcP1* seems to be functional, and is inducible by glucose. Our results in *S. peucetius* var. *caesius* correlate with the expression experiments for *glcP1* reported for *S. coelicolor* (van Wezel et al. 2005), supporting an inducible glucose transport in both species.

Similar to glucose, galactose transport in *S. peucetius* var. *caesius* was stimulated 4-fold in mycelia grown in the presence of this sugar. However, the level of galactose incorporation was almost 15 times less compared to that observed for glucose (0.25 μmol/mg dry cell weight galactose vs 3.84 μmol/mg dry cell weight glucose). This suggests that, in *S. peucetius* var. *caesius*, glucose is more easily utilized than galactose. In *S. coelicolor*, galactose transport appears to be constitutive (Hodgson 1982). Genes for the utilization of galactose have been identified in *S. lividans*. The products of the *galKE1T* operon in *S. lividans* mediate the funneling of galactose into glycolysis. The promoter region of the *galKEIT* operon has been analyzed with respect to its role in glucose repression and seems also to be glucose sensitive (Mattern et al. 1993). Homologs of these galactose genes have also been detected in *S. coelicolor* (Adams et al. 1988). However, the galactose transporter-encoding gene has so far not been elucidated.

In addition to the induced uptake of galactose, a basal incorporation was also detected. Basal transport of galactose seems to be constitutive and makes up 36% of the total galactose uptake observed in induced cells. In *S. coelicolor*, other groups have reported constitutive uptake of xylose, lactose and mannitol, and two genes (*rbsH and rbs 3*) potentially involved in ribose incorporation have been identified (Bertram et al. 2004).

It is interesting to note that although glucose and galactose (both lactose degradation products) were easily transported by the original *S. peucetius* var. *caesius* strain, lactose incorporation was minimal (almost 100-fold less than that of glucose). In *S. lividans*, Eckhardt et al. (1987) found that, in the presence of lactose, β-galactosidase is synthesized and excreted into the fermentation medium to hydrolyze the disaccharide, resulting in the production of glucose and galactose. The presence of these sugars in the medium may explain the partial inhibitory effect on anthracycline biosynthesis exerted by this disaccharide.

In *S. peucetius* var. *caesius*, we found that both basal and induced incorporation of galactose were suppressed when the microorganism was grown in the presence of glucose, suggesting a repressive effect on galactose transport and supporting yet again the greater hierarchical position of glucose as the easiest utilizable carbon source. In addition to its possible repressive effect, glucose almost completely inhibited galactose transport, resembling the inducer exclusion mechanism described for low G-C Gram positive bacteria. In these bacteria, inducer exclusion involves allosteric control by HPr(Ser-P) or control via HPr(His~P)-dependent phosphorylation. Preferential utilization of glucose has also been described in *S. coelicolor*. An example of this occurs with glycerol transport. It seems that two inducible transport systems for glycerol exist, one of which is inhibited and repressed by glucose, the other being only repressed by this carbohydrate (Hodgson 1982).

Additional evidence in favor of the importance of sugar transport in sensitivity to CCR in *S. peucetius* var. *caesius*, was obtained by using a mutant (Dog^R), whose synthesis of anthracyclines is insensitive to catabolic repression by glucose. When we measured glucose and galactose transport in this mutant, we observed a reduced incorporation of each sugar of 48 and 81%, respectively. This decrease in intracellular concentration of sugars could explain, at least in part, the basis for the observed resistance. As a consequence, basal incorporation of galactose also showed a lower sensitivity to the negative effects of glucose.

The deficiency in the incorporation of these sugars, as well as sensitivity to glucose, were corrected when the Dog^R mutant was transformed with the *SCO2127* region of *Streptomyces coelicolor*. The above mentioned results were quite unforeseen considering that this region does not seem to code for a glucose permease (Angell et al. 1994; Bertram et al. 2004). The question arises of how *SCO2127* mediates its stimulating effect on glucose and galactose uptake. Our data from the transformed strains suggests that this region is expressed and may function as a trans-acting factor in glucose metabolism. Recently, we have obtained experimental evidence suggesting that *SCO2127* also functions as a putative transcriptional activator of Glk (Guzmán et al. 2005). The obvious consequence of these actions, the stimulation of both glucose transport and Glk, is the synthesis of catabolites that elicit CCR in this microorganism. In agreement with this possibility, among several products of glucose metabolism, fructose 1,6 bis-phosphate and phosphoenolpyruvate exert CCR on anthracycline formation in *S. peucetius* var. *caesius* (Ramos et al. 2004) with fructose 1,6 bis-phosphate being the most effective. This effect resembles that reported for fructose 1,6 bis-phosphate on phosphorylation of Hpr kinase from *Bacillus subtilis* as a preliminary step in CCR (Jault et al. 2000).

In conclusion, the results obtained in this work support glucose as both the most easily metabolizable carbon source and the sugar most capable of exerting the greatest repression over anthracycline biosynthesis. In consequence, glucose prevented the suppressive effect of galactose by suppressing incorporation of the latter into the cell. Additionally, these data suggest the participation of an integral regulatory system that is initiated by an increase in incorporation of repressive sugars, as well as their metabolism, as

a requisite for the establishment of the phenomenon of CCR in *S. peucetius* var. *caesius*.

Acknowledgements We are indebted to Marco A. Ortíz Jiménez for strain preservation studies. This work was partially supported by grant IN-202903 from Dirección General de Asuntos del Personal Académico UNAM, México. Silvia Guzmán was the recipient of a doctoral fellowship from Consejo Nacional de Ciencia y Tecnología, México and from Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México.

References

Adams CW, Fornwald JA, Schmidt FJ, Rosenberg M, Brawner ME (1988) Gene organization and structure of the *Streptomyces lividans gal* operon. J Bacteriol 170:203–212

Angell S, Schwartz E, Bibb JM (1992) The glucose kinase gene of *Streptomyces coelicolor* A3(2): its nucleotide sequence, transcriptional analysis and role in glucose repression. Mol Microbiol 6:2833–2844

Angell S, Lewis CG, Buttner MJ, Bibb JM (1994) Glucose repression in *Streptomyces coelicolor* A3(2): a likely regulatory role for glucose kinase. Mol Gen Genet 244:135–143

Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C, Spalla C (1969) Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from *Streptomyces peucetius* var. *caesius*. Biotechnol Bioeng 11:1101–1110

Balts RH, Matsushima P (1981) Protoplast fusion in *Streptomyces*: conditions for efficient genetic recombination and cell-regeneration. J Gen Microbiol 127:137–146

Bertram R, Schlicht M, Mahr K, Nothaft H, Saier MH Jr, Titgemeyer F (2004) In silico and transcriptional analysis of carbohydrate uptake systems of *Streptomyces coelicolor* A3(2). J Bacteriol 186:1362–1373

Crandall LW, Hamill RL (1986) Antibiotics produced by *Streptomyces*: major structural classes. In: Queener SW, Day LE (eds) The bacteria, vol 9. Academic, New York, pp 355–402

Dekleva ML, Titus JA, Strohl WR (1985) Nutrient effects on anthracycline production by *Streptomyces peucetius* in a defined medium. Can J Microbiol 31:287–294

Eckhardt T, Strickler J, Gorniak L, Burnett WB, Fare LR (1987) Characterization of the promoter signal sequence and amino terminus of a secreted β-galactosidase from *Streptomyces lividans*. J Bacteriol 169:4249–4256

Escalante L, Ramos I, Imriskova I, Langley E, Sanchez S (1999) Glucose repression of anthracyclines formation in *Streptomyces peucetius* var. *caesius*. Appl Microbiol Biotechnol 52:572–578

Frost MG, Moss DA (1987) Production of enzymes by fermentation. In: Rehm HJ, Reed G (eds) Biotechnology, vol 7a. VCH, Weinheim, pp 108–110

Guzmán S, Carmona A, Escalante L, Imriskova I, López R, Rodríguez-Sanoja R, Ruiz B, Servín-González L, Sánchez S, Langley E (2005) Pleiotropic effect of the *SCO2127* gene on the glucose uptake, glucose kinase activity and carbon catabolite repression in *Streptomyces peucetius* var. *caesius*. Microbiology (in press)

Hodgson DA (1982) Glucose repression of carbon uptake and metabolism in *Streptomyces coelicolor* A3(2) and its perturbation in mutants resistant to 2-deoxyglucose. J Gen Microbiol 128:2417–2430

Imriskova I, Langley E, Arreguín-Espinoza R, Aguilar G, Pardo JP, Sanchez S (2001) Purification and characterization of glucose kinase from *Streptomyces peucetius* var. *caesius*. Arch Biochem Biophys 394:137–144

Jault JM, Fieulaine S, Nessler S, Gonzalo P, Di Pietro A, Deutscher J, Galinier A (2000) The HPr kinase from *Bacillus subtilis* is a homo-oligomeric enzyme which exhibits strong positive cooperativity for nucleotide and fructose 1,6-bisphosphate binding. J Biol Chem 275:1773–1780

Kendall K, Ali-Dunkrah U, Cullum J (1987) Cloning of the galactokinase gene (galK) from *Streptomyces coelicolor* A3(2). J Gen Microbiol 133:721–725

Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical *Streptomyces* genetics. John Innes Foundation, Norwich

Kwakman JHJM, Postma PW (1994) Glucose kinase has a regulatory role in carbon catabolite repression in *Streptomyces coelicolor*. J Bacteriol 176:2694–2698

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chemistry 193:265–275

Mattern SG, Brawner ME, Westpheling J (1993) Identification of a complex operator for *galP1*, the glucose-sensitive, galactose-dependent promoter of the *Streptomyces* galactose operon. J Bacteriol 175:1213–1220

Nothaft H, Dresel D, Willimek A, Mahr K, Niederweis M, Titgemeyer F (2003) The phosphotransferase system of *Streptomyces coelicolor* is biased for *N*-acetylglucosamine metabolism. J Bacteriol 185:7019–7023

Ramos I, Guzmán S, Escalante L, Imriskova I, Rodríguez-Sanoja R, Sanchez S, Langley E (2004) The glucose kinase alone cannot be responsible for carbon source regulation in *Streptomyces peucetius* var. *caesius*. Res Microbiol 155:267–274

Rose AH (1979) Production and industrial importance of secondary products of metabolism. In: Rose AH (ed) Economic microbiology, vol 3. Academic, New York, NY, pp 1–33

Saier MH Jr (1996) Cyclic AMP-independent catabolite repression in bacteria. FEMS Microbiol Lett 138:97–103

Saier MH Jr, Chauvaux S, Deutcher J, Reizer J, Ye JJ (1995) Protein phosphorylation and regulation of carbon metabolism in Gram-negative versus Gram-positive bacteria. Trends Biochem Sci 20:267–271

Segura D, González R, Rodríguez R, Sandoval T, Escalante L, Sanchez S (1996) *Streptomyces* mutants insensitive to glucose repression showed deregulation of primary and secondary metabolism. Asia Pac J Mol Biol Biotechnol 4:30–36

Titgemeyer F, Walkenhorst J, Reizer J, Stiver MH, Cui X, Saier MH Jr (1995) Identification and characterization of phosphoenolpyruvate:fructose phosphotransferase systems in three *Streptomyces* species. Microbiology 141:51–58

Wezel GP van, Mahr K, König M, Traag BA, Pimentel-Schmitt EF, Willimek A, Titgemeyer F (2005) GlcP constitutes the major glucose uptake system of *Streptomyces coelicolor* A3(2). Mol Microbiol 55:624–636