Controlling growth and morphogenesis of the industrial enzyme producer Streptomyces lividans

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Chapter 5

Identification of strong constitutive promoters for protein expression in *Streptomyces lividans* 1326.

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Manuscript in preparation

ABSTRACT

A growing number of Gram-positive bacteria are considered as effective hosts for the production of industrial relevant enzymes. In the era of high throughput genome sequencing, many new genes for enzymes are identified. With the expected concomitant increase in the use of heterologously produced enzymes in industry, the optimization of the hosts for all aspects related to production becomes more and more attractive. One of the platforms for the production of enzymes and also secondary metabolites is *Streptomyces lividans*. However, the number of expression vectors with strong promoters is limited. Here we present a short pipeline to identify and apply new naturally occurring promoters in *Streptomyces* that form good alternatives to those currently in use. The strong constitutive promoter P_{ermE} was used as the benchmark. RNA-Seq and DNA-microarray data were analyzed, which resulted in 15 candidate promoters. These promoters were then screened using the *lux* genes as reporters. The most attractive candidate promoters were validated in an expression system based on the secreted laccase SLAC in *Streptomyces lividans* 1326. This resulted in the identification of three promoter sequences ($P_{sco1947}$, $P_{sco4253}$ and $P_{sco3484}$) with higher or comparable strength than the benchmark P_{ermE}.

INTRODUCTION

Streptomycetes are Gram positive, soil dwelling bacteria, which are well known for their ability to produce the vast majority of the antibiotics available on today's market and have a great potential in the production of enzymes and therapeutic proteins (Vrancken and Anné 2009; Anné et al. 2012).

Several aspects should be taken into consideration when selecting a bacterial host for heterologous protein production. Among them, the availability of proven expression systems plays a substantial role. The expression system should provide high level transcription and efficient translation and secretion (Nakashima et al. 2005; Vrancken et al. 2010). Plasmids derived from the high copy number pIJ101 are generally preferred, as they are present in the cell in up to 300 copies per chromosome (Kieser et al. 2000). For plasmid maintenance, a selectable marker is required, which is typically a gene conferring antibiotic resistance. To avoid the addition of antibiotics during fermentations, alternatives such as toxin/antitoxin systems have been developed, *e.g.* for the production of a xylanase and an amylase in *S. lividans* (Sevillano et al. 2013). At a transcriptional level, strong promoters that do not affect the physiology and morphology of the host when present in high copy are required. Furthermore, optimal ribosome binding sites for efficient ribosome recruitment, appropriate signal sequences and optimized codon usage contribute to maximal production.

In this study, we have identified strong and constitutive promoters obtained from *Streptomyces* genomes as valuable alternatives to the current small selection of available sequences. The promoter of the erythromycin resistance gene from *S. erythraeus* (P_{ermE}) is commonly used (Bibb et al. 1985). However, it does not always perform reliably in all strains and conditions (Zhou et al. 2011). An alternative, the constitutive promoter from the subtilisin inhibitor *vsi* from *S. venezuelae,* has also be used for protein expression (Lammertyn et al. 1997). Some inducible promoters such as the thiostrepton-inducible promoter P_{tipA} from *S. lividans* (Murakami et al. 1989) or the xylan-inducible promoter P_{xysA} from *S. halstedii* (Ruiz-Arribas et al. 1997),

which are important for the expression of toxic proteins, have been used in protein production studies but have the drawback of requiring an inducer during industrial production. Moreover, the addition of thiostrepton induces the expression of stress-related proteins in *Streptomyces* (Holmes et al. 1993), while P_{xysA} leads to significant expression only after prolonged fermentation times.

The elements that define a strong promoter have been studied in *Streptomyces* but not completely understood (Strohl 1992; Bourn and Babb 1995). Over 60 different genes for σ factors have been identified in *S. coelicolor* (Bentley et al. 2002), some of which are strictly regulated by development such as *bldN* and *sigF* (Kelemen et al. 1996; Bibb et al. 2000). In contrast, the *E. coli* genome only encodes seven σ factors (Pérez-Rueda and Collado-Vides 2000). Attempts to isolate a strong promoter from a synthetic library of randomized promoter sequences underlined the importance of guanine residues at specific position within the regulatory regions, but failed to produce promoters stronger than the currently available ones (Seghezzi et al. 2011).

The engineering of known promoters can produce a remarkable improvement in strength. A combination of rational and random mutagenesis of the strictly regulated promoter P_{kasO} resulted in a de-repressed strong promoter sequence (Wang et al. 2013). The opposite engineering was shown recently to work as well in *Streptomyces*. Introduction of riboswitches in between the promoter sequence and the translational start codon mediated ligand-dependent expression of reporter genes (Rudolph et al. 2013).

In this work, we analyzed the global transcription profiles from liquid- and solid-grown cultures of two different streptomycetes, *S. coelicolor* M145 (Świątek et al. 2013) and *S. lividans* 1326 (Dwarakanath et al. 2012) and identified new strong promoters. To screen the activity of those promoters, a reliable and quick reporter system with a good level of sensitivity and an easy temporal recording is needed. The *redD*, *xylE*, *egfp* and *lux* promoter-probe systems are commonly used for analysis of promoter activity. RedD is the transcriptional activator of the biosynthetic pathway for the pigmented antibiotic undecylprodigiosin (Red) in *Streptomyces* (Takano et al. 1992). The *redD* gene has been used as a reporter (van Wezel et al. 2000c) as it

allows a direct pigmentation of the colonies that can be followed in time, and is in contrast to most antibiotics not secreted, but the limited sensitivity of the detection method hampers an accurate quantification. The *xylE* gene from *Pseudomonas putida* encodes catechol dioxygenase, which converts the colorless substrate catechol into the bright yellow compound benzoquinone (Ingram et al. 1989). The requirement of a substrate that needs to be sprayed directly onto the plate is a disadvantage of this system. Cloning different promoters in front of the *egfp* gene from the jellyfish *Aequorea Victoria* (Chalfie et al. 1994) allows assessing gene expression in space and time, but is not suitable for large scale screenings.

In this work, we chose the LuxCDABE system optimized for GC rich bacteria (Craney et al. 2007) to test our selection of putative strong promoters. This system consists of the operon from *Photorhabdus luminescens*, that includes the luciferase genes *luxA* and *luxB* in addition to three genes for the production of the substrate tetradecanal (*luxC*, *luxD*, *luxE*) which is synthesized directly in the cytosol and does not need to be added separately. The result is a spontaneous emission of light at 490 nm, potentially suitable for screening large number of samples in 96-well plates and including the collection of temporal data.

Among the 15 promoters analyzed, three were equivalent or stronger in comparison to P_{ermE} and were successfully tested for the production of a small laccase (SLAC) in *S. lividans*.

RESULTS AND DISCUSSION

Putative constitutive strong promoters

In this study, we used a modified version of the *lux* reporter plasmid, namely pMU1S*_mmrt, and a laccase reporter system to assess the strength of promoters selected on the basis of genome-wide transcript analysis data (in-house RNA-Seq and microarray analysis).

A list of putative strong promoters was compiled on the basis of our RNA-Seq data of *S. lividans* 1326 in liquid-grown cultures (Dwarakanath et al. 2012). The best hits in terms of transcriptional activity were filtered for ribosomal protein synthesis genes, because these genes are known to have a strict and growth dependent transcription control (Lindahl and Zengel 1982). Only the promoter upstream of *rpsP* ($P_{sco5591}$) was kept in the selection as an internal control. 15 promoters were selected, the 13 best hits from *S. lividans* and in addition the two best hits of genes present in *S. coelicolor* but not in *S. lividans* (Table 1 and Table S1). The latter data were taken from microarray data of *S. coelicolor* M145 grown on minimal media agar plates with mannitol (15 w/v) as the sole carbon source (Świątek et al. 2013). In the final list, five genes encode stress-related proteins, namely the cold-shock proteins CspD, CspG, CspA and the heat-shock proteins GroEL2 and GroES, while the others encode various membrane proteins and enzymes. Little is known about the regulation of these sequences (Table S2). When the regulatory elements are not described in literature, the sequences were analyzed by BPROM Softberry (http://linux1.softberry.com) for the presence of canonical -10/-35 sequences, conforming to the consensus for the household sigma factor σ^{70}. This identified seven out of 15 sequences as likely σ^{70}-like promoters (Table S2).

Table 1. Selection of 15 potential strong promoters and relative expression levels from in-house transcription data (RNA-Seq and microarray analysis).

Annotation	SCO Gene #	SLI Gene #	sequence relative to start codon	RPKM
Cold shock protein CspD	SCO4505	SLI4786	-200/-22	40657
Membrane protein	SCO6624	SLI6984	-182/-15	14184
60 kDa chaperonin 2 GroEL protein 2	SCO4296	SLI4533	-200/-25	10076
Cold-shock protein CspG	SCO3731	SLI3978	-191/-12	9099
Cold shock protein CspA	SCO0527	SLI0486	-324/-16	7184
DNA-binding protein Hbsu	SCO2950	SLI3296	-184/-23	6658
10 kDa chaperonin GroES protein	SCO4761	SLI5031	-300/-81	5850
Uncharacterized protein SCO4253	SCO4253	SLI4489	-212/-15	2466
30S ribosomal protein 16S, RpsP	SCO5591	SLI5878	-277/-16	5167
Glyceraldehyde-3-phosphate dehydrogenase GAPDH (EC 1.2.1.12)	SCO1947	SLI2261	-236/-25	4322
ATP synthase subunit a	SCO5367	SLI5636	-200/-13	1679
Thiosulfate sulfurtransferase (EC 2.8.1.1)	SCO4164	SLI4405	-200/-27	3970
Membrane protein	SCO7636	SLI7864	-300/-10	43
Sugar binding protein	SCO3484	-	-278/-14	-
Hydrolase	SCO3487	-	-280/-10	-

Vector optimization

The vector used in this study is a derivative of pMU1, designated pMU1S* (Craney et al. 2007, JR Nodwell pers comm.). First, *S. lividans* 1326 was transformed with the empty vector and tested for background transcription when grown on several solid media (Fig. S1). The vector itself already gave high luminescence from the 30 hr time point onwards. This luminescence was assumed to be caused by promoter activity originating from the vector sequences upstream of the *lux* genes. Analysis for the presence of a putative promoter by BPROM Softberry revealed putative promoter sequences upstream of the transcriptional terminator (-10 box AGTTAGGCT and -35 box TTTTTT). To reduce transcriptional readthrough, the transcriptional terminator of the methylenomycin resistance gene of *S. coelicolor* (*mmrT*) was amplified from pMT3003 (Paget et al. 1994) and cloned as an EcoRV-BamHI fragment in the multiple cloning site of pMU1S*, generating plasmid pMU1S*_mmrt. The new

vector showed much lower background luminescence on different solid media (Fig. S1) and was therefore used in all subsequent experiments.

To eliminate translational effects, the RBS from pIJ8660 cloned in front of *luxC* in the original vector was substituted with the strong RBS from *S. ramocissimus* elongation factor *tuf1*, preceded by a 16 nucleotide linker essential for the correct recognition (Vijgenboom et al. 1994; Motamedi et al. 1995). The reference P_{ermE} including the *tuf1* RBS was taken from pHM10a (Motamedi et al. 1995). This construct typically results in high levels of protein expression (van Wezel et al. 2000).

Promoter screening

All promoter sequences were cloned in pMU1S*_mmrt directly upstream of *luxCDABE*. *S. lividans* transformants containing the constructs with the *lux* genes under the control of one of the selected promoters were first screened by growing them in 96-well microtitre plates containing minimal agar medium supplemented with mannitol (1% w/v). Luminescence was measured at eight hour intervals over a period of 72 hours (Fig. S2). All putative promoter sequences that did not result in luminescence higher than 4000 cps during exponential growth were discarded. The seven strongest promoters were $P_{sco0527}$, $P_{sco1947}$, $P_{sco3484}$, $P_{sco3487}$, $P_{sco4505}$, $P_{sco4253}$ and $P_{sco5591}$ and these were selected for further analysis (Fig. 1A). The transformants were grown in liquid minimal medium supplemented with 0.5% mannitol and 0.5% glucose (Fig. 1B). The luminescence was measured at 10 time points during exponential growth (14-23 hr) and normalized for biomass. Comparison of the luminescence/biomass values (14-20h) showed that four promoters had similar or higher strength than P_{ermE}, namely $P_{sco1947}$ (about the same strength), $P_{sco4253}$ (+33%), $P_{sco5591}$ (+125%) and $P_{sco3484}$ (+154%) (Fig. 1C). The other three promoters ($P_{sco0527}$, $P_{sco3487}$ and $P_{sco4505}$) were discarded.

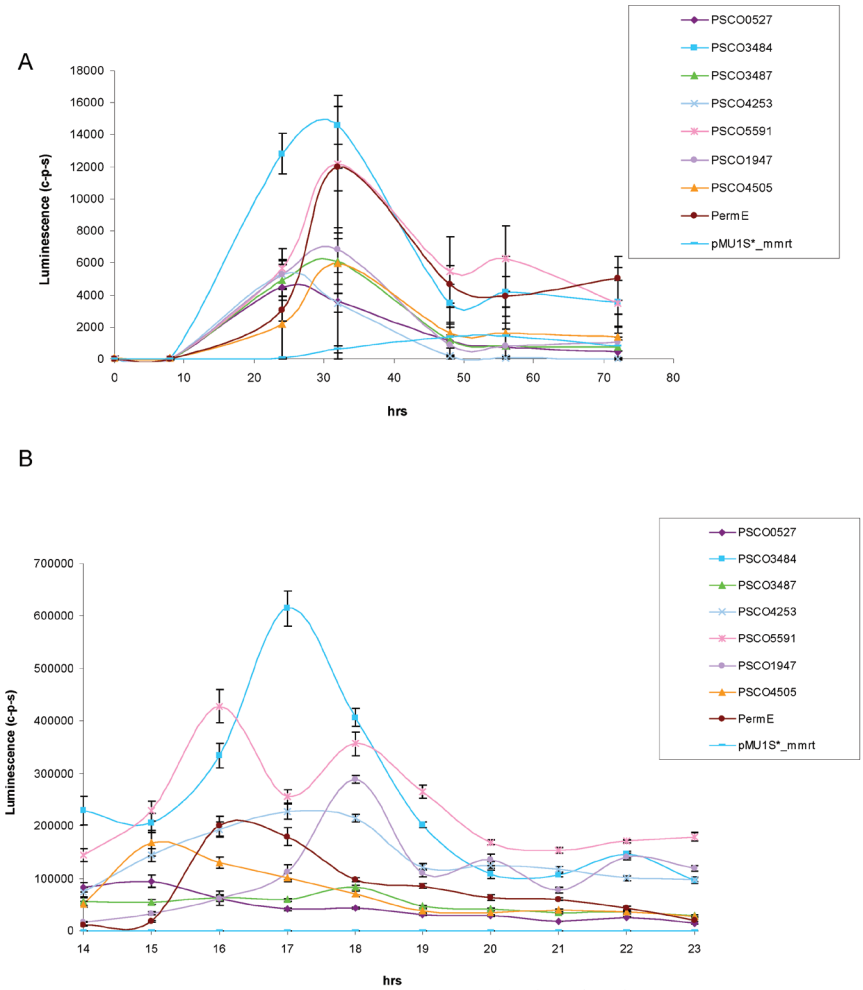

Figure 1 Pre-screening of the selected promoters. The data for the seven strongest promoters are shown together with the luminescence of the benchmark P_{ermE} and the empty vector. The strains are grown on solid MM + 1% mannitol (A) and liquid NMMP + 0.5% mannitol and 0.5% glucose (B).

	Average luminescence/biomass 14-20 hrs (%)	highest value (%)
PermE	100	100
Psco1947	108	145
Psco3484	254	308
Psco4253	133	114
Psco5591	225	179
negative control	3	0

Table 2 Comparison of promoters $P_{SCO1927}$, $P_{SCO3484}$, $P_{SCO4253}$ and $P_{SCO5591}$ from liquid culture data between 14 and 20 hr of growth. The luminescence obtained with P_{ermE} was set as 100%.

Validation of the promoters

To assess if the promoters could potentially find application in expression vectors, the four selected promoters were used to drive the transcription of a small laccase gene, encoding SLAC of *S. coelicolor* (Machczynski et al. 2004). *S. lividans* transformants containing the different promoter-*slac* expression constructs were grown in liquid TSBS and samples were taken at 24 and 48 hr. The amount of SLAC present in the spent medium was determined by an in-gel enzymatic detection, visualizing the active SLAC fraction (Fig. 2A and C) and by Western blot analysis (Fig. 2B and D), reflecting the total protein produced.

The activity assay showed that $P_{sco1947}$-*slac* resulted in SLAC activity comparable to P_{ermE} after 24 hr of growth, with an increase of 67% after 48 hr, while $P_{sco4253}$-*slac* and $P_{sco3484}$-*slac* resulted in slightly higher (+10%) or comparable expression of SLAC when enzyme levels were measured after 48 hr. Similar results were obtained with the Western blot analysis for $P_{sco1947}$-*slac* construct. The total SLAC levels for $P_{SCO4253}$-slac and $P_{sco3484}$-*slac* as detected in the Western blots are higher than those measured by the in-gel assay. So part of the enzyme produced in transformants with these constructs is not active. However, the difference between the protein production assays is much smaller after 48 hours of growth. Nevertheless, it should be noted that $P_{sco3484}$ was almost three-fold stronger when the transcriptional level was analyzed with the bioluminescence assay and that the transformants carrying $P_{sco3484}$-*slac* were strongly retarded in growth. This might be explained by a metabolic imbalance, with a deviation of the flux towards heterologous protein production versus biomass formation, resulting in growth impairment (D'Huys et al. 2011). In addition, the promoter derived from rpsP ($P_{sco5591}$) did not show any protein production despite the high transcriptional levels shown in the lux experiment. This difference might depend on the fact that the *slac* reporter is inserted in a multicopy vector while the lux construct is present in a single copy integrated in the genome. However, this demonstrates once more that promoters derived from ribosomal protein or rRNA genes typically are not suitable for use in multicopy expression vectors.

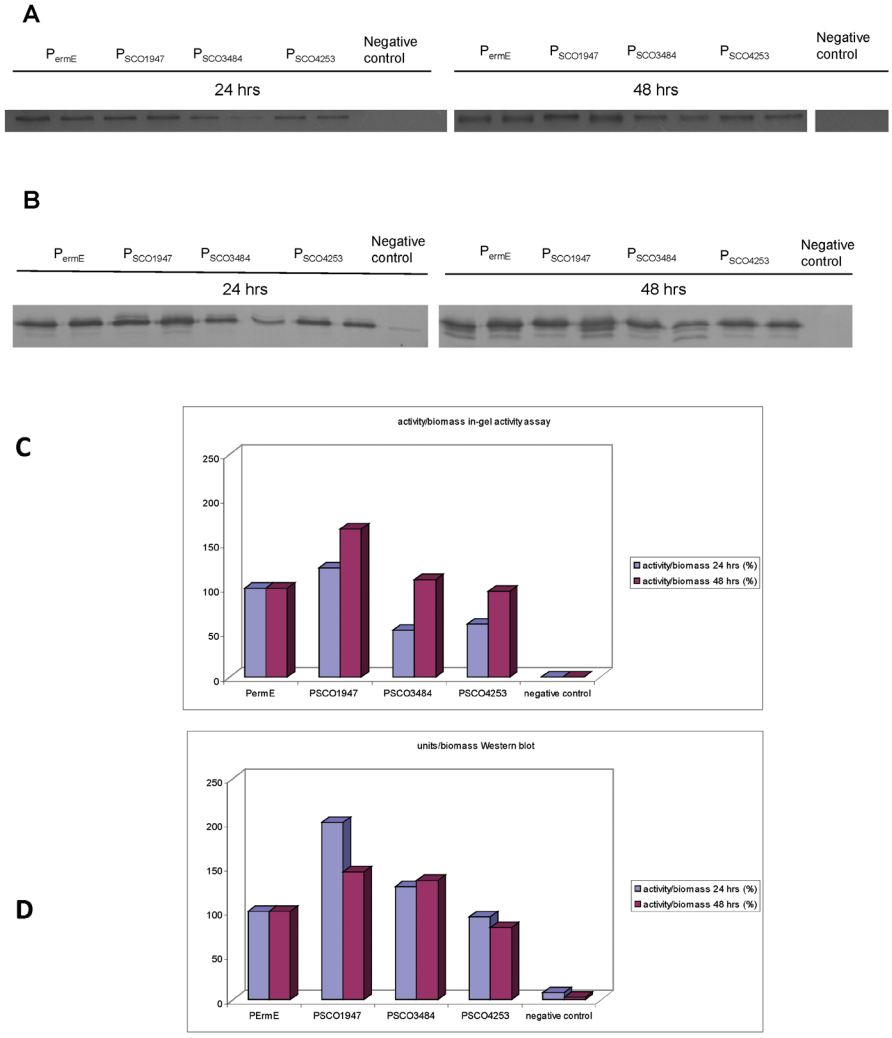

Figure 2 In-gel SLAC activity assay with DMPPDA 0.125 mg/ml and 1-naphtol 0.125 mg/ml (A) and Western blot with polyclonal antibodies raised in rabbits and GARAP as secondary antibody (B). The quantification of the signals corresponding to active SLAC as determined by the in-gel assay and the total amount fo SLAC as detected by Western blot are shown in panels C and D Band intensities were normalized for biomass and the amount of (active) protein obtained in the P_{ermE} construct was set at 100%.

The SLAC assays are all carried out on laboratory scale and in rich liquid medium, mimicking to some extend the conditions in industry were complex media are preferred. However, production pilots in a fermentation setup with optimized growth conditions and fermentation parameters are required to properly validate the potential of expression systems.

CONCLUSION

We have identified three promoters with potential use in expression systems. The transcriptional activity of these promoters could also be correlated to high level protein expression, using the versatile SLAC protein as a reporter. Currently, a limited number of promoter-vector combinations are available in *Streptomyces*. The promoters identified in this study are a welcome addition to the selection and will provide more flexibility for the design of protein production platforms. Further design of these new promoters by random or Selex methods as well as optimizing growth conditions should be employed to obtain even better levels of protein production.

MATERIALS AND METHODS

Promoter amplification and cloning in lux constructs

A modified version of plasmid pMU1 (Craney et al. 2007) named pMU1S* (JR Nodwell, personal communication) was used in this study. All 15 promoter sequences were amplified by PCR with the *S. lividans* 1326 genome as template, except for $P_{sco3484}$ and $P_{sco3487}$, which were amplified from *S. coelicolor* M145 genomic DNA. The region to amplify was selected as a 200-300bp sequence upstream of the annotated translational start site for each gene, including the putative -35 and -10 regions. The ribosome binding site was identified and substituted by a 16 nucleotide linker and the RBS of *tuf1* (TACAGAACCACTCCACAGGAGGACC) included in the reverse primer of each construct. The constructs and primers used in this study are listed in Table S3 and S4. The promoters were cloned in pMU1S*_mmrt as BamHI-NdeI fragments, generating 15 different *lux* constructs. These plasmids were introduced in *S. lividans* 1326 by protoplast transformation (Kieser et al. 2000). Transformants carrying the promoterless pMU1S*_mmrt plasmid were used as negative control and transformants carrying P_{ermE} as reference (benchmark).

Bioluminescence assay

Bioluminescence assays on solid media were performed in 96 well plates made of white polystyrene (Greiner-bio-one) filled with 200 µl of different media, including MS, R5 and Minimal Medium + 1% mannitol. Eight wells per construct were inoculated with 1000 spores and the plate was incubated at 30 °C. Measurements of the eight replicates were done every 8 hr for 72 hr with a GloMax 96 Microplate Luminometer and luminescence is expressed in counts per second (cps).

For the assay in liquid cultures, one flask per construct carrying 50 ml of liquid minimal medium (NMMP + 0.5% mannitol and 0.5% glucose) was inoculated with $2.5*10^8$ spores and incubated at 30 °C in a shaking incubator. After 14 hrs of growth, sampling of each culture was done every hr up to 23 hrs of growth with eight replicates. A 1.5 mL sample was taken and the mycelium was harvested by

centrifugation and used for the biomass determination. The dry weight of each sample was determined after overnight drying at 100 ^{0}C. Eight samples of 100 µL were taken for the bioluminescence measurements. These samples were loaded in eight wells of a white polystyrene 96 well plate and bioluminescence was determined in a GloMax 96 Microplate Luminometer.

Plasmids for SLAC expression

The efficiency of the promoters in protein production was carried out with a selection of the four strongest promoters and P_{ermE} as reference. They were cloned as EcoRI-NdeI fragments in plasmid pSLAC, carrying the ORF of *slac* (SCO6712) with an NdeI site overlapping with the translational start codon and 400 nucleotides downstream. The vector used is a modified version of pHJL401 lacking all NdeI sites (pHJL401N$^-$). The constructs and primers used in this study are listed in Table S3 and S4. After protoplast transformation of *S. lividans* 1326, two independent transformants were used for the enzymatic assay. Transformants carrying the promoterless pSLAC plasmid were used as negative control.

SLAC/laccase in-gel activity assay

The transformants were grown in 10 ml TSBS with 25 µM Cu(II) and 2 µg/ml thiostrepton. About 5*10^7 spores were inoculated and the flasks incubated at 30 ^{o}C in a shaking incubator. Samples of 1 ml were taken after 24 and 48 hr of growth, centrifuged for 10 min and the pellet incubated overnight at 100 ^{o}C for dry weight determination. Ten µl of the supernatant were mixed with ten µl of SDS PAGE loading buffer without the addition of β-mercaptoethanol and loaded on a 12.5% polyacrylamide gel. Enzymatic activity was essentially determined according to Endo et al. (2003) and Machczynski et al. (2004). Following electrophoresis, the gel was soaked for 1 hr in 100 mM Na-phosphate buffer pH 6.8 at room temperature on a rocking platform and for 1 hr at 30 ^{o}C. Subsequently, the subtrates, 0.125 mg/ml DMPPDA and 0.125 mg/ml 1-naphtol in phosphate buffer were added. SLAC activity

becomes visible within minutes as clear blue/purple bands in the gel. A digital image was taken and the band intensities were analyzed with the software ImageJ (Schneider et al. 2012). For the calibration curve, two to 25 µl of the most concentrated sample were loaded, revealing a linear range.

Western blot

Samples of the spent medium were mixed 1:1 with loading buffer including β-mercaptoethanol and heated at 95 ^{0}C for 4 minutes. Equal amounts of each sample were loaded on a 12.5% polyacrylamide SDS-PAGE gel. After electrophoresis, proteins were transferred to Hybond-P membrane (Amersham) using the Biorad blotting system. The membranes were washed with PBS and incubated in 5% low fat baby milk powder (Frisolac) in PBS for 30 minutes followed by overnight incubation with a 1:5000 diluted polyclonal antibodies raised against SLAC in rabbits. SLAC was detected using an anti–rabbit IgG alkaline phosphatase secondary antibody (sigma A8025), diluted 1:5000 and BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/ Nitro blue tetrazolium) as substrates. Band intensities were determined with ImageJ and the gel analysis option. Gels were standardized using 2 pmol of purified SLAC and one of the samples as internal controls in each Western Blot experiment. The amount of SLAC detected was normalized for the biomass (g/L). For the calibration curve, SLAC was purified as a N-terminal truncated form following expression in E. *coli* an stored as a 5 µM stcok at -20 ^{0}C (Machczynski et al 2004). Serial dilution were run on SDS PAGE followed by Western blotting. NBT/BCIP signals were quantified with ImageJ and the calibration curve showed a linear response up to 3 pmol of SLAC.

SUPPLEMENTAL MATERIAL

Table S-1 The best expression hits obtained from RNA-Seq data ordered by expression level.

***S.coelicolor* M145**	***S.lividans* 1326**	**RPKM**	**Function**
SCO4505	SLI4786	40657	Cold shock protein CspD
SCO4635	SLI4906	37093	LSU ribosomal protein L33p @ LSU ribosomal protein L33p, zinc-dependent
SCO4653	SLI4924	19581	LSU ribosomal protein L7/L12 (P1/P2)
SCO4662	SLI4936	17428	Translation elongation factor Tu
SCO3906	SLI4164	15191	SSU ribosomal protein S6p
SCO4652	SLI4923	14885	LSU ribosomal protein L10p (P0)
SCO6624	SLI6984	14184	hypothetical protein
SCO4725	SLI4994	12641	Translation initiation factor 1
SCO4716	SLI4985	11909	SSU ribosomal protein S8p (S15Ae)
SCO4721	SLI4990	11528	LSU ribosomal protein L15p (L27Ae)
SCO4702	SLI4971	10854	LSU ribosomal protein L3p (L3e)
SCO4717	SLI4986	10637	LSU ribosomal protein L6p (L9e)
SCO4713	SLI4982	10483	LSU ribosomal protein L24p (L26e)
SCO4296	SLI4533	10076	chaperonin GroEL
SCO4661	SLI4935	9424	Translation elongation factor G
SCO4701	SLI4970	9381	SSU ribosomal protein S10p (S20e)
SCO4711	SLI4980	9375	SSU ribosomal protein S17p (S11e)
SCO4730	SLI4999	9232	LSU ribosomal protein L17p
SCO3731	SLI3978	9099	Cold shock protein CspG
SCO4714	SLI4983	8983	LSU ribosomal protein L5p (L11e)
SCO3909	SLI4167	8730	LSU ribosomal protein L9p
SCO4706	SLI4975	8576	SSU ribosomal protein S19p (S15e)
SCO4659	SLI4933	8390	SSU ribosomal protein S12p (S23e)
SCO4709	SLI4978	8172	LSU ribosomal protein L16p (L10e)
SCO4719	SLI4988	8051	SSU ribosomal protein S5p (S2e)
SCO4720	SLI4989	8046	LSU ribosomal protein L30p (L7e)
SCO4660	SLI4934	8044	SSU ribosomal protein S7p (S5e)
SCO3908	SLI4166	7728	SSU ribosomal protein S18p @ SSU ribosomal protein S18p, zinc-dependent
SCO4704	SLI4973	7614	LSU ribosomal protein L23p (L23Ae)
SCO4708	SLI4977	7220	SSU ribosomal protein S3p (S3e)
SCO0527	SLI0486	7184	Cold shock protein CspA

SCO1998	SLI2315	7085	SSU ribosomal protein S1p
SCO4718	SLI4987	6987	LSU ribosomal protein L18p (L5e)
SCO4712	SLI4981	6979	LSU ribosomal protein L14p (L23e)
SCO4727	SLI4996	6970	SSU ribosomal protein S13p (S18e)
SCO4707	SLI4976	6742	LSU ribosomal protein L22p (L17e)
SCO2950	SLI3296	6658	Non-specific DNA-binding protein HBsu

Table S-2. Classification of sequences according to their similarities with the σ-70 like promoters. Known elements of regulation are listed.

SCO Gene #	SLI Gene #	-35/-10 regions (based on Strohl et al.)	-35/-10 regions (Softberry)	σ70-like promoter	Regulators
SCO4505	SLI4786	-160/-182	-60/-83	yes	
SCO6624	SLI6984	-135/-161	-	yes	
SCO4296	SLI4533	-159/-183	-		HrcA/CIRCE regulon (Duchene et al. 1994)
SCO3731	SLI3978	-	-57/-84	yes	
SCO0527	SLI0486	-	-		
SCO2950	SLI3296	-149/-167			developmentally regulated (Salerno et al, 2009)
SCO4761	SLI5031	-131/-155	-133/-155	yes	HrcA/CIRCE regulon (Duchene et al. 1994)
SCO4253	SLI4489	-	-		controlled by SCO4263, BldA dependent (Kim et al, 2005)
SCO5591	SLI5878	-59/-83	-135/-184	yes	
SCO1947	SLI2261	-	-		
SCO5367	SLI5636	-	-		
SCO4164	SLI4405	-140/-163	-		
SCO7636	SLI7864	-	-		
SCO3487	-	-61/-84	-61/-84	yes	possible BldA regulation (Temuujin et al 2012)
SCO3484	-	-134/-156	-96/-120	yes	

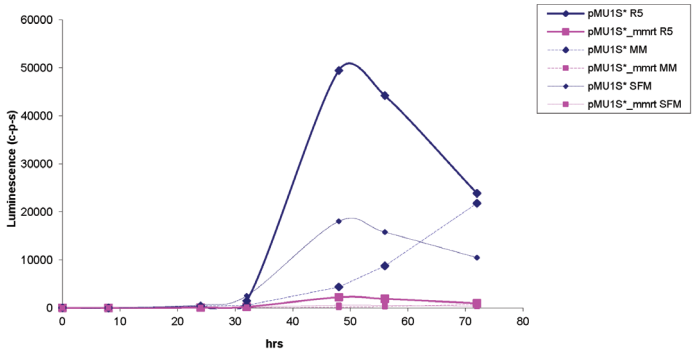

Figure S-1. Comparison of background light emission between pMU1S* and pMU1S*_mmrt on different solid media. An increase in emission can be observed starting at 30 hr for pMU1*, while pMU1S*_mmrt shows a remarkable reduction in background.

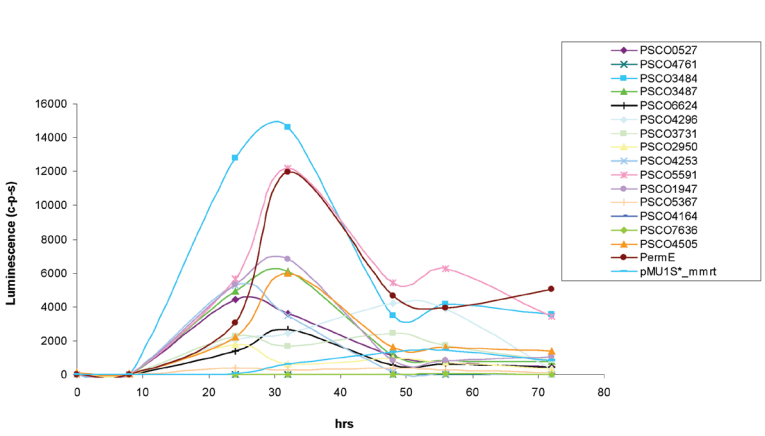

Figure S-2. Pre-screening of the 15 selected promoters compared with the benchmark P_{ermE} and the empty vector. Transformants were grown on MM agar plates with mannitol (1% w/v).

Table S-3. Plasmid used in this study

Name	Description	Reference
pMU1S*	pIJ8660 derivative carrying the luxCDABE cluster from *Photorhabdus luminescens*	(derived from Craney et al., 2007)
pMU1S*_mmrt	pMU1S* derivative with additional mmrT transcriptional terminator upstream of the MCS	This work
pTZ18R	pUC18 derivative, *E. coli* cloning vector	Pharmacia, Sweden
p18ERM	pTZ18R carrying P_{ermE} from er gene of *S. erythraeus* + 16 nucleotide linker sequence and RBS of *tuf1* from *S. ramocissimus*	(derived from Motamedi et al., 1995)
pHJL401	*E.coli-Streptomyces* shuttle vector, TsrR, AmpR	(Larson and Hershberger, 1986)
pHJL401N-	pHJL401 derivative without NdeI sites	This work
pSLAC	pHJL401N- carrying the *slac* gene and 400 downstream nucleotides from *S. coelicolor* cloned as EcoRI/NdeI-HindIII	This work

Table S-4. Oligonucleotides used in this study

Name	Sequence 5'-3'	Restriction sites
mmrt FW	GCG GAT ATC GAA CGC CGC AGC GCC GTC AC	EcoRV
mmrt RV	CGC GGA TCC GGT CGA TAC CCG GAG TGC GTG	BamHI
4253F	GCG GAA TTC GGA TCC GAC AGT CGA CAC AAG ACG TTG AAT C	EcoRI-BamHI
4253R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGG TAC GAG ACA GGA CGC C	HindIII-NdeI
5591F	GCG GAA TTC GGA TCC GGT CGG CGC GGG AAT GAG CTG	EcoRI-BamHI
5591R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGT TTT GAC GTG GTT GGG CAC GG	HindIII-NdeI
1947F	GCG GAA TTC GGA TCC GAC GAG TGA ATC CCG GTG TGC G	EcoRI-BamHI
1947R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGC CCG ATG TGC CGG CGA G	HindIII-NdeI
4505F	GCG GAA TTC GGA TCC TCT TGA CCT CTG TTG CGC TCG G	EcoRI-BamHI
4505R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGTT GCC CTG CTC CAG AAC CAG	HindIII-NdeI
6624F	GCG GAA TTC GGA TCC GTG CAG TAG AGT GAC TTG TGC TG	EcoRI-BamHI
6624R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGT GGT GAG CAG GAA TGG TGG	HindIII-NdeI
4296F	GCG GAA TTC GGA TCC CCC GAG AGG CGC TTG CAC TC	EcoRI-BamHI
4296R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGT GAT TCC TTC GGA CCG CGC	HindIII-NdeI
3731F	GCG GAA TTC GGA TCC GCC GTC CCG GGA ATA TTC CC	EcoRI-BamHI

3731R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGT ACG GTG CTC GGA GTT CAC C	HindIII-NdeI
2950F	GCG GAA TTC GGA TCC GGG TGC CGG ATT GGC TTT ACC	EcoRI-BamHI
2950R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGCC GTT GAG GCG TGC CAC	HindIII-NdeI
5367F	GCG GAA TTC GGA TCC TGT GAA GTC CTG CTA TCG TCC G	EcoRI-BamHI
5367R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AAGC GTG GCG CAT GGA TAC GG	HindIII-NdeI
4164F	GCG GAA TTC GGA TCC ACG AAG CGG CGG GCA GTG	EcoRI-BamHI
4164R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGGG CGT GCG GTG AGA AGG	HindIII-NdeI
4761PF	GCG GAA TTC GGA TCC TCG AGG ACG AGG CCG TCC	EcoRI-BamHI
4761R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGA CCT GCC CGT CGC GTA G	HindIII-NdeI
3487PF	GCG GAA TTC GGA TCC CTC ATC TTG TCG TCG CAG CC	EcoRI-BamHI
3487R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AAT CTG GGA AGG TGC GCA GAG G	HindIII-NdeI
3484PF	GCG GAA TTC GGA TCC CGT CGA CCA GAT AGA GGG CC	EcoRI-BamHI
3484R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT AGAG CGT CGT TGC ATC GGG	HindIII-NdeI
7636PF	GCG GAA TTC GGA TCC CCG GAA CTC CGC GGA GCC	EcoRI-BamHI
7636R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT ATA CGT GCA CGC CGC CCG G	HindIII-NdeI
0527F	GCG GAA TTC GGA TCC CTC CGA CTC CGT GGG TGG ACT C	EcoRI-BamHI
0527R_RBS	CGC AAG CTT CAT ATG GGT CCT CCT GTG GAG TGG TTC TGT ACCG TTA TCG GAT TCG CAC CGC G	HindIII-NdeI