An induction of Undecylprodigiosin Production from *Streptomyces coelicolor* by Elicitation with Microbial Cells Using Solid State Fermentation

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Abstract

Red pigmented undecylprodigiosin produced by *Streptomyces coelicolor* (A3)2 is a promising drug owing to its characteristics of antibacterial, antifungal, immunosuppressive and anticancer activities. The culture of *S. coelicolor* in liquid medium produces mainly the blue pigmented actinorhodin and only low quantities of undecylprodigiosin. From an industrial point of view, it is necessary to find a strategy to improve undecylprodigiosin production. The present study provides evidence that cultivation of *S. coelicolor* on solid substrate resulted in a reversal in this pattern of antibiotic production as the production of undecylprodigiosin was significantly increased and actinorhodin was completely suppressed. Four different solid substrate (wheat bran, soya bean ground, rice husk and ground corn) were tested for their ability to support the maximal production of undecylprodigiosin in solid state fermentation. Wheat bran showed the highest production of undecylprodigiosin, starting from the first day of incubation at a moisture level of (1:1 weight: volume) and reaching its maximum of 16 mg/gds on the fourth day. In addition, we report the exploitation of the interspecies interaction in order to enhance undecylprodigiosin production by introducing live or dead cells of *E. coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae*, separately, to *Streptomyces coelicolor* solid substrate fermentation. Our results revealed a significant increase in the production of undecylprodigiosin in the elicited cultures compared with control. The maximum enhancement occurred in the culture elicited with the live cells of *B. subtilis* with an increase of 2-fold compared with control.

Keywords: Undecylprodigiosin; *Streptomyces coelicolor*; Solid state fermentation; Elicitation

تحفيز انتاج الانديسايل بروديجيوسين من بكتريا *Streptomyces coelicolor* بواسطة الخلايا المايكروبيه باستخدام تخمرات الحاله الصلبه

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الخلاصة

اكتسبت الصبغة الحمراء الانديسيل بروديجيوسين Undecylprodigiosin المنتجه من بكتريا A3(2) *Streptomyces coelicolor* اهتماما متزايدا نتيجة لفعاليتها التثبيطيه العاليه ضد مختلف انواع البكتريا والفطريات بالاضافة الى فعالتيها ضد الخلايا السرطانية . تنتج هذه الصبغة في الاوساط السائله بكميات قليلة جدا مقارنتة مع الصبغه الزرقاء اكتينورهودين التي تنتج بكميات اكبر والتي تعتبر اقل اهميه نتيجة لفعاليتها المايكروبيه الضعيفة. من الناحيه الصناعيه هنالك ضروره لايجاد طريقه فعاله لتطوير انتاجية الانديسيل بروديجيوسين. تقدم الدراسه الحاليه دليل على ان تنمية *Streptomyces coelicolor* في الوسط الصلب انتج تغير في طراز انتاج المضاد الحيوي حيث ازداد انتاج الانديسيل بروديجيوسين بشكل واضح وتوقف انتاج الاكتينورهودين بشكل كامل. تم اختبار اربعة اوساط صلبة في الانتاج تضمنت نخالة الحنطه و دقيق فول الصويا و وسحالة الرز ومخلفات الذرة واختبار قدرتها على دعم انتاج الاعلى للانديسل بروديجيوسين في وسط التخمرات الصلبه. وسط نخالة الحنطه كان الافضل في الانتاج ومن اليوم الاول وبنسبة ترطيب (1:1) (وزن: حجم) حيث بلغ تركيز الصبغه الاعلى المنتجه 16 ملغم/ غم وسط صلب في اليوم الرابع. بالاضافة الى ذلك تم اختبار امكانية زيادة الانتاج عن طريق استغلال التداخل المايكروبي باضافة خلايا مايكروبيه حية وميته من , *E. coli, Bacillus subtilis* *Saccharomyces cerevisiae* وبشكل منفصل الى وسط االنتاج لمزرعة *Streptomyces coelicolor* . النتائج اظهرت زياده واضحه في انتاج الانديسيل بروديجيوسين في الاوساط المحفزه مقارنة مع السيطره. اقصى انتاج وجد في المزرعه التي تم تحفيزها بخلايا بكتريا *Bacillus subtilis* مع زياده في الانتاج بمقدار ضعفين مقارنتة مع وسط السيطره.

Introduction

Undecylprodigiosin is a red pigmented, cell wall-associated antibiotic that belongs to a group of polypyrrole bioactive compounds called prodiginines [1]. It is chemically similar to prodigiosin produced by *Serratia marcescens* and it is thought that both compounds have a similar biosynthetic pathway [2, 3]. During the past thirty years, researchers have shown an increased interest in prodiginines compounds due to their immunosuppressive and anticancer properties in addition to antimicrobial activities [1].

Compared to prodigiosin, undecylprodigiosin is one of the least studied prodiginines. In 1989, the immunosuppressive activity of undecylprodigiosin on cytotoxic T-lymphocytes was discovered [4]. Later, the capability of this compound to inhibit the generation of human B and T lymphocytes was confirmed [5, 6]. In 2007, the selective apoptotic effect of undecylprodigiosin on breast carcinoma cells was found, and consequently, it was suggested 'as a novel anticancer drug for the treatment of breast cancer' [7]. However, far too little attention has been paid to maximize the productivity of undecylprodigiosin from *Streptomyces coelicolor*.

Solid state fermentation was used widely for the production of various enzymes and secondary metabolites that generally associated with the stationary phase of the microbial growth. Although the common approach for the production of these compounds is the submerged liquid fermentations, production "has been shown to be less efficient than solid state fermentation" [8]. It is worthy mentioned that solid state fermentation potentially provide superior productivity that require simple technology and low capital costs compared with submerged fermentation. Therefore, it may represent the technology that can be applied for providing the increasing global demand for secondary metabolites, particularly antibiotics. In this context, high yield of penicillin in a short time period was achieved by solid state fermentation [9, 10]. The antifungal peptide cyclosporine A was successfully produced by *Tolypocladium inflatum* on wheat bran [11]. In addition, the broad-spectrum antibiotics tetracycline [12], Cephamycin C [13] and the anti-fungal antibiotic iturin [14] are other examples of antibiotics that produced by solid state fermentation. However, these antibiotics are still produced by liquid fermentations albeit production by this method has been shown to be less efficient than solid state fermentation.

Microorganisms in nature exist in complex mixtures of populations that lead to different interactions and responses among them. Scientists believe that these interactions and responses are the driving forces for

the production of antibiotics [15]. Therefore, mimicking the natural environmental setting of the microorganisms in the laboratory by co-culturing the microbial species that may interact in nature should give a more accurate picture of the microorganism's biosynthetic potential. In this field, most research in the literature is focused on exploiting inter-species interactions to induce the unexpressed biosynthetic pathways for new bioactive compounds [16] or to improve the productivity of the antibiotic-producing strains [17, 18]. Previously, we reported that *Streptomyces coelicolor* increased its antibiotic production when elicited with *Bacillus Subtilis* [17], *E. coli* [19] and dead cells of *Bacillus Subtilis* and *Staphylococcus aureus* [20].

Although there are some publications in the literature on the use of solid state fermentation in the production of antibiotics, this work is the first attempt on the use of such process with *Streptomyces coelicolor* to produce undecylprodigiosin. Furthermore, another novel aspect of this work is the exploitation of bacterial inter-species interactions in solid state fermentation in order to elicit *Streptomyces coelicolor* to increase the production of undecylprodigiosin.

Materials and Methods

Microorganism

MT1110 strain of *Streptomyces coelicolor* A3 (2) was used in this work. This strain is SCP1-, SCP2 – plasmids-free and was derived from the wild type strain 1147 (20). It was kindly provided by Professor Ferda Mavituna of the School of Chemical Engineering and Analytical Science, The University of Manchester, UK.

E. coli, Bacillus subtilis and *Saccharomyces cerevisiae* were used as elicitor bacteria which were already available in the Department of Biotechnology, College of Science, University of Baghdad.

Spore inoculum preparation

S. coelicolor was initially cultivated on mannitol soya flour (MS) agar slant for 10-14 days at 30°C from the original culture on MS agar. After growth and spore formation, 5ml of sterile distilled water was added and the growth was scraped gently by a loop to release the spores into the water which were then collected in a sterile tube. This spore suspension was centrifuged at 4000 rpm for 10min to collect the spores. Next, the spores were washed and re-suspended in 1ml of sterile distilled water and counted using a haemocytometer. In all experiments, the number of spores in this inoculum was kept to be approximately 10^8 spores /ml.

Cultivation conditions

Ten grams of solid substrate was placed in 250 ml Erlenmeyer flasks and 10 ml of tap water was added to produce moisture level (1:1) (w/v). The flasks were then autoclaved at 121C, 15 lbs for 20 min. Three ml of spore suspension contain approximately 10^8 spores/ml was added and flasks were then incubated at 28 ± 2 C for 5 days. Samples were taken always at the same time throughout the batch cultivation for the analyses of undecylprodigiosin.

Selection of the substrate

Wheat bran, soya bean ground, rice husk and ground corn were checked for their suitability for undecylprodigiosin production. The substrate supporting maximum production of undecylprodigiosin was selected for further study.

Effect of initial moisture Content

The influence of initial moisture content of the solid substrate on the production of undecylprodigiosin was investigated at different moisture levels (1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3 W/V) of wheat bran using tap water before autoclaving.

Elicitation experiments

Inocula of *E. coli* and *Bacillus subtilis* were prepared as follows: a few loopfuls of each bacterium growth from an overnight culture on LB agar was inoculated into a 250 ml Erlenmeyer flask containing 50 ml of Luria-Bertani (LB**)** broth and incubated at 37°C for 24 hours. After the incubation, a haemocytomtere was used to adjust the number of cells to be approximately 1×10^7 cells/ml by adding fresh sterile LB broth if necessary.

Then, the inoculum of each elicitor was centrifuged at 13000 rpm for 10 min. If dead cells were required, the culture flask was placed in boiling water for 30 min before separating by centrifugation. Live

and heat-killed cells of the elicitor were then washed and re-suspended in equal volume (culture volume before centrifugation) of normal saline. Inoculum of *Saccharomyces cerevisiae* was prepared by following the same procedure using sabouraud medium instead of LB medium and incubation temperature at 30C° [18].

In the elicitation experiments, each elicitor (live or heat killed cells) was added to *S. coelicolor* culture at zero time at a level of 2.5 ml/ 10 g substrate. For more reliability, all elicitation experiments were accompanied with a pure culture of *S. coelicolor* which will be referred to as the control and each run was conducted either in triplicate or duplicate and the results were represented as the arithmetic average.

Undecylprodigiosin determination

Fermented matter was extracted with methanol at room temperature on an orbital shaker at 180 rpm for 3 h. The extract was centrifuged at 10,000g and the absorbance at 530 nm of the supernatant was determined after removing substrate and cells debris. The concentration of undecylprodigiosin was calculated by using molar extinction coefficient (E_{530} =100150 M^{-1} cm^{-1}) [21].

Results and Discussion

Streptomyces sp is a filamentous bacterium that usually grows in a liquid culture in the form of mycelial strands normally aggregating in pellets. The pellets are composed of mycelia of different ages at different nutritional and growth stages. This heterogeneity in the culture conditions makes obtaining a meaningful physiological data difficult [22]. In addition, increasing the medium viscosity due to the metabolites secretion as well as the high requirements for mixing and oxygen transfer efficiency are considerable difficulties usually associated with the growth of actinomycetes in liquid medium. Though, these difficulties may be overcome through using solid state fermentation based on the fact that the morphology of *Streptomyces* mycelium is well suited to spread and invasive growth on the solid substrate.

In general the type and nature of substrate, moisture level/water activity and inoculum size are the important factors that play a crucial role in the growth and activity of the microorganism in a solid-state culture and consequently production of metabolites. Therefore, these factors were investigated in this study to identify the optimal conditions for undecylprodigiosin production as shown below.

In order to determine the solid substrate that support the maximal production of undecylprodigiosin, *S. coelicolor* was grown in different media **(**Wheat bran, soya bean ground, rice husk, ground corn). As can be seen in figure.1, wheat bran showed the highest production of undecylprodigiosin. The concentration of undecylprodigiosin in this substrate after 5 days of incubation was 12.9 mg/gram of dry substrate (gds). On the other hand, although rice husk provides a nutritionally complete medium for growth, *S. coelicolor* produced less undecylprodigiosin (6.23 mg/gds) compared with wheat bran. No production of undecylprodigiosin however, was observed in soya bean ground and ground corn as the growth of *S. coelicolor* was faint.

In the wheat bran medium, production of undecylprodigiosin was begun at the end of the first day of incubation , then increased dramatically throughout the fermentation period and reached its maximum of 16.06 mg/gds on the fourth day of the incubation figure. 2. During the incubation period, the colour of the solid substrate was turned red associated with the accumulation of undecylprodigiosin on the solid substrate.

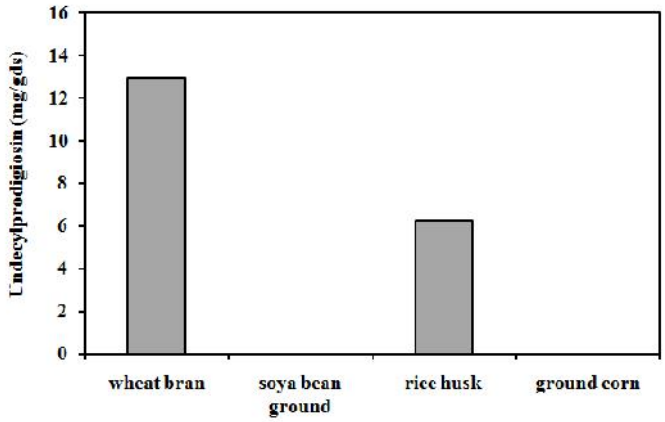

Figure 1-Production of undecylprodigiosin by *S. coelicolor* in solid state fermentation using different substrates at 28^0C after 7 days of incubation.

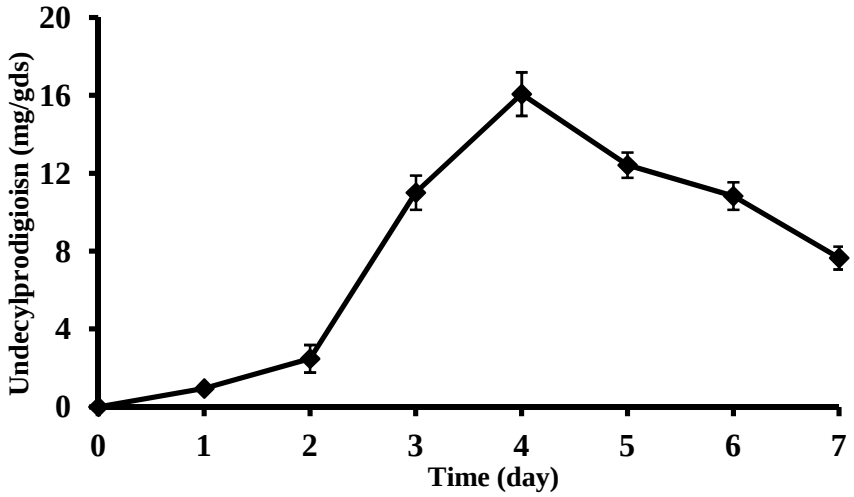

Figure 2-Effect of fermentation period on the production of undecylprodigiosin by *S. coelicolor* using wheat bran as substrate at 28^0C.

In the defined liquid medium, pure cultures of *Streptomyces coelicolor* produced mainly actinorhodin and only low quantities of undecylprodigiosin.. The production of these antibiotics usually started as the cells entered the exponential growth phase. Undecylprodigiosin was produced first on day two, followed by actinorhodin about 30 hours later. At the end of the second day, the concentration of undecylprodigiosin was 0.1 mg/L and rose to the maximum of 0.96 mg/L on the fifth day of the incubation. Onset of actinorhodin production occurred on the third day and reached its maximum of 5.35 mg/L on the seventh day [19]. However, undecylprodigiosin is more important due to its antimicrobial and anticancer activities therefore, enhancing the production of undecylprodigiosin by *S. coelicolor* represent an important challenge. The most striking result to emerge from this work is that, production of actinorhodin was completely suppressed and *S. coelicolor* produced only undecylprodigiosin in the wheat bran medium. Williamson [1] mentioned that the biosynthetic pathway of undecylprodigiosin in *S. coelicolor* involved incorporation of some amino acids such as proline and methionine that serve as an essential precursor for the production of the red pigment. According to the literature, wheat bran is normally contains high level of these amino acids [23] which might be the reason for the production of undecylprodigiosin observed in wheat bran.

Antibiotics are synthesized via dedicated biosynthetic pathways that involve several precursors formed from the catabolism of carbon substrates. Undecylprodigiosin and actinorhodin are both derived at least in part, from the same precursor acetyl-CoA (www.KEGG.com). As it is well known, the metabolism in living cells is a highly conserved process in terms of energy usage [24]. Thus, a possible interpretation for the suppression in the production of actinorhodin observed in the solid state fermentation may be the result of increase in the production of undecylprodigiosin because of the implications on the metabolic costs of producing two compounds simultaneously.

Initial moisture level is an important parameter in solid state fermentation which can directly affect the growth and metabolite production. Studies on the effect of moisture have shown that at high moisture level a reduction in the porosity of the substrate can be occurred as the substrate becomes agglomerated which lower the oxygen diffusion rates and generally decreasing gaseous exchange. Consequently, the rate of substrate degradation is reduced which lead to reduce the growth and as a result metabolite production. On the other hand, if the moisture level is too low, the microbial growth is usually reduced because the substrate is become less accessible as it does not swell.

Based on the results presented in figure 3, maximum production of undecylprodigiosin was obtained in culture with initial moisture level of 1:1 (W/V). Concentration of undecylprodigiosin in this culture was 14.2 mg/gds on day four of the incubation. When the initial moisture level was less or more than 1:1 (W/V), undecylprodigiosin production was significantly decreased.

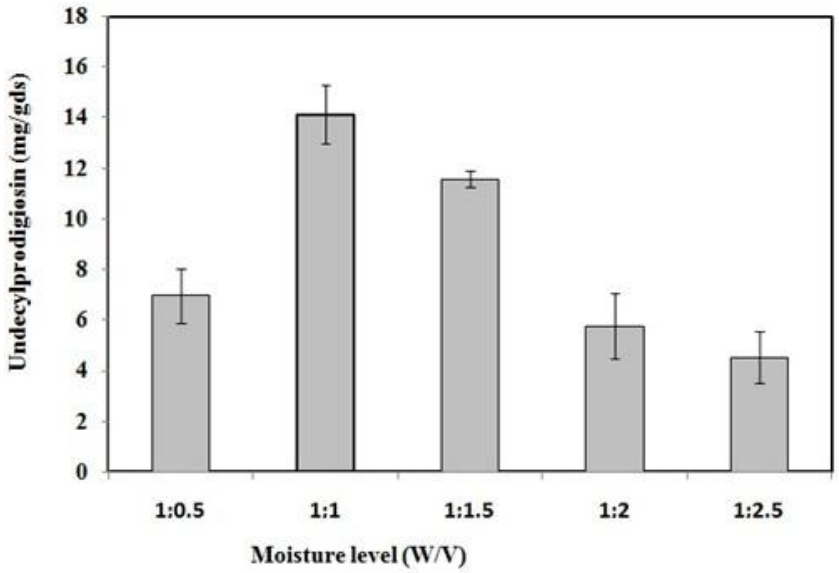

Figure 3-Effect of the moisture level (W/V) on the production of undecylprodigiosin by *S. coelicolor* using wheat bran as a substrate.

Elicitation experiments

Microorganisms normally co-exist with other species in nature; they have developed complex metabolic and physiological responses as a result of such inter-species interactions. Our strategy for the elicitation of *S. coelicolor* is based on utilizing some aspects of these interactions by introducing bacterial cells to *S. coelicolor* cultures. We already tested successfully this strategy in submerged fermentation in *S. coelicolor* culture that revealed an alteration in the antibiotic production pattern, such that undecylprodigiosin production was significantly enhanced and actinorhodin decreased [17; 19], Hence, this work was designed to examine the suitability of this strategy in solid state fermentation for enhancing antibiotic production, particularly, undecylprodigiosin.

Streptomyces species are soil dwelling bacteria which grow as complex mycelia of branching filaments that attach to or penetrate the solid substrate such as the tissues of dead organisms [24]. Greater competition can be occurred in soil among the various types of microbial species that can lead to deplete

the nutritional resources. For that reason, different strategies might be followed by the soil microorganisms in order to survive in such environment, one of which is the production of antibiotics. Elicitation is based on mimicking the presence of a second microorganism in the fermentation medium that act as a competitor in order to stimulate the antibiotic producer organism into switching on its secondary metabolism that yields a novel product of antibiotics [16] or increase the known compounds as obtained in this study. Three microorganisms; the Gram positive *Bacillus subtilis,* Gram negative *E. coli* and the eukaryotic microorganism *S. cerevisiae*; were chosen as the likely competitors for *S. coelicolor*. The selected elicitors are usually found in the soil and therefore, it is expected that *S. coelicolor* has developed an evolutionary recognition mechanism for them that may help to make an effect in the antibiotic production by *S. coelicolor*.

Several inoculation levels of the elicitors were tested ranging from 0.5 to 5 ml/ 10 g substrate each contains a fixed concentration of cells (approximately 10^7/ml). Results showed that addition of 3 ml of elicitor inoculum or more could affect the growth of *S. coelicolor* and production of undecylprodigiosin under the experimental conditions used in this work (data not shown). Accordingly, 2.5 ml/ 10 g substrate of live elicitor cells containing approximately 10^7/ml was added to the solid substrate immediately after the inoculation with *S. coelicolor* spores. Furthermore, for a realistic comparison, the same inoculation levels were used for the addition of heat-killed elicitor cells.

Addition of live cells elicitor

Our results revealed that the production of undecylprodigiosin was notably enhanced in the cultures supplemented with live cells of the elicitors compared with the control. The biosynthesis of undecylprodigiosin in the elicited cultures was begun earlier at the end of the first day of incubation and about 25-35 hours before its normal production in the pure culture reaching its maximum on day three.

Figure 4 shows that the maximum enhancement of undecylprodigiosin was observed in culture elicited with live cells of *B. subtilis* compared with those elicited with *E. coli* and *S. cerevisiae*. Concentration of undecylprodigiosin on the third day of incubation in the cultures elicited with *E. coli*, *B. subtilis* and *S. cerevisiae* were 20.1, 23.1 and 18.8 mg/gds. Production of undecylprodigiosin in the pure culture started after two days of incubation and reached its maximum concentration of 11.7 mg/gds on day four of incubation. Elicitation with live cells of *E. coli B. subtilis* and *S. cerevisiae* therefore, corresponds to an increase of 1.7, 2, 1.6-fold in the maximum undecylprodigiosin concentration compared with the pure culture.

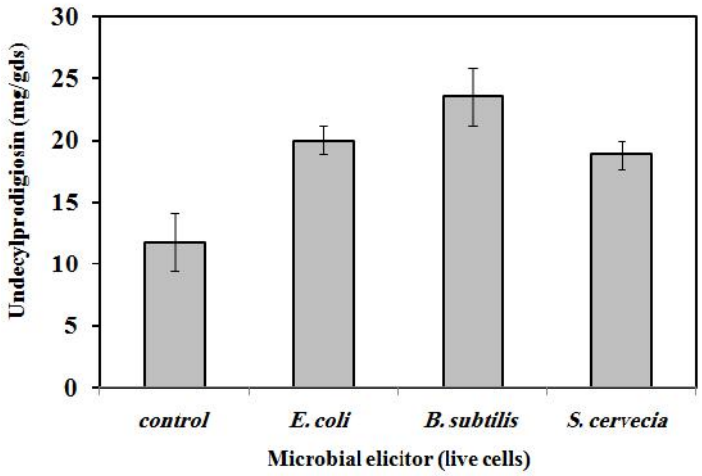

Figure 4-Production of undecylprodigiosin by *S. coelicolor* elicited with live cells of *E. coli*, *B. subtitles* and *S. cerevisiae* using wheat bran as a substrate.

Addition of dead cells elicitor

Interestingly, we found that heat-killed cells of *E. coli, Bacillus subtilis and S. cerevisiae* had the same role as live cells in terms of their effect on undecylprodigiosin production by *S. coelicolor*. As can be seen in figure 5, production of undecylprodigiosin was higher in the elicited cultures compared with the pure culture. Similar to the cultures elicited with live cells, an earlier onset of undecylprodigiosin production was observed at the end of the first day of incubation reaching its maximum on day three. Maximum enhancement of undecylprodigiosin was observed in culture elicited with heat-killed cells of *S. cerevisiae* compared with those elicited with *E. coli* and *Bacillus subtilis*. Concentration of undecylprodigiosin on the third day of incubation in the cultures elicited with *E. coli*, *B. subtilis* and *S. cerevisiae* were 13.65, 17.65 and 19.62 mg/gds respectively, resulting in 1.16, 1.5 and 1.66-fold increase compared with the pure culture of *S. coelicolor*.

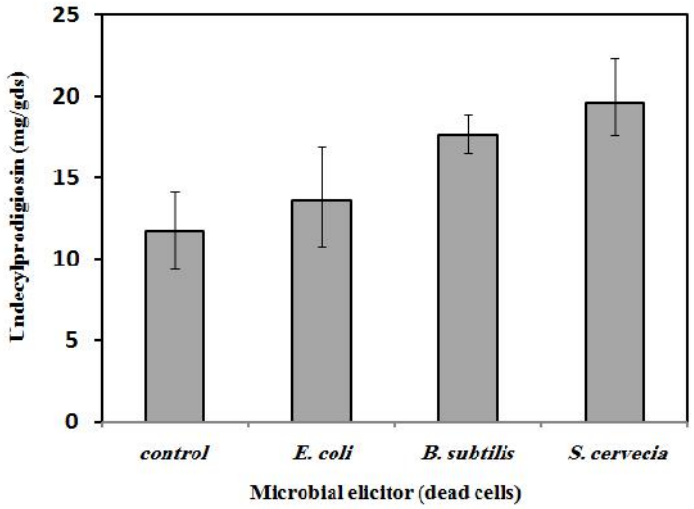

Figure 5-Production of undecylprodigiosin by *S. coelicolor* elicited with heat killed cells of *E. coli*, *B. subtilis* and *S. cerevisiae* using wheat bran as a substrate.

Based on the results, the elicitation resulted in an earlier onset of undecylprodigiosin under the conditions employed in this work. Previous studies have reported that an earlier production of undecylprodigiosin can be occurred in the liquid culture during the exponential growth phase as a result of some environmental and physiological influences such as nutritional stress, changes in growth rate or signaling molecules [25; 26]. However, no mention was found in the literature on such phenomenon in the solid state fermentation.

In an interesting method, Yang and co workers [27] developed an imaging mass spectrometry to study the chemical communication between two neighboring populations of *Streptomyces coelicolor* and *Bacillus subtilis* grown on nutrient agar. The main objective of their research was based on capturing the metabolic exchange between these bacteria using thin layer agar natural product imaging. They discovered that the production of prodiginines by *Streptomyces coelicolor* was inhibited as a result of producing the non-ribosomal peptide bacillaene by *Bacillus subtilis*. However, in the absence of bacillaene, prodiginines were produced earlier than their normal production in the pure culture. In another study, using the same technique, Watrous [28,29] reported that *Streptomyces coelicolor* increased production of some secondary metabolites particularly actinorhodin when interacted with a mutant strain of *Bacillus subtilis.* Though, production of actinorhodin was inhibited upon interaction with a wild type of *Bacillus subtilis.*

In our experiments, the heat-killed cells of *E. coli*, *Bacillus subtilis* and *S. cerevisiae* had the same effect as live cells to elicit *S. coelicolor.* Certainly, their presence in the *S. coelicolor* fermentation culture could not cause any nutritional stress or production of compounds that may cause the elicitation. Furthermore, since live cells of *E. coli*, *Bacillus subtilis* and *S. cerevisiae* is usually grow poorly in the solid state

fermentation, it can be deduced that the mechanism of interaction in this case was not mediated via a compound produced by the elicitor bacteria.

Usually, when growth of bacteria ceases due to unsuitable cultivation conditions, cells may lyse because of the autolytic enzymes [30]. Moreover, in order to prepare inocula of dead cells, heat was used to kill the elicitor cells which may damage the bacterial cell wall as well as the outer membrane, enzymes, proteins and nucleic acids. In view of that, one possible interpretation of the mechanism of elicitation may be as follows: live and heat killed cells of *E. coli*, *Bacillus subtilis* and *S. cerevisiae* may have lysed during *S. coelicolor* cultivation on the solid substrate and hence contributed some undecylprodigiosin stimulants or compounds that may act as precursors for the product.

Alternatively, another possible interpretation for the mechanism of the elicitation may be a physical direct cell-to-cell contact between *S. coelicolor* and the live or heat-killed cells of the elicitor bacteria. Although *E. coli*, *Bacillus subtilis* and *S. cerevisiae* in some cases were used as dead cells, *S. coelicolor* may have recognized some proteins or receptors on the surface of these cells which may not have been affected by the heat. In this context, Oh [15] observed an induction of biosynthesis of four novel diterpenoids in a co-culture of a marine bacterium and the marine fungus *Libertella*. The authors found no evidence for signaling molecules mediated this interaction; therefore, they suggested that the mechanism of interaction was due to cell-cell direct contact.

In conclusion, in general, the common strategy for producing antibiotics is submerged liquid fermentation, though yields is likely to be low in relation to the cost and energy input. In liquid culture, *S. coelicolor* produces mainly the weak antibiotic actinorhodin and only low quantities of undecylprodigiosin which is more valuable due to its anticancer properties as well as antimicrobial activity. Based on the results obtained in this work, *S. coelicolor* produced a considerable amount of undecylprodigiosin in solid state fermentation using wheat bran as a substrate with no production of actinorhodin. In addition, the present study provides additional evidence with respect to the strategy of exploiting the interspecies interactions to enhance antibiotic production that previously tested effectively in liquid culture. This strategy was successful for undecylprodigiosin in solid state fermentation such that production of this antibiotic was increased by 2-fold compared with that in pure culture of *S. coelicolor*.

The findings from this study make a contribution to the growing body of literature on using solid state fermentation as an alternative strategy for the production of antibiotics from *Streptomyces* sp.

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References:

1. Williamson, N.R.; Fineran, P.C, Leeper F.J. and Salmond G.P.C. **2006**. The biosynthesis and regulation of bacterial prodiginines. *Nat. Rev. Microbiol*. 4(12):887-899.

2. Feitelson, J.S. and Hopwood, D.A. **1983**. Coning of a *Streptomyces* gene for an O-methyltransferase involved in antibiotic biosynthesis. *Mol. & Gen. Genet*. 190(3):394-398.

3. White, J. and Bibb, M. **1997.** bldA dependence of undecylprodigiosin production in Streptomyces coelicolor A3(2) involves a pathway-specific regulatory cascade. *J. Bacteriol.* 179(3):627-633.

4. Nakamura, A.; Magae, J.; Tsuji, R.F.; Yamasaki, M. and Nagai, K. **1989**. Suppression of cyto-toxic T-cell induction invivo by prodigiosin 25-C. *Transplant.* 47(6):1013-1016.

5. Mortellaro, A.; Songia, S.; Gnocchi, P.; Ferrari, M.; Fornasiero, C.; D'Alessio, R.; Isetta, A.; Colotta, F. and Golay, J. **1999.** New immunosuppressive drug PNU156804 blocks IL-2-dependent proliferation and NF-kappa B and AP-1 activation. *J. Immunol*. 162(12):7102-7109.

6. Songia, S.; Mortellaro, A.; Taverna, S.; Fornasiero, C.; Scheiber, E.A.; Erba, E.; Colotta, F.; Mantovani, A.; Isetta, A.M. and Golay, J. **1997**. Characterization of the new immunosuppressive drug undecylprodigiosin in human lymphocytes - Retinoblastoma protein, cyclin-dependent kinase-2, and cyclin-dependent kinase-4 as molecular targets. *J. Immunol*. 158(8):3987-3995.

7. Ho, T.F.; Ma CJ, Lu CH, Tsai YT, Wei YH, Chang JS, La JK, Cheuh PJ, Yeh CT, Tang PC and others. **2007**. Undecylprodigiosin selectively induces apoptosis in human breast carcinoma cells independent of p53. Toxicol. *Appl. Pharmacol*. 225(3):318-328.

8. Robinson T, Singh D, Nigam P. **2001**. Solid-state fermentation: a promising microbial technology for secondary metabolite production. *Appl. Microbiol. Biotechnol*. 55:284–289.
9. Barrios-Gonzalez J, Tomassini A, Viniegra-Gonzalez G, Lopez L, **1988**. Penicillin production by solid state fermentation. *Biotechnol. Lett*. 10:793–798
10. Barrios-Gonzalez J, Castillo TE, Mejia A. **1993**. Development of high penicillin-producing strains for solid state fermentation. *Biotechnol. Adv*. 11:525–537
11. Ramana Murthy MV, Mohan EVS, Sadhukhan AK. **1999**. Cyclosporin A production by *Tolypocladium inflatum* using solid state fermentation. *Proc. Biochem*. 34:269–280.
12. Yang SS, Ling MY. **1989.** Tetracycline production with sweat potato residue by solid state fermentation. *Biotechnol. Bioeng*. 33:1021–1028.
13. Kota KP, Sridhar P. **1998**. Solid state cultivation of *Streptomyces clavuligerus* for producing cephamycins C. *J. Sci. Ind. Res.* 57:587–590.
14. Balakrishnan K, Pandey A. **1996**. Production of biologically active secondary metabolites in solid state fermentation. *J. Sci. Ind. Res*. 55:365–372.
15. Oh DC, Jensen PR, Kauffman CA, Fenical W. **2005**. Libertellenones A-D: Induction of cytotoxic diterpenoid biosynthesis by marine microbial competition. Bioorgan *Med. Chemist*. 13(17):5267-5273.
16. Pettit RK. **2009.** Mixed fermentation for natural product drug discovery. *Appl. Microbiol. Biotech.* 83(1):19-25.
17. Luti KJK, Mavituna F. **2011a**. *Streptomyces coelicolor* increases the production of undecylprodigiosin when interacted with *Bacillus subtilis*. *Biotechnol. Lett*. 33:113-118.
18. Luti KJK, Yonis RW. **2013**. Elicitation of *Pseudomonas aeruginosa* with live and dead microbial cells enhances phenazine production. *Rom. Biotechnol. Let.* 18 (6): 7169-7178.
19. Luti KJK, Mavituna F. **2011b**. Elicitation of *Streptomyces coelicolor* with *E. coli* in a bioreactor enhances undecylprodigiosin production. *Bioch. Engineer. J.* 53,281–285.
20. Luti KJK, Mavituna F. **2011c**. Elicitation of *Streptomyces coelicolor* with dead cells of *Bacillus subtilis* and *Staphylococcus aureus* in a bioreactor increases production of undecylprodigiosin. *Appl. Microbiol. Biotechnol*. 90:461-466.
21. Kang SG, Jin W, Bibb M, Lee KJ.**1998**. Actinorhodin and undecylprodigiosin production in wild-type and relA mutant strains of Streptomyces coelicolor A3(2) grown in continuous culture. *Fems Microbiol. Lett*. 168(2):221-226.
22. Doull JL, Vining LC. **1989**. Culture conditions promoting dispersed growth and biphasic production of actinorhodin in shaken cultures of Streptomyces coelicolor A3(2). *FEMS Microbiol Lett* 53(3):265-8.
23. Stevenson L, Phillipsi F, O'Sullivan K, Walton J. **2012.** Wheat bran: its composition and benefits to health, a European perspective. Int. J. Food Sc. Nut.63(8): 1001–1013
24. Sanchez S, Demainb A. **2002**. Metabolic regulation of fermentation processes. Enzy. Microb. Technol. 31 (2002): 895–906.
25. Chater KF. **2006.** *Streptomyces* inside-out: a new perspective on the bacteria that provide us with antibiotics. Philosophical Transactions of the Royal Society B-Biological Sciences 361(1469):761-768.
26. Bibb M. **1996.** The regulation of antibiotic production in *Streptomyces coelicolor* A3(2). *Microbiol*-Uk 142:1335-1344.
27. Sevcikova B, Kormanec J. **2004**. Differential production of two antibiotics of Streptomyces coelicolor A3(2), actinorhodin and undecylprodigiosin, upon salt stress conditions. *Arch. Microbiol*. 181(5):384-389.
28. Yang YL, Xu YQ, Straight P, Dorrestein PC. **2009**. Translating metabolic exchange with imaging mass spectrometry. *Nat. Chem. Biol*. 5(12):885-887.

29. Watrous J, Hendricks N, Meehan M, Dorrestein PC. **2010**. Capturing Bacterial Metabolic Exchange Using Thin Film Desorption Electrospray Ionization-Imaging Mass Spectrometry. *Analyt. Chemist*. 82(5):1598-1600.
30. Forsberg C, Rogers HJ. **1971**. Autolytic enzymes in growth of bacteria. *Nat.* (London) 224(5282):272-273.