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Shifting production of rifamycin-SV by mutant of *Amycolatopsis mediterranei* tolerant to benzyl alcohol

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Abstract

Benzyl alcohol is a toxic hydrophobic compound for the microorganisms but using at specific concentration, 0.25%, a mutant of *A. mediterranei*-CBS-42575 was selected which has the maximum metabolism shifting of rifamycin-B from 1460 into 95(mg l^{-1}) into rifamycin-SV upto 4.896 (g l^{-1}). At the optimum fermentation differentiation between *A. mediterranei*-CBS- 42575 and its benzyl alcohol mutant in kinetic analysis was established, the resulted values for the growth yield coefficient $Y_{X/S}$ was decreased from 0.4663 for the parent strain into 0.311 and 0.3675 (g cells g glucose^{-1} l^{-1}) for the mutant in the shake flasks and the fermentor respectively. Also Monod growth saturation coefficient (Ks) recorded values, 1.01 for the parent strain while 0.95 and 1.57 (g S l^{-1}) for the mutant in the shake flasks and the fermentor respectively. Also, $Y_{P/X}$ was 0.373 for the parent strain and increased upto 1.0756 and 0.98 (g rif.-SV g cells^{-1}) for the mutant in the shake flasks and the fermentor respectively. Also, the maximum of yield coefficients of refamycin-SV, $Y_{P/S,}$ were obtained, 0.2523 and 0.3144 (g rif.-SV g glucose^{-1}) for the mutant in the shake flasks and fermentor respectively.

Key words: Rifamycin-SV, *Amycolatopsis mediterranei,* benzyl alcohol, hydrophobic compounds, fermentation, kinetic analyses.

Introduction

The rifamycin is an ansamycin compound which produced by submerged fermentation of *Amycolatopsis (A.) mediteranei* species using organic or semi-synthetic media (Sensi *et al.* 1960, Margalith and Pagani 1961). Eight rifamycins were discovered, important them is SV. Several chemically modified rifamycins, such as rifampicin, have been used since the mid-1960s for the treatment of tuberculosis and other mycobacterial infections, leprosy and AIDS-related mycobacterial infections (Sepkowitze et al. 1995). Furthermore, rifamycins were used in combination with chemotherapy for cancer treatment (Fardel et al. 1995). Rifamycin-S considers a key intermediate for a variety of transformation reaction leading to the highly active rifamycin-SV (Birner et al. 1972). The biosynthesis involve a polyketide intermediate synthesized with chain extension of an unusual starter unit 3-amino-5 hydroxy benzoic acid (AHBA) in all the other ansamycin-producing actinomycetes which arises from the aminoshikimats path way and recognize as the starter unite (Admiraal et al. 2002, Yu et al. 2004). To improve the production of rifamycins, uracil at 2% was enhanced the production of rifamycin-B and -SV in the presence of yeast extract by *Amycolatopsis. meditrranei*-CBS-42575 and –MV35 in shake flasks (El-Shahed and Farid 1994, Krishma et al. 2003). Grafe et al. (1986) isolated mutants of *Streptomyces noursei* resistant to benzyl alcohol and consolidated that the effect occurred because of the changes of lipid function cell wall at the level of pleiotropic gene function.

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Different bacteria have been isolated and characterized can be adapted in the presence of organic solvents (Iskan and Jan 2004). It is apparent that the cells adjust their metabolism to the change in function from growth to secondary metabolite production (Borodina et al. 2005). In this paper procedure was adapted to select the benzyl alcohol tolerant mutant of *Amycolatopsis mediterranei* to shift rifamycin-B to rifamycin-SV and the variations of kinetic were studied.

Materials and Methods

Rifamycins producing strains and culture media

Amycolatopsis mediterranei-ATCC-21271 and *Amycolatopsis mediterranei*-CBS-42575 were used in this study. The strains were grown and maintained on slants of ISP medium no.5 which incubated at 28 – 30 °C for 8-12 days. For long preservative suspension of spores were lyophilized in skim milk.

Selection tolerant mutant of hydrophobic compounds

Hydrophobic aromatic compounds such as; benzyl alcohol, dinitrosylasylic acid, chromotropic acid and dinitrophenyl amine were used with concentrations (1.0 % w or v/v). Each concentration were dissolved while benzyl alcohol were suspended in water and sterilized with hydrophobic membrane filter (0.22 um) then mixed well with agar medium at 55 °C to obtain the proper concentration, then the mixture poured in the plate. After solidification, 15 µl of mycelium suspension of used strain was spread with sterilized policeman glass rode then the plates incubated at 28-30 °C and the growth was observed through the incubation time which extended up to 12 days. After that, the different colonies of *A. mediterranei* strains were picked up and maintained on ISP -5 till examined to produce the different rifamycins.

Tolerant mutant of A. Mediterranei-CBS-42575 to benzyl alcohol

This experiment was designed to find an optimum, nontoxic range of benzyl alcohol concentrations that induce only some alteration of morphological characters of *A. mediterranei*-CBS under steady-state conditions allow to pick up the potent colonies shift rif-B into rif-SV. An aqueous solution 20% benzyl alcohol was sterilized with hydrophobic membrane filter and adapted with 20 ml sterilized agar ISP-5 medium to obtain different concentrations, 0.05, 0.1,0.15, 0.20, 0.25, 0.30, 0.40, 0.50 and 0.60.%, mixed well and was poured in sterilized Petri-dishes (9 mm diameter). After solidification, 15 µl of cell suspension of dilutions 10^{-4} and 10^{-5} of *A. mediterranei*-CBS were spread with policeman glass rode and incubated at 28-30 °C for 8 – 21 days. The resistant colonies picked up from the higher concentrations of used benzyl alcohol and slanted on ISP medium-5 as aforementioned. Seed medium of the resistant colonies were prepared to select the potent mutant shifted the different rifamycins B, S into SV in shake flasks using the rifamycin production medium (EL-Shahed and Faried 1994). The parent strain was also fermented at the same rifamycins production conditions as a control.

Culture Morphology

The differences in color and cultural characters of different colonies were observed with Tresener and Backus (1963) and computerized software color wheel.

Rifamycin B and SV fermentation in shake flasks

A. mediterranei-CBS either parent strain or its benzyl alcohol mutant was fermented using the production medium recommended by EL-Shahed and Faried (1994). The fermentation was carried out at 28 °C and 200 rpm in an orbital rotary shaker and every 12 hours during the fermentation, two flasks were received to determine the cell dry weight, final pH value, rifamycins –B or -SV yield and reducing sugars (glucose). After that, 20 ml of each flask were lyophilized and extracted with 5 ml methanol to detect rifamycin derivatives with TLC technique.

Rifamycin SV fermentation process in 7l bioreactor

500 ml of inoculia were used to inoculate 4.5 l of the rifamycins production medium (EL-Shahed and Faried 1994), sterilized in jar fermentor 7 l capacities. In batch cultivation was carried out in fermentor (New Brunswick Sci.Comp.) at controlled temperature, 28 °C and the initial pH was 6.8 without control in the fermentation process while the agitation at 200 rpm was applied with a constant aeration of 1:1

(v/v) and soy bean oil used as antifoam. Samples of about 50 ml were received approximately every 12 hours during the fermentation to determine the cell dry weight (CDW), final pH value, rifamycin-SV yield (g l^{-1}) and reducing sugars as glucose (g l^{-1}), after 120 hours the fermentation broth was harvested.

Analytical chemistry

Detection of rifamycins on TLC

50 ml fermented broth (one flask) was filtrated and lyophilized then dissolved in methanol : chloroform (1:1, v/v) and the solvents were evaporated under vacuum then the residual re-solved in 10 ml of the same solvent system. The mixture was spotted on TLC almonium sheets silica gel 60F254 (Merck) using rifamycin B and SV which kindly donated by Dr.L.Cellai, Institute of strutturistica, Seoul Korea and maintained at -20°C. The spots were developed and detected on TLC according to individual colors with R_f values recorded according to Seong et al. (1985).

Mycelial growth

Cell growth (CDW) was determined by weight the residual mater of 50 ml of fermented broth using Whatman No.1 filter paper after dryness for constant weight at 60 °C.

Determination of rifamycins

Rifamycins-B and -SV were estimated spectrophotometrically by a method of Pasqualucci et al. (1970)

Reducing sugars

Reducing sugars in the fermented broths were determined calorimetrically according to Nelson (1944) and modified by Somogyi (1952) using D-glucose as a standard.

Bioprocess fermentation kinetic

Growth kinetic

The growth kinetics was analyzed in batch fermentation as a closed system and substrate limited and the fermentation of Sinclair and Cantero (1990) was used to estimate these parameters μ, μ_{max} , k_s , q_p , $Y_{x/s}$,$Y_{p/s}$ and $Y_{p/x}$.

Production kinetic

The production of rifamycin-SV and other experimental results were analyzed by Claudiu et al. (2001) and Luodoking and Piritlia (1959) while the correlation coefficients were estimated mathematically by software.

Substrate kinetic

The substrate kinetic substitution was provided by Sinclair and Cantero (1990)

Results and Discussions

Morphological characters of Amycolotapsis mediteranei (ATCC- and CBS-) strains tolerant to hydrophopic compound

Improvement of most industrial strains has been accomplished by mutation (Holt and Sanders 1986). The results in Table 1 revealed that the strains displayed the same characters of their growth at the same concentrations of hydrophobic compounds. The morphological and culture characters of the tested strains were varied specially by *A. mediteranei* CBS-strain using benzyl alcohol which recorded Flat edge with brown folded center and red edge and dark red on reverse while *A. mediteranei*-ATCC strain was Hygroscopic flat and brownish on reverse. The soluble pigments were indicated to effects of hydrophopic compounds especially on *A. mediterranei* CBS-strain where a dark red pigment of rifamycin-SV production was secreted in the agar medium and a reddish color with using chromotropic acid. The differences of morphological characters of the mutant produced rifamycin-SV were recorded of an Australian isolate of *Nocardia mediterranei* produced only Rifamycin-SV by Birner et al. (1972). The

effect of benzyl alcohol on the mutant of *A. mediterranei* CBS-strain may be depended on redistribution of glucose-6-phosphate dehydrogenase a typical cytoplasmic enzyme both in the low- and high-production strain. The redistribution of the enzymes is discussed with respect to tetracycline over-production (Erban et al. 1987). Also the dramatic changes in the *Streptomyces coelicolor* colony's appearance reflect the differentiation processes taking place in the colony as that produces complex sporulation structures on its surface and, underneath, an array of antibiotic compounds, some of which are brightly colored (Pang et al. 2004). In addition to morphological differentiation (aerial mycelium and spore formation), *Streptomycetes* display a physiological differentiation related to the synthesis of a wide variety of secondary metabolites. This complex life cycle and differentiation undoubtedly require various levels of regulation and signal transduction mechanism (Zhangl et al. 2000). These fascinating aspects of amycolotopsis biology are yielding intriguing glimpses of the underlying mechanisms regulating development. Therefore, the mutant of *A. mediterranei*-CBS strain was satisfied to shift rifamycin-B into rifamycin-SV.

Table 1. Effect of hydrophobic compounds on morphological characters of *Amycolatopsis mediterranei* ATCC-21271 and *Amycolatopsis mediterranei CBS*-42575.

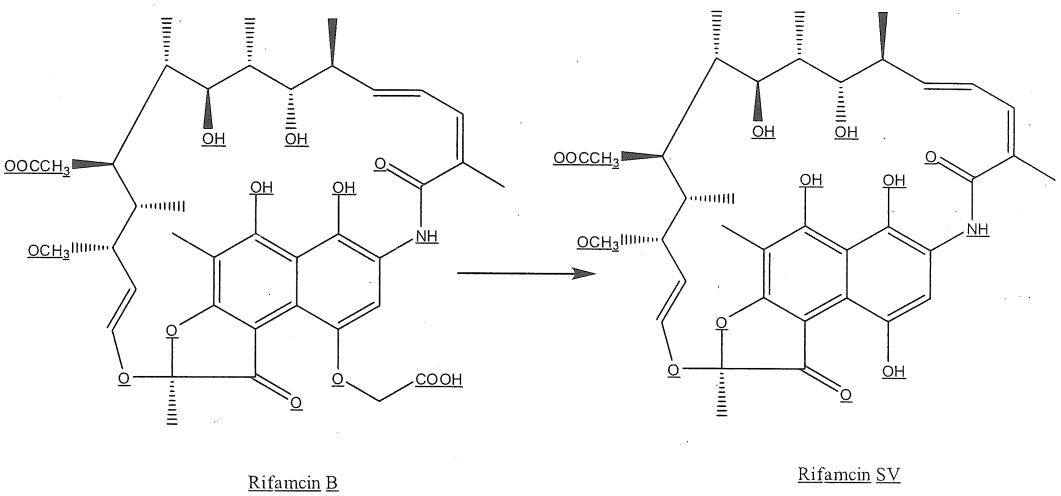
Hydrophobic compounds (0.1%)	*Amycolotopsis mediterranei ATCC--21271*			*Amycolotopsis mediterranei CBS*-42575.		
	Growth	**Aerial mycelium (Colony) color**	**Soluble pigment**	**Growth**	**Colony color (aerial and substrate mycelium)**	**Soluble pigment**
Control	Good	Bage to yellowish and pall orange of substrate mycelium	Yellow	Good	Folded orange and brown on reverse	amber
Chromotropic	Good	Orange and amber of substrate mycelium	yellowish	good	Folded-orange and yellowish on reverse	reddish
Nitrophenol	Poor	Hygroscopic and colorless on reverse	none	moderate	Bage to yellowish and brownish of substrate mycelium	yellow
Benzyl alcohol	Poor	Hygroscopic flat and brownish on reverse	Yellow	Moderate	Flat edge with brown folded center and red edge and dark red on reverse	dark red
Dinitrosalycilic acid	Good	yellow and orange on reverse	yellow	good	Orange-folded and brownish on reverse	yellow
Salycilic acid	Good	Brownish and yellow on reverse	orange	Good	Brown folded in center-flat edge and yellow on reverse	brownish

Effect of Hydrophobic compounds shifting rifamycin-SV production by A. mediterranei-CBS and -ATCC strains

The tolerant of *A. mediterranei*- CBS and –ATCC for hydrophopic compounds tested were slanted on agar medium for 5-8 days and cell suspension were fermented separately in shake flasks contained production medium where rif-B was shifted to rfamycin-SV (Figure 1) in the fermentation broth.

The production of rifamycin-B and rif–SV were differed when isolates of *A. mediterrane*-CBS and –ATCC tolerated to hydrophopic compounds were fermented in the production medium in comparing to the using parent strains (control without hydrophobic compounds) (Figure 2).

Evidently the mutant of *A.mediterrane*-CBS-42575 tolerated to benzyl alcohol was highly decreased the metabolic of rif.-B from 1455 to 761 (mg l^{-1}) and shifted into rif.-SV from 3043 into 4196 (mg l^{-1}) comparing to the parent strains (control) and other isolates of the other used hydrophobic compounds with *A. mediterranei*–CBS and ATCC strains.



Rifamcin B

Rifamcin SV

Figure 1. Chemical structure of Rifamycins B and SV.

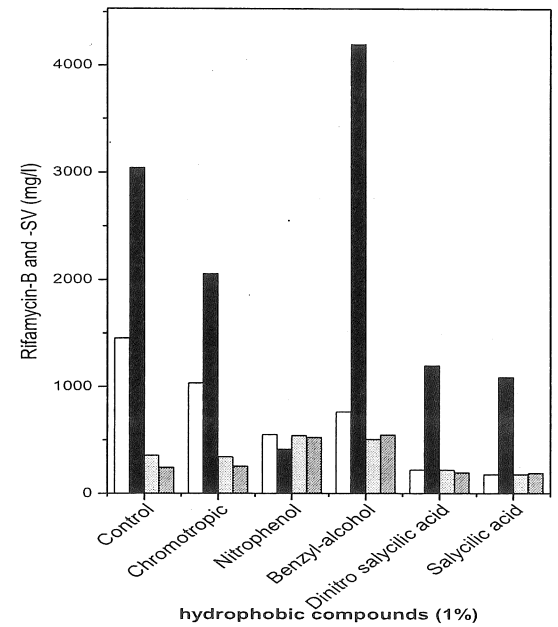


Fig.(2): production of rifamycins B and SV by *Amy.mediterranei* ATCC and CBS strains by different hydrophobic compounds

rifamycin-B-(CBS-strain). rifamycin-SV-(CBS-strain)

famycin-B-(ATCC-strain), famycin-SV-(ATCC-strain)

Whilst, *A. mediterrane*-CBS-42575 was characterized with more rifamycins production than *A. mediterranei-ATCC-strain* either as a parent strain or its mutants tolerant to the used hydrophobic compounds. Yeast extract was reported to play a regulatory role in the production of rifamycin-B by *Nocardia* (Kawaguchi et al. 1984). The proper mutant was stabilized many times on the same medium, incubated at 28 °C for 7-9 days. The regulatory process is modeled by assuming that the uptake of a substrate depends on the level of a key enzyme or a set of enzymes, which may be inducible (Bapat et al. 2005). The variations in the tolerant mutants of *A. mediterrane*-CBS-42575 of benzyl alcohol which may be effected the cell membrane of the tolerant mutant, these may play important role in the transport of nutrients and products

into/from the cytoplasm as well as some bio-signals regulating morphological differentiation in mutant of *A. mediterranei*-CBS of the microbial pheromones (B-factor) (Ohnishi et al. 2005).

Selection mutant of A. mediterranei-CBS-42575 tolerant to benzyl alcohol

The local anesthetic benzyl alcohol, well known as a potent fluidizing agent reduced the lag period (Friedlander et al. 1987). Svobodova et al. (1995) used these characteristics to explain well the beneficial influence of benzyl alcohol on cold-shock recovery of *B. subtilis*. It is not clear whether the greater competence for complementation observed in the *A. mediterranei* mutants is accidental or has evolved due to a long-term molecular adaptation between substrates and the enzymes exposed to the rifamycin-producing environment (Yu et al. 1999). The results in Table 2 of this study define the adaptation of procedure at different benzyl alcohol concentrations to select mutant of *A. mediterranei*-CBS-42575 from agar media shifted rif-B into the maximum rif-SV production. These adaptation suppress the effect of the solvents on the membrane stability changes, different bacteria have been isolated and characterized can be adapted in the presence of organic solvents (Iskan and Jan 2004). The maximum of rif-B was decreased from 1460 (control) to 95 (mg l^{-1}) which shifted into rif-SV from 3100 up to 4896 (mg l^{-1}) was obtained from *A. mediterranei*- CBS-42575 colonies selected from agar cultures at 0.25% of benzyl alcohol concentration (Table 2) which had survival about 5-8 colonies. The maximum shifting of rif.-B to rif.-SV was characterized with the growth of mutant (TDW) about 213 mg l^{-1}. The pH values of the mutants were shifted towards acidic range, 6.00 with increasing of benzyl alcohol concentrations into 0.4 % concentration, above this concentration the growth was hygroscopic till inhibited. Some observations can be made for metabolic shift in *Streptomyces lividens* which produces actinorhodin (Rossa et al. 2003) where NADPH is regarded as the cofactor necessary for biosynthesis of several antibiotics such as β-lactams , polyketides (rifamycins) and glycopeptide where NADPH is produced in the PP and ED pathway (Borodina et al. 2005). Furthemore, the aromatic nucleus for all ansamycins is probably built up starting from 3-amino-5-hydroxybenzoic acid (AHBA). Starting from this molecule, which is presumably activated as coenzyme A, the entire aliphatic bridge is synthesized by a multifunctional polyketide synthase (Jackie and Khosla 1995). All changes observed in the morphological pattern of *A. mediterranei*-CBS-42575 in the presence of benzyl alcohol, which affect the physical properties of the cell membrane may also be related the ability of the microorganism to shift the metabolic pathway of rif.B into the maximum of rif.SV in vitro assays. In fact, numerous morphological and physiological changes are induced by chemical and physical changes in the local environment. Such adaptive responses involve chemical perception and information processing that transiently alter gene expression patterns so as to protect against environmental threats (Delisa and Bentley 2002).

Fermentation production of rifamycin-SV

In this study, the inoculums were enough and the cell growth enter directly to the exponential phase which compact with the stationary phase reached the maximum growth (X_{max}) of the parent strain (control) in shake flasks, 7.98 g cells l^{-1} at 72 hrs from the beginning of fermentation (Table 3 and Figure 3).

Table 2. Effect of different concentrations of benzyle alcohol on *Amy. mediterrane*-CBS 42575 to select mutant shifting rifamycin-B to Rifamycin-SV.

Benzyle alcohol concentration In agar plate (v %)	*Amycolatopsis mediterranei* CBS-42575.				
	Numbers of mutant (colony/15µl)	Final pH	TDW (mg l^{-1})	Rifamycin-B (mg l^{-1})	Rifamycin-SV (mg l^{-1})
Control	65	6.75	345	1460	3100
0.05	22	6.70	322	841	3241
0.10	12	6.65	305	761	4190
0.15	11	6.65	276	654	4465
0.20	8 -12	6.58	253	110	4679
0.25	5 -8	6.50	213	95	4896
0.30	3	6.52	203	75	3065
0.35	2	6.43	78	46	657
0.40	1	6.00	45	10	65
0.50	00	--	--	--	--
0.60	00	--	--	--	--

Table 3. Kinetics and correlations coefficient of *Amycolotopsis mediterranei CBS*-42575 and its benzyl alcohol mutant in batch Fermentation in shake flasks and fermentor.

Kinetic Parameter	*Amycolotopsis mediterranei CBS*-42575 in shake flasks	Mutant of *Amycolotopsis mediterranei CBS*-42575 tolerant to benzyl alcohol	
		shake flasks	fermentor
X_{max} (g l^{-1})	7.98	5.230	6.120
μ_{max} (h^{-1})	0.05	0.056	0.05
k_s (mg glucose l^{-1})	1.01	0.95	1.57
$Y_{x/s}$ (g cells g glucose^{-1})	0.4663	0.311	0.3675
$Y_{p/x}$ (g Rif.-SV g cells^{-1})	0.373	1.0756	0.98
$Y_{p/s}$ (g Rif.-SV g glucose^{-1})	0.151	0.2523	0.3144

At the same time, X_{max} of the mutant in the fermentor batch and shake flasks were 6.12 and 5.23 g cells l^{-1} respectively. The relationship between trophophase and ideophase varied from organism to organism and from one set of cultivation conditions to another; thus, secondary metabolism may tightly coupled to biomass accumulation, or it may be delayed until the stationary phase (Krumphanzl 1989). As expected, the carbon source (glucose) was consumed in the fermentor more than in shake flasks recorded residual values, 4.756 and 4.867 and 5.72 (g glucose l^{-1}) for the parent strain and the mutant in fermentor and shake flasks respectively. The production of rifamycin-SV was detected and determined at the beginning of the fermentation because of the inoculums and adherent to the growth of the high tolerant mutant of *A. mediterranei*-CBS and increased gradually by increasing the growth (Figure 2) reached the maximum at 96 h with values, 5.784, 4.859 and 3.126 (g rif.SV l^{-1}) for the fermentation of mutant in fermentor, shake flasks and the parent in the shake flasks respectively, after that the growth enters the decline phase and the production of rif.-SV was decreased.

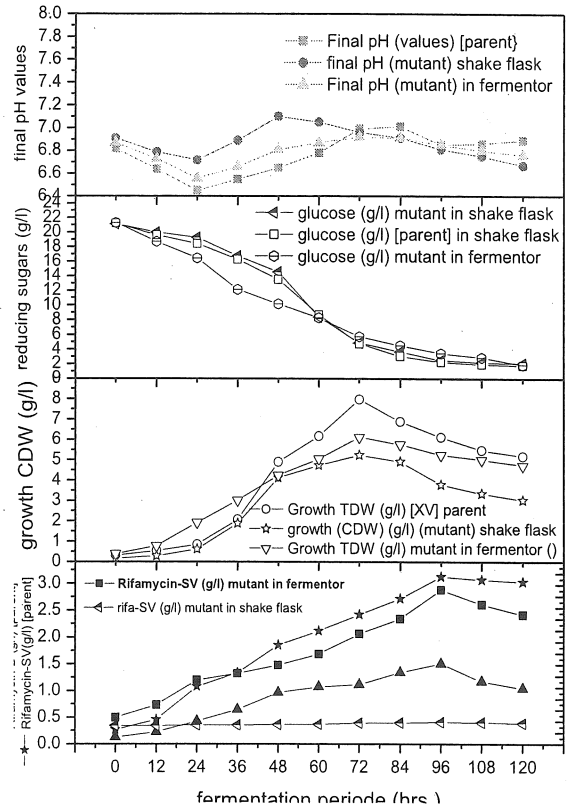


Figure 3. Effect of different period on the production of rifamycin-SV by Amycolatopsis mediterranei CBS-42575 and I ts benzyl alcohol mutant in batch fermentation using shake flasks and fermentor.

The final pH values indicated the catabolism of the ingredients in the fermentation medium by the parent and mutant strain of *A. medaterranei*-CBS, the pH was shifted from range, 6.82-6.91 towards range, 6.18- 6.66 in the first 24 h then elevated again in the second 24 h towards neutral range, 6.92-7.1 by the parent strain and the mutant in the fermentor and shake flasks respectively. Although, the glucose was decreased through the fermentation period, the pH values still steady state at neutral range through the fermentation period by the metabolic conditions. The pH values of shake flasks were more systematic than its behavior in the fermentor. The mutant can be differed than the parent strain due to its higher capacity of lipid formation this mutant was able to maintain its lipid content within a physiologically acceptable range even when more concentrations of benzyl alcohol were added to agar optimum medium (Grafe et al. 1986).

Kinetic of A. mediterranei-CBS and its benzyl alcohol mutant in batch fermentation

In batch fermentation using optimized rifamycin-SV production medium we can be compared between the parent of CBS-strain and its mutant of tolerant to benzyl alcohol, the tolerant CBS-strain of benzyl alcohol showed a classical growth trend where the used inoculums size was enough. The results in Table 3 and Figure 4 show variation between the parent *A. mediterranei*-CBS-strain and its mutant of tolerant to benzyl alcohol in batch fermentation wither in the growth behavior or kinetics. The specific growth rate (μ) of the microorganism continues decelerating. Frequently, the variation of μ_{max} was observed at the same time, 36 h of the parent *A. mediterranei*-CBS-strain, 0.0495 less than its mutant 0.056 h^{-1} in shake flasks

while the μ_{max} of the mutant in the fermentor, 0.038 h^{-1} at 48 h. until all of the available limiting substrate is metabolized before the rate has been become negative values fig. (3). Growth is no longer sustainable and the cells enter the stationary phase. At this point, the overall growth rate has declined to zero and there is no net change in cell biomass (rate of cell division equals rate of cell death). However, the microorganisms are still metabolically active, involved in metabolizing intracellular storage compounds, utilizing nutrients released from lysed cells, and in some cases producing secondary metabolites. The fermentation started where the cells entered directly to exponential growth phase which extended about 72 hrs has X_{max}, 7.98, (g cells l^{-1}) for the parent strain in the shake flask and 5.23 and 6.12 (g cells l^{-1}) for the benzyl alcohol mutant in the shake flasks and fermentor respectively, then the parent or mutant entered the stationary phase which extended about 24 h after that the decline phase took place at 96 hrs from the beginning of the fermentation. In this study Monod equation was used to express the cell growth rate Table 3, the results show the μ_{max} was 0.05 (h^{-1}) for the batch fermentation of parent strain in the shake flask, while μ_{max} was 0.065 and 0.05 (h^{-1}) for the mutant in the shake flasks and fermentor respectively.

Substrate consumption kinetic in batch fermentation

The growth concentration (dry weight) was adherent by decreasing of reducing sugars (glucose), the consumption of carbon sources was to supply cell growth, the maintenance of active cells and formation of rifamycin-SV (product).The results in table (3) indicated that the initial carbon source (glucose) was 21.2 and 20.05 (g l^{-1}) estimated as a total reducing sugars of medium contains for fermentor and shake flask respectively. The kinetic analysis resulted that the growth yield coefficient $Y_{X/S}$ values were 0.4663, 0.311 and 0.3675 (g cells g $glucose^{-1}$ l^{-1}) for the parent strain and the mutant in the shake flasks and the fermentor respectively. Also the saturation constant (Ks) recorded values, 1.01, 0.95 and 1.57 (mg l^{-1}) (Table 3) for the parent strain and the mutant in the shake flasks and the fermentor respectively. Microorganisms are able to adapt to growth at different extracellular substrate concentrations by drastically adjusting their key kinetic properties in Monod terms μ_{max} and k_s (Kovarova-Kovar and Egli 1998) and switch transport system affinity was observed for different sugars and changes can include the modification of outer membrane components (Ferenci 1996).

Kinetic of rifamycin-SV production in batch fermentation

An important parameter is the yield coefficient (Y), which is determined on the basis of the quantity of rare limiting nutrient, normally the carbohydrate source, converted into the microbial product (Table 3 and Figure 4).

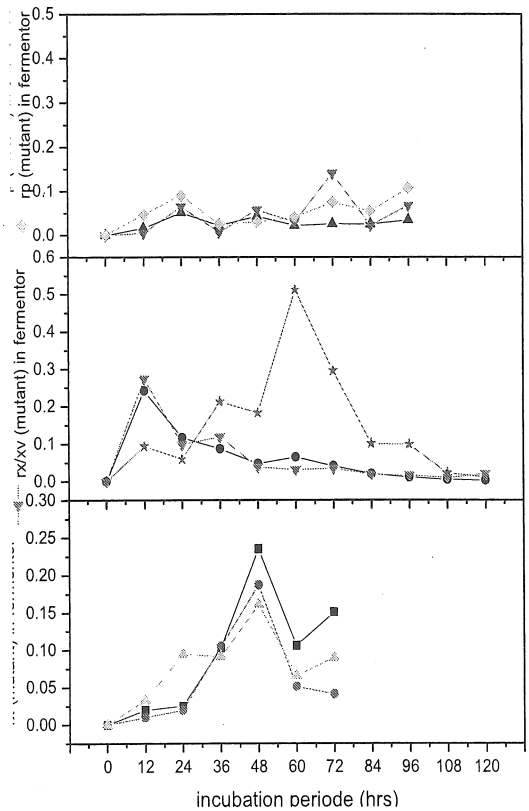


Figure 4. Kinetic parameters of rifamycin-SV production by *Amycolatopsis mediterranei* CBS-42575 and its benzyl alcohol mutant in batch fermentation using shake flasks and fermentor.

The production of rif.-SV by parent strain or benzyl alcohol mutant of *A. mediterranei*-CBS in batch fermentation was adherent to the growth and glucose consumption rate through the exponential phase, the yield coefficient of rif-SV, $Y_{P/X}$ were 0.373, 1.0756 and 0.98 (g rif.-SV g cells^{-1}) for the parent strain and the mutant in the shake flasks and the fermentor respectively. While the $Y_{P/S}$ was increased gradually recorded values, 0.151, 0.2523 and 0.3144 (g rif.-SV g glucose^{-1}) for the parent strain and the mutant in the shake flasks and the fermentor respectively. Satisfactory agreement between the resulted values and equation prediction for the production of rif.-SV through the growth rate and the carbon substrate was suitable. Although, the relation between the growth and the production of rif.-SV not enough where using the mixed carbon sources such as (glucose and starch) were effective for the growth and the production of rif.-SV from glucose only (El-Shahed and Faried 1996).

Conclusion

In strain improvement programs, a strain producing a high titer is usually the desired goal. The tremendous increases in fermentation productivity and the resulting decreases in costs have come about mainly by mutagenesis and screening / selection for higher microbial strains and the application of recombinant DNA technology (Jose and Arnold 2006). The tolerance of organic solvents can be used to select the highly potent strain for the production or shifting of rifamycins / secondary metabolites where in this case the benzyl alcohol tolerant mutant of

A.mediterranei-CBS-42575 may be affected on the physiological differentiation of its cell membrane. So, Kinetic analysis of the yield coefficient parameters was estimated within the expected production range to emphasis the stability of the benzyl alcohol mutant of *A. medaternei*-CBS-strain at different batch fermentation procedures was occurred.

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