Trends in Applied Sciences Research

ISSN 1819-3579

Academic
Journals Inc.

www.academicjournals.com

Trends in Applied Sciences Research 2 (6): 472-482, 2007
ISSN 1819-3579
© 2007 Academic Journals Inc.

Application of Fractional Factorial Design for the Development of Production Media for the Pikromycin Macrolide Family by *Streptomyces venezuelae*

Yasser R. Abdel-Fattah
Department of Bioprocess Development,
Genetic Engineering and Biotechnology Research Institute,
Mubarak City for Scientific Research and Technology Applications,
New Burg El-Arab City, Universities and Research Institute District,
P.O. Box 21934, Alexandria, Egypt

Abstract: Twelve and fourteen membered ring macrolides produced by *Streptomyces venezuelae* are characterized by their effectiveness on respiratory pathogens in comparison with erythromycin antibiotic. In the present study, the effect of culture conditions on the production of three macrolides of this family, namely: Pikromycin, methymycin and neomethymycin were evaluated. Plackett-Burman experimental design based on numerical modeling was implemented in 20 trials matrix to design a pre-optimized media for the three antibiotics using some pharama grade components in comparison with SCM medium. Among the examined culture conditions, sodium nitrate and unrefined hydrolyzed soy protein were found stimulating the production of all three macrolides. In addition, inoculum size and soluble starch showed significant increase of pikromycin production, while xylose improved production of methymycin. In all studied macrolides, higher temperature inhibited their accumulation in fermentation broth. The pre-optimized medium, based on statistical analysis, showed a production of 123 mg L^{-1} pikromycin, which is more than two times the basal SCM medium.

Key words: Macrolides production, medium optimization, Plackett-Burman design, *Streptomyces venezuelae*

INTRODUCTION

Streptomycetes and related actinomycetes continue to be prolific sources of novel secondary metabolites with a range of biological activities that may ultimately find application as anti-infectives, anti-cancer agents or other pharmaceutically useful compounds. The production of secondary metabolites by the Gram-positive mycelial streptomycetes generally coincides with, or slightly precedes, the development of aerial hyphae in surface-grown cultures. In liquid-grown cultures, it is generally confined to stationary phase and it is frequently assumed to result from nutrient limitation (Bibb, 2005).

Pikromycin was the first isolated macrolide antibiotic and got its name from an apparent bitter taste (Brockmann and Hekel, 1951). From a biosynthetic point of view, the pikromycin series includes the 12 and 14-membered ring macrolides as represented in Fig. 1. This group of macrolides has a very attractive characteristic, where they do not induce the erythromycin family of resistance that is widely distributed in respiratory pathogens (Kamimiya and Weisblum, 1997). More recently, pikromycin-related semisynthetic ketolide antibiotics have demonstrated promising potential in combating multi-drug-resistant pathogens (Agouridas *et al*., 1998). Methymycin and neomethymycin are produced by *Streptomyces venezuelae* (Donin *et al*., 1953-1954; Perlman and O'Brien, 1954), including *S. venezuelae* ATCC 15439, *S. venezuelae* ATCC 15068 and *S. venezuelae* SC 2366, while the 14-membered ring macrolides pikromycin (Brockmann and Hekel, 1951) and narbomycin (Corbaz *et al*., 1955) are

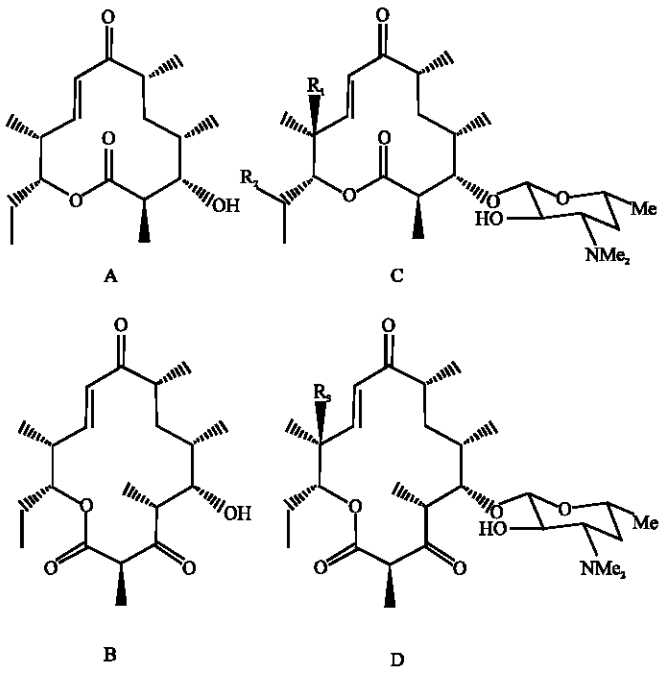

Fig. 1: Structures of 10-deoxymethynolide (A), narbonolide (B), methymycin (C, R_1 = OH and R_2 = H), neomethymycin (C, R_1 = H and R_2 = OH), pikromycin (D, R_3 = OH) and narbomycin (R_3 = H)

produced by a number of species, including *Streptomyces felleus* (NRRLB-16606), *Streptomyces flavochromogenes* (Ömura *et al*., 1976), *Streptomyces narbonensis* and *Streptomyces zaomyceticus* (Maezawa *et al*., 1973). It was later discovered that certain *S. venezuelae* strains that produce methymycin and neomethymycin also produce the 14-membered ring macrolides pikromycin and narbomycin (Hori *et al*., 1971; Maezawa *et al*., 1973), which provided the first clue that these macrolides are related in their biogenesis.

Methymycin and neomethymycin are derived from the 12-membered ring macrolactone 10-deoxymethynolide (Lambalot and Cane, 1992), while pikromycin and narbomycin are derived from the 14-membered ring macrolactone narbonolide (Maezawa *et al*., 1973). Besides the macrolactone backbone, the pikromycin series of macrolides contain a single sugar appendage, desosamine and are hydroxylated at one of several specific positions on the macrolactone ring (Fig. 1).

Streptomyces venezuelae ATCC 15439 is unusual in that it generates both 12 and 14-membered ring macrolide antibiotics, methymycin and neomethymycin in the first case and pikromycin in the other (Xue *et al*., 1998; Xue and Sherman, 2001). There are no reports on optimization of the nutritional and environmental parameters affecting the production of the different polyketides in *S. venezuelae* wild type, where only two or three media were used routinely for production purposes. As a result of the importance of this family of antibiotics, it was striving to formulate and design media for production using pharma grade components. When the target of the optimization processes is a secondary metabolite, usually slowly-metabolized nitrogen sources such as peptonized milk, cotton seed flour (Pharmamedia), skim milk and soybean meal (or soy bean flour) are favored. Thus, the task of the present work was to investigate effects of these slowly utilized nitrogen sources by the statistical optimization methods for the accurate evaluation of the fermentation physiology of

the producing microorganism. For that purpose, a Plackett-Burman fractional factorial design was implemented. Moreover, supplementation of some additions to the medium will be inspected for their effect on the productivities.

MATERIALS AND METHODS

The present study was carried out in the Department of Bioprocess Development, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Applications during the period January-May 2006.

Microorganism and Media

Streptomyces venezuelae ATCC 15439 was obtained as a freeze-dried pellet from the American Type Culture Collection (ATCC), where it was maintained and propagated at 30°C on sporulation agar plates (per liter): yeast extract 1 g; beef extract 1 g; tryptone 2 g; glucose 10 g; $FeSO_4$ trace; agar 15 g. For vegetative mycelial growth SGGP liquid medium, (per liter): Tryptone 4 g; yeast extract 4 g; $MgSO_4$ 0.5 g; glucose 10 g; glycine 2 g; potassium phosphate buffer 0.01 M, pH 7.0, was used. For the sake of comparing production with different statistical experimental design trials, macrolides production was conducted in SCM medium, which contains (per liter) the following: Bacto-Soytone 20 g; soluble starch 15 g; morpholinepropanesulfonic acid 10.5 g; yeast extract 1.5 g and $CaCl_2$ 0.1 g.

Production and Extraction of Macrolides

Cultures of *S. venezuelae*, grown in production media, for 48 h at 30°C and 200 rpm shaking, were centrifuged for 10 min at 5000 rpm and the supernatant was extracted with chloroform twice and left over night. The organic phases were combined, concentrated by rotary evaporation till dryness then dissolved in 200 μL methanol for HPLC analysis.

HPLC

For determination of pikromycin and its family, a method based on High-performance Liquid Chromatography (HPLC), Mass Spectrometry (MS) was used. Measurements were carried out on an Agilent Technologies 1100 series LC/MSD system that comprised a capillary pump, a micro-vacuum degasser, an autosampler, a thermostatted column compartment and an MSD detector. The LC-MSD system was used with an electrospray ionization source. Complete system control and data evaluation were done on an Agilent Technologies ChemStation for LC-MS. The HPLC separation was carried out on an Asahipak ODP-50, 5 μm column (125 by 2.0 mm; Agilent Technologies) with an ammonium formate buffer-acetonitrile mobile phase mixture. Electrospray ionization-MS in single-ion monitoring mode was used for detection. An authentic sample of pikromycin was kindly provided by Dr. David Sherman Laboratory (Department of Microbiology and Biological Process Technology Institute, University of Minnesota, Minneapolis, USA) and quantitative determination was done based on standard curve. For neomethymycin and methymycin the peaks were identified by mass spectrometry and peak area was used to control the quantitative measurements.

Fractional Factorial Design

Plackett-Burman experimental design (Plackett and Burman, 1946) was applied to investigate the significance of various medium components on PGA production. Fourteen culture variables were tested in two levels: −1 for low and +1 for high level based on Plackett-Burman matrix design, which is a fraction of a two-level factorial design and allows the investigation of n-1 variables in at least n-experiments. Table 1 represents the lower and higher levels of each variable. In this study, the independent variables were screened in 20 combinations according to the matrix shown in Table 2. The main effect of each variable was calculated simply as the difference between the average of

Table 1: Variables and their levels employed in Plackett-Burman design for screening of culture conditions affecting on pikromycin, methymycin and neomethymycin production by *S. venezuelae*

Variables	Variable code	Value	
		-1	1
Tryptone peptone	X_1	0	3%
SE70BT*	X_2	0	3%
Yeast extract	X_3	0	3%
CNE50M*	X_4	0	3%
WGE80M*	X_5	0	3%
$(NH_4)_2SO_4$	X_6	0.5	2.50%
$NaNO_3$	X_7	0	0.10%
Soluble starch	X_8	0.5	2.50%
Xylose	X_9	0.5	2.50%
Glucose	X_{10}	0.5	2.50%
Inoculum size	X_{11}	1	5%
Temperature	X_{12}	30°C	37°C
Aeration	X_{13}	No baffles	3 baffles
pH	X_{14}	6	8

*SE70BT is an unrefined hydrolyzed soy protein, CNE50M is a refined hydrolyzed cotton seed, WGE80M is a refined hydrolyzed wheet protein

Table 2: Plackett-Burman experimental design for evaluation of factors affecting macrolides production by *S. venezuelae*

Trials	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	Pikromycin (mg L^{-1})	Methymycin (PA)**	Neomethymycin (PA)**
1	1	1	−1	−1	1	1	1	1	−1	1	−1	1	−1	−1	8.47	2.81E+06	4.43E+06
2	1	−1	−1	1	1	1	1	−1	1	−1	1	−1	−1	−1	31.51	6.88E+06	4.01E+06
3	−1	−1	1	1	1	1	−1	1	−1	1	−1	−1	−1	−1	6.67	3.58E+06	2.14E+06
4	−1	1	1	1	1	−1	1	−1	1	−1	−1	−1	−1	1	5.21	6.62E+06	4.65E+06
5	1	1	1	1	−1	1	−1	1	−1	−1	−1	−1	1	1	6.03	2.89E+06	3.96E+06
6	1	1	1	−1	1	−1	1	−1	−1	−1	−1	1	1	−1	5.57	4.18E+06	2.51E+06
7	1	1	−1	1	−1	1	−1	−1	−1	−1	1	1	−1	1	13.84	2.15E+06	2.03E+06
8	1	−1	1	−1	1	−1	−1	−1	−1	1	1	−1	1	1	20.81	1.69E+06	2.79E+06
9	−1	1	−1	1	−1	−1	−1	−1	1	1	−1	1	1	−1	3.57	2.26E+06	2.19E+06
10	1	−1	1	−1	−1	−1	−1	1	1	−1	1	1	−1	−1	14.15	2.05E+06	2.26E+06
11	−1	1	−1	−1	−1	−1	1	1	−1	1	1	−1	−1	1	123.25	7.17E+06	6.49E+06
12	1	−1	−1	−1	−1	1	1	−1	1	1	−1	−1	1	1	12.94	5.02E+06	3.78E+06
13	−1	−1	−1	−1	1	1	−1	1	1	−1	−1	1	1	1	9.04	2.03E+06	1.43E+06
14	−1	−1	−1	1	1	−1	1	1	−1	−1	1	1	1	1	14.86	2.10E+06	2.01E+06
15	−1	−1	1	1	−1	1	1	−1	−1	1	1	1	1	−1	12.41	1.92E+06	2.78E+06
16	−1	1	1	−1	1	1	−1	−1	1	1	1	1	−1	1	12.60	2.32E+06	2.11E+06
17	1	1	−1	1	1	−1	−1	1	1	1	1	−1	1	−1	10.76	3.54E+06	3.37E+06
18	1	−1	1	1	−1	−1	1	1	1	1	−1	1	−1	1	9.38	2.72E+06	3.96E+06
19	−1	1	1	−1	−1	1	1	1	1	−1	1	−1	1	−1	152.90	2.94E+07	2.25E+07
20	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	12.20	0.00E+00	0.00E+00

**PA = Determinations and analysis was carried out based on peak area

measurements made at high setting (+1) and the average of measurements observed at low setting (−1) of that factor.

Plackett-Burman experimental design is based on the first order model (equation 1):

$$Y = \beta_0 + \Sigma \beta_i x_i \quad (1)$$

Where, Y is the predicted response, β_0, β_i are constant coefficients and x_i is the coded independent variables estimates or factors.

Analysis of Data

The data on the pikromycin, methymycin, neomethymycin production, were subjected to statistical analysis. Essential Experimental Design free software (Steppan *et al*., 1999) was used for data analysis, determination of coefficients, as well as polynomial model reduction. Factors having highest t-value and confidence level over 83% were considered to be highly significant on macrolides production.

RESULTS AND DISCUSSION

Growth and Pikromycin Production by *S. venezuelae* in SCM and SGGP Media

In a preliminary experiment, *S. venezuelae* ATCC 15439 was grown in SCM and SGGP media, the commonly used media for production of this macrolide, for 64 h to evaluate the production pattern of pikromycin in these media and determine production kinetics.

Figure 2 shows that pikromycin production started in both media in the idiophase (late exponential) where it reached its maximum after 48 h. Afterwards, pikromycin concentration was decreased in the culture supernatant of SCM medium, although it was more or less constant in SGGP medium. In general, SCM medium showed more growth and volumetric production of pikromycin (48.3 mg L^{-1}) than in SGGP medium (38 mg L^{-1}).

The kinetics of cell growth and pikromycin production in these two media showed that *S. venezuelae* grew exponentially with specific growth rate of 0.23 and 0.57 h^{-1} in SGGP and SCM media, respectively. During the exponential growth phase fair production of pikromycin was determined in both media. After 18 h of growth, pikromycin production was increased to reach its maximum volumetric production rate of 2.4 and 3.1 mg L^{-1} h^{-1} after 21 h in SGGP and SCM media, respectively. Although, the difference in the volumetric production as well as the rate of pikromycin production is not significant between these two media; the SCM medium will be used in the further experimental design as a control and comparison medium.

Evaluation of Culture Condition Affecting the Production of 12 and 14-membered Ring Macrolides in *S. venezuelae*

Screening should be performed when the investigator is faced with a large number of factors and is unsure which settings are likely to produce optimal or nearly optimal responses. Identification of the key response(s) and all possible process factors is a crucial step in experimental design

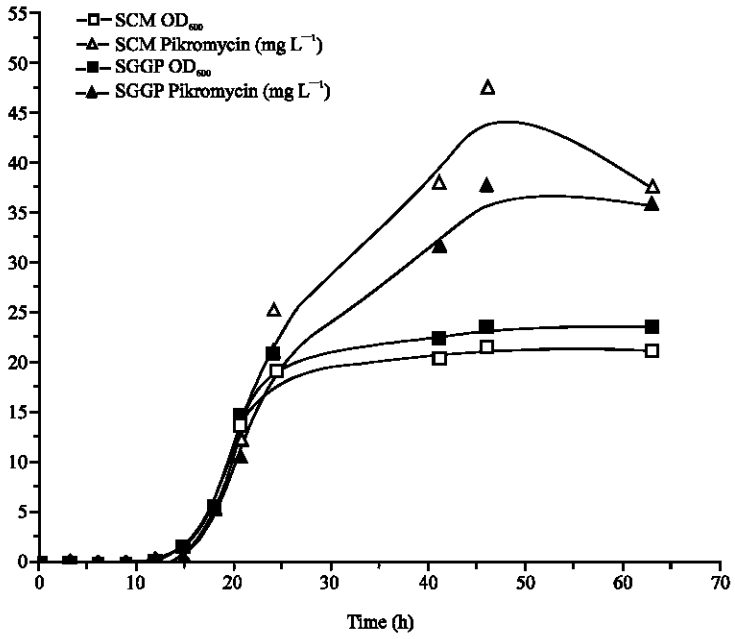

Fig. 2: Growth (OD_{600}) and pikromycin production (mg L^{-1}) by *S. venezuelae* in SCM and SGGP media

methodology. Factor level selection can be a difficult part of the experimental process. Experience, prior experimentation and literature can be valuable resources for choosing factor settings (Strobel and Sullivan, 1999).

In order to take the advantage of applying fractional factorial experiment by designing the least number of trials, it was planned to measure different responses representing two 12-membered ring

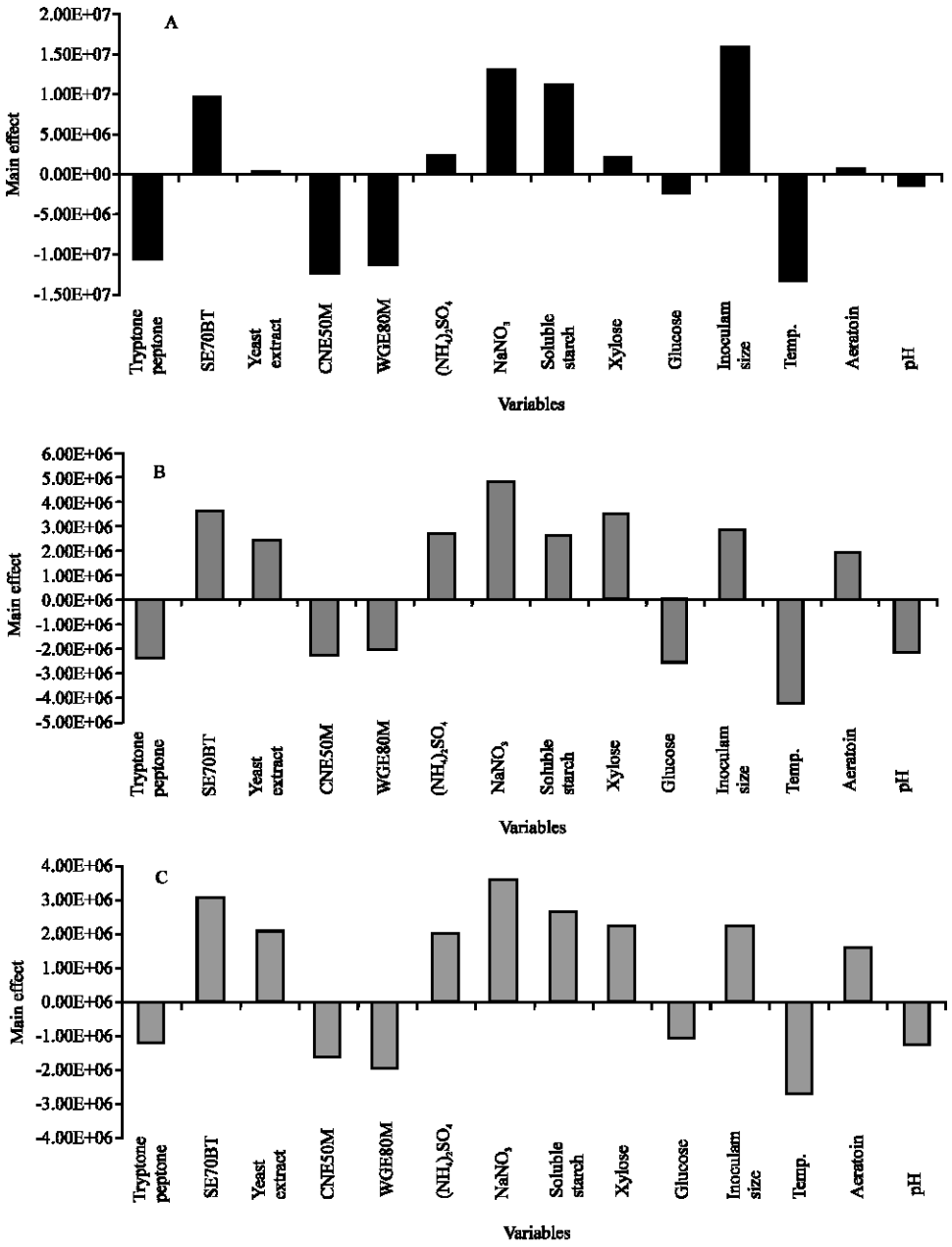

Fig. 3: Effect of environmental factors on pikromycin (A), methymycin (B) and neomethymycin (C) by *S. venezuelae* ATCC based on the results of Plackett-Burman experiment

(methymycin and neomethymycin) and one 14-membered ring (pikromycin) to evaluate the significance of different culture conditions on their co-production. In this experiment, twenty different medium formulae have been designed to examine the significance of 14 culture conditions on the production of methymycin, neomethymycin and pikromycin by applying Plackett-Burman (1946) design. Settings of the examined 14 independent variables are shown in Table 1. The experiments are carried out according to the experimental matrix presented in Table 2, where pikromycin (mg L^{-1}), methymycin and neomethymycin (PA) production are the measured responses.

A wide variation in pikromycin production results (3.57-152.9 mg L^{-1}), methymycin ($0-2.94\times10^{7}$) and neomethymycin ($0-2.25\times10^{7}$) was shown, reflecting the importance of medium optimization to attain high yield of a particular product. The main effects of examined factors on pikromycin, methymycin and neomethymycin were calculated and presented graphically in Fig. 3. The main effect of each variable is calculated simply by measuring the difference between the average of measurements made at high settings (+1) and the average of measurements observed at low settings (−1) of that factor. It is also worthwhile to mention that the main effect pattern of the three macrolides is the same with slight differences in the amplitude of variables, which supports the idea that they were produced by single pathway that includes metabolic branch.

Based on analysis of regression coefficients and t-value of 14 ingredients (Table 3), inoculum size, sodium nitrate, soluble starch and unrefined hydrolyzed soy protein were the most significant factors increasing pikromycin, whereas temperature, refined hydrolyzed cotton seed, refined hydrolyzed wheat protein and tryptone peptone were the most significant factors decreasing pikromycin production. For methymycin and neomethymycin it was found that sodium nitrate and unrefined hydrolyzed soy protein were the most significant factors increasing both 12-memberd ring macrolides, where xylose was contributed positively in methymycin production. On the other hand, the most significant factor inhibiting production of the 12-membered ring macrolides was growth temperature. It has been reported by Sujatha *et al*. (2005), that sodium nitrate was one of the most promising nitrogen sources in the production of polyketide antibiotic SBR-22. Farid *et al*. (2000) proved that sodium nitrate was the best inorganic nitrogen source for the production of natamycin antibiotic by *S. natalensis*. The positive significant effect of the unrefined soy protein hydrolysate among the tested organic nitrogen sources could be attributed to its low rate of metabolism by the producing strain. Being unrefined, i.e., containing some trace element contents; led to slowing the utilization of such

Table 3: Statistical analysis of Plackett-Burman design showing coefficient values, t and p-values for each variable

	Pikromycin (mg L^{-1})			Methymycin (PA)			Neomethymycin (PA)		
Variables	Coefficients	t-stat	p-value	Coefficients	t-stat	p-value	Coefficients	t-stat	p-value
Intercept	24.31			4.57E+06			3.97E+06		
Tryptone peptone	−10.96	−1.78	0.135	−1.18E+06	−1.07	0.335	−6.58E+05	−0.79	0.465
SE70BT	9.91	1.61	0.169	1.77E+06	1.60	0.170	1.45E+06	1.74	0.142
Yeast extract	0.26	0.04	0.967	1.17E+06	1.06	0.337	9.95E+05	1.19	0.286
CNE50M	−12.89	−2.09	0.091	−1.10E+06	−1.00	0.364	−8.59E+05	−1.03	0.350
WGE80M	−11.76	−1.91	0.115	−9.94E+05	−0.90	0.409	−1.02E+06	−1.23	0.274
$(NH_4)_2SO_4$	2.33	0.38	0.721	1.33E+06	1.21	0.281	9.47E+05	1.14	0.307
$NaNO_3$	13.34	2.16	0.083	2.32E+06	2.10	0.090	1.74E+06	2.09	0.091
Soluble starch	11.24	1.82	0.128	1.26E+06	1.15	0.304	1.28E+06	1.54	0.184
Xylose	1.90	0.31	0.771	1.72E+06	1.56	0.180	1.06E+06	1.27	0.261
Glucose	−2.22	−0.36	0.733	−1.26E+06	−1.15	0.304	−5.64E+05	−0.68	0.529
Inoculum size	16.40	2.66	0.045	1.36E+06	1.23	0.273	1.06E+06	1.28	0.258
Temperature	−13.92	−2.26	0.073	−2.11E+06	−1.92	0.114	−1.40E+06	−1.68	0.154
Aeration	0.58	0.09	0.929	9.38E+05	0.85	0.434	7.61E+05	0.91	0.403
pH	−1.51	−0.25	0.816	−1.10E+06	−0.99	0.366	−6.48E+05	−0.78	0.472

nitrogen source which in general beneficial for secondary metabolite production. Although glucose was considered as the best carbon source favoring the production of macrolide polyketides (Farid *et al*., 2000; Sujatha *et al*., 2005), in the present study it was inhibitory to the production of pikromycin family. This could be attributed to the ease of utilization of a simple carbon source like glucose that leads to more production of biomass over secondary metabolites. Desai *et al.* (2002) observed that high levels of macrolide aglycones production was attained at low pH (5.5) rather than high pH (6.5); while maximum antibiotic production was attained at pH 7.2 as claimed by Sujatha *et al.* (2005). These results are in agreement with results of this study, as lower levels of pH was favoured for the production of the three produced macrolides.

The t-test for any individual effect allows an evaluation of the probability of finding the observed effect purely by chance. Some investigators find that confidence levels greater than 70% are acceptable (Stowe and Mayer, 1966).

In these experiments, variables with confidence levels exceeding 80% were considered as significant. The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2. The determination coefficients R^2 of the full model for pikromycin, methymycin and neomethymycin were 0.87, 0.83 and 0.83, respectively.

Neglecting the insignificant terms ($p>0.17$), the model equations for the produced macrolides can be written as:

$$Y_{pikromycin} = 24.3-11\ X_1 + 9.9\ X_2-12.9\ X_4-11.8\ X_5 + 13.3\ X_7 + 11.2\ X_8 + 16.4\ X_{11}-13.9\ X_{12}$$
$$Y_{methymycin} = 4568665.3 + 1769659.3\ X_2 + 2317740.8\ X_7 + 1719664.7\ X_9 - 2114339.9\ X_{12}$$
$$Y_{neomethymycin} = 3969726.8 + 1452227.6\ X_2 + 1740575.4\ X_7 - 1397456.7\ X_{12}$$

One of the advantages of the screening designs is that they allow ranking the effects created by experimental variables, even if they were of different categories. Ranking is developed independent on either the nature of variable or its sign. Figure 4 shows the ranking of factor estimates in a Pareto plot. Factors ranking is the percent of effect of each variable, represented by the main effect, over the sum of all variables effect. The Pareto plot displays the magnitude of each factor estimate and is a convenient way to view the results of a Plackett-Burman design (Strobel and Sullivan, 1999).

Effect of DMSO on the Production of Pikromycin

Stimulatory effect of Di-methylsufoxide (DMSO) on the production of different secondary metabolites has been previously reported, where a significant enhancement in the production of microbial metabolites such as chorismate, peptide and polyketide antibiotics has been recognized (Chen *et al*., 2000).

In this experiment, the effect of DMSO on cellular productivity of pikromycin was investigated, where SCM medium was supplemented with different concentrations of DMSO (1-5%) (Fig. 5). Increasing the concentration of DMSO up to 3% led to three fold increase of pikromycin production, while more than 3% of DMSO concentration led to slight decrease of pikromycin titer in culture supernatant. Growing on solid medium supplemented with different concentrations of DMSO showed alteration of growth and spore formation.

DMSO(an antioxidant) has been demonstrated to have various physiological effects ranging from analgesic, to anti-inflammatory and in addition it is widely used as a vehicle to promote drug penetration and delivery (Jacob and Herschler, 1986). Among the known cellular effects, DMSO has been shown to modulate nuclear factor-kappa B and cytokine activation in lipopolysaccharide-treated macrophages (Kelly *et al*., 1994). These properties have been attributed to the dipolarity of DMSO. However, whether this dipolar property plays a role in its effect on secondary metabolite production by microorganisms can not be stated (Chen *et al*., 2000).

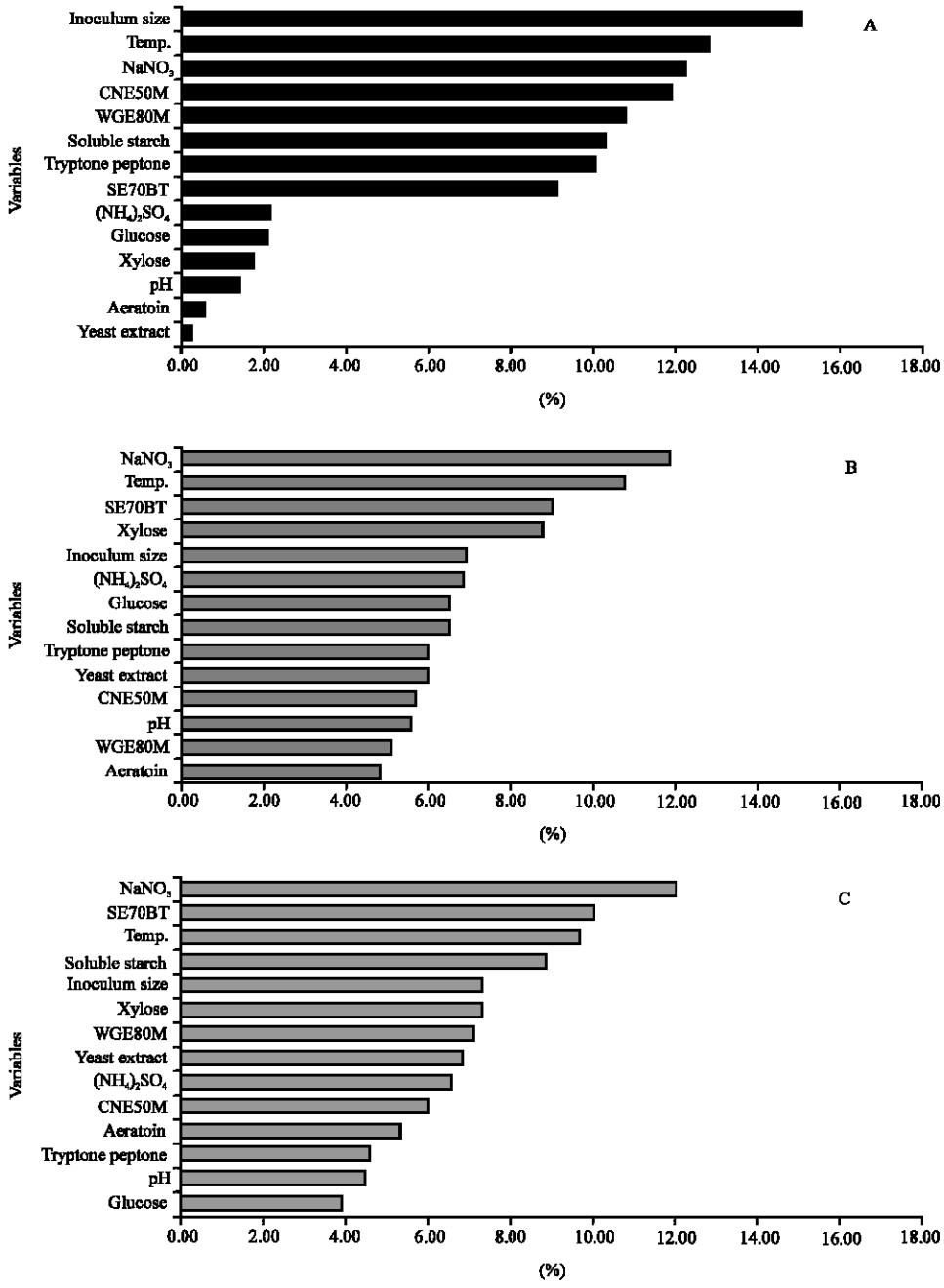

Fig. 4: Pareto plot of factors on pikromycin (A), methymycin (B) and neomethymycin (C) by *S. venezuelae* ATCC based on the results of Plackett-Burman experiment

The practical implication of this experiment is that the addition of a small concentration of DMSO as a component of the fermentation medium elicits physiological and biochemical changes in the growing cells, including secondary metabolite production. This simple enrichment strategy could have

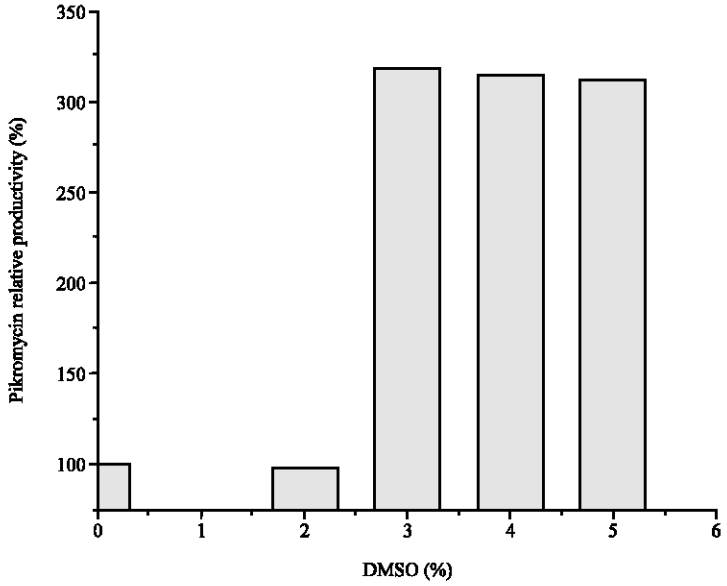

Fig. 5: Effect of different DMSO concentrations on pikromycin production after 48 h cultivation

value in expanding the available molecular diversity and increasing yields of minor metabolites from natural isolates or from genetically engineered recombinant strains.

REFERENCES

Agouridas, C., A. Denis, J.M. Auger, Y. Benedetti, A. Bonnefoy, F. Bretin, J.F. Chantot, A. Dussarat, C. Fromentin, S.G. D'Ambrieres, S. Lachaud, P. Laurin, O. Le Martret, V. Loyau and N. Tessot, 1998. Synthesis and antibacterial activity of ketolides (6-O-methyl-3-oxoerythromycin derivatives) a new class of antibacterials highly potent against macrolide-resistant and susceptible respiratory pathogens. J. Med. Chem., 41: 4080-4100.

Bibb, M.J., 2005. Regulation of secondary metabolism in Streptomycetes. Curr. Opin. Microbiol., 8: 208-215.

Brockmann, H. And W. Hekel, 1951. Pikromycin, a bitter-taste antibiotic from Actinomycetes. Chem. Ber., 84: 284-288.

Chen, G., G.Y.S. Wang, X. Li, B. Waters and J. Davies, 2000. Enhanced production pf microbial metabolites in the presence of dimethyl sulfoxide. J. Antibiotics, 53: 1145-1153.

Corbaz, R., L. Ettlinger, E. Gäumann, W. Keller, F. Kradofer, E. Kyburz, L. Neipp, V. Prelog, R. Reusser and H. Zahner, 1955. Stoffwechselprodukte von actinomyceten. Narbomycin. Helv. Chim. Acta, 38: 935-942.

Desai, R.P., T. Leaf, E. Woo and P. Licari, 2002. Enhanced production of heterologous macrolide aglycones by fed-vatch cultivation of *Streptomyces coelicolor*. J. Ind. Microbiol. Biotechnol., 28: 297-301.

Donin, M.N., J. Pagano, J.D. Dutcher and C.M. McKee, 1953-1954. Methymycin a new crystalline antibiotic. Antibiot. Annu., 1: 179-189.

Farid, M.A., H.A. El-Enshasy, A.I. El-Diwany and A.E. El-Sayed, 2000. Optimization of the cultivation medium for natamycin production by *Streptomyces natalensis*. J. Basic Microbiol., 3: 157-166.

Hori, T., I. Maezawa, N. Nagahama and M. Suzuki, 1971. Isolation and structure of narbonolide, narbomycin aglycone from *Streptomyces venezuelae* and its biological transformation into picromycin via narbomycin. J. Am. Chem. Soc. Chem. Commun., 92: 304-305.

Jacob, S.W. and R. Herschler, 1986. Pharmacology of DMSO. Cryobiology, 23: 14-27.

Kamimiya, S. and B. Weisblum, 1997. Induction of ermSV by 16-membered-ring macrolide antibiotics. Antimicrob. Agent Chemother., 41: 530-534.

Kelly, K.A., M.R. Hill, K. Youkhana, F. Wanker and J.M. Gimble, 1994. Dimethyl sulfoxide modulates NF-kappa B and cytokine activation in lipopolysaccharide-treated murine macrphages. Infect. Immunol., 62: 3122-3128.

Lambalot, R.H. and D.E. Cane, 1992. Isolation and characterization of 10-deoxymethynolide produced by *Streptomyces venezuelae*. J. Antibiot., 45: 1981-1982.

Maezawa, I., T. Hori, A. Kinumaki and M. Suzuki, 1973. Biological conversion of narbonolide to picromycin. J. Antibiot., 26: 771-775.

Õmura, S., H. Takeshima, A. Nakagawa and J. Miyazawa, 1976. The biosynthesis of picromycin using ^{13}C enriched precursors. J. Antibiot., 29: 316-317.

Perlman, D. and E. O'Brien, 1954. Microbiological production of methymycin and related antibiotics. Antibiot. Chemother., 4: 894-898.

Plackett, R.L. and J.P. Burman, 1946. The design of optimum multifactorial experiments. Biometrika, 33: 305-325.

Steppan, D., J. Werner and B. Yeater, 1999. Essential regression and experimental design software. http://www.geocities.com/SiliconValley/Network/1032.

Stowe, R.A. and R.P. Mayer, 1966. Efficient screening of process variables. Ind. Eng. Chem., 58: 36-40.

Strobel, R.J. and G.R. Sullivan, 1999. Experimental Design for Improvement of Fermentations. In: Manual of Industrial Microbiology and Biotechnology. Demain, A.L. and J.E. Davies (Eds.), ASM Press, Washington, pp: 80-93.

Sujatha, P., K.V.V.S.N. Bapi Raju and T. Ramana, 2005. Studies on a new marine Streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Stephylococcus aureus*. Microbiol. Res., 160: 119-126.

Xue, Y., L. Zhao, H.W. Liu and D.H. Sherman, 1998. A gene cluster for macrolide antibiotic biosynthesis in *Streptomyces venezuelae*: Architecture of metabolic diversity. Proc. Natl. Acad. Sci. USA., 95: 12111-12116.

Xue, Y. and D.H. Sherman, 2001. Biosynthesis and combinatorial biosynthesis of pikromycin-related macrolides in *Streptomyces venezuelae*. Metab. Eng., 3: 15-26.