Journal of Applied Pharmaceutical Science Vol. 8(02), pp 128-135, February, 2018
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8220
ISSN 2231-3354

Induction of novel mutants of *Streptomyces lincolnensis* with high Lincomycin production

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ARTICLE INFO

Article history:
Received on: 07/08/2017
Accepted on: 25/09/2017
Available online: 27/02/2018

Key words:
Improvement, Lincomycin, *S. lincolnensis*, Mutants, RAPD-PCR

ABSTRACT

Different mutants of *Streptomyces lincolnensis* were selected after mutagenesis and screened for Lincomycin production. Out of 60 obtained mutants after ultra-violet (UV) irradiation, 34 of these exhibited higher Lincomycin production (from 7.69 to 84.62%) than wild type strain. Through mutagenesis by ethyl methan sulfonate (EMS), out of 60 obtained mutants after EMS treatment 48 of these exhibited higher Lincomycin production (from 7.69 to 146.15%) than original strain. The superior mutant M-40-18 produced Lincomycin 146.15% higher than original wild strain. Furthermore, the random amplified polymorphic DNA (RAPD) protocol by polymerase chain reaction (PCR) machine on some selected superior mutants lead to correlate the genetic characteristics of these mutants with the results obtained from the Lincomycin productivities in comparison with the original strain. The variations in RAPD profiles confirmed the occurrence of genetic variation induced in *S. lincolnensis* genotype after mutagenesis.

INTRODUCTION

Actinobacteria are a group of Gram-positive bacteria which produce a variety of secondary metabolites with high pharmacological and commercial interest (Anandan *et al.*, 2016). Almost 80% of the world's antibiotics are known to be derived from Actinobacteria, mostly from the genera *Streptomyces* and *Micromonospora* (Jensen *et al.*, 1991; Hassan, 2011). Around 7600 compounds are produced by *Streptomyces* species, many of which are secondary metabolites that are potent antibiotics, which has made streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical fields (Ramesh and Mathivanan, 2009; Jensen *et al.*, 2007). *Streptomyces* species have been extensively studied since they have been recognized as important sources of antibiotics. In fact, about 60% of the antibiotics used in agriculture and horticulture have been obtained from *Streptomyces* spp. (Hwang *et al.*, 2001). Lincomycin was isolated in 1962 from a soil actinomycete found near Lincoln, Nebraska, which gave origin to its name (Stratton, 1998). The actinomycete was identified as *Streptomyces lincolnensis* var. *lincolnensis*, a new streptomycete species. Lincomycin proved to be a member of a new class of antibiotics characterised by an alkyl 6-amino-6,8-dideoxy-1-thio-D-erythro-α-D-galacto-octopyranoside joined with a proline moiety by an amide linkage (Spizek and Rezanka, 2004a). It is applied for the treatment of diseases caused by Gram-positive bacteria (Spizek and Rezanka, 2004b). Lincomycin block the growth mainly of Gram-positive bacteria. It is used in both human and veterinary medicine. Their mechanism of action is via inhibition of protein synthesis in sensitive Gram-positive microorganisms. It is inactive against Gram-negative bacteria but it's active against anaerobic bacteria and some protozoa (Spizek *et al.*, 2004a).

Many studies have been established to improve the fermentation performance of lincomycin by environmental conditions (Kuo *et al.*, 1992; Spizek and Rezanka, 2004b; Semenova *et al.*, 1994; Choi and Cho, 2004; Young *et al.*, 1985). On the other hand, other advanced studies have been established to improve the fermentation performance of lincomycin by genetic improvement protocols (Jin *et al.*,

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2002a, b). The screening of the fermentative performance after mutagenesis can be occurred to obtain the best strains for Lincomycin production (Xuewei *et al.*, 2016).

The technique of RAPD has been shown to be more benefit in detection and assay of genetic variability of the different bacterial isolates. Many studies cite the RAPD as ideal protocol for assay of genomic variability. This method has been used to compare intra- and interspecific differences in bacteria (Williams *et al.*, 1990; Ikeh 2003). Current literature did not reveal any use of mutagenesis by UV and EMS to improve Lincomycin production by *Streptomyces lincolnensis* NRRL 2936. Therefore, this work was established to take the benefit of mutagenesis in *S. lincolnensis* to obtain lincomycin overproducing strains. So, the goal of the present work was to choose of mutagenesis and RAPD protocols to enhancement the Lincomycin production by *S. lincolnensis* NRRL 2936.

MATERIALS AND METHODS

Materials

Microorganisms

The *Streptomyces lincolnensis* NRRL 2936 was obtained from Northern Regional Research Laboratories (NRRL), Peoria, IL, USA and used for lincomycin production. *Bacillus subtilis* NRRL 543 was used as the test organisms for lincomycin assay. This strain was obtained NRRL also.

Culture media

The culture of *S. lincolnensis* was maintained on spore medium slants (Zhe and Ruishen, 1998) at 28°C and was subcultured at monthly intervals.

Methods

Culture conditions and inoculum preparation

The production of Lincomycin were studied in the basal medium (Young and Kempe, 1985) consisting of: glucose 30 g/L, Na_2-Citrate. $2H_20$ 3.4 g/L, NH_4NO_3, 2.5 g/L, K_2HPO_4, 5.0 g/L, $MgSO_4$. $7H_201$ g/L, $ZnSO_4$. $7H_2O$ 1 mg/L, Fe_2SO_4. $7H_20$ 1 mg/L, and NaCl 0.5 g/L and the medium was adjusted to pH 7.2 and sterilized. For the development of inoculum, 20 ml of Seed medium (Zhe and Ruishen, 1998) broth was placed in a 50-ml Erlenmeyer flask, the pH was adjusted to 7.0, and the medium was sterilized. It was inoculated with a well-sporulated slant culture of *S. lincolnensis* (8 days old) and kept at 180 rpm on a rotary shaker for 60 h at 28°C. A 0.5-ml portion of this broth was used to inoculate 50 ml of the production medium contained in a 250-ml Erlenmeyer flask. The flasks were kept on 250 rpm a rotary shaker. Incubation temperature was 28°C.

UV-mutagenesis

The spores of *Streptomyces lincolnensis* from old slants (8 days) were suspended in sterile distilled water and exposed to UV-light for 0, 6, 12 and 15 minutes at a distance of 20 cm. After irradiation, the treated spore suspensions were protected from light for two hours. Appropriate dilutions were spread on spore medium and incubated at 28 ºC for three days. The growing colonies were transplanted on spore medium slants for the forward assay of lincomycin productivities.

EMS-mutagenesis

The spores of *Streptomyces lincolnensis* from old slants (8 days) were suspended in sodium phosphate buffer (0.1M, pH 7.0) and treated with 200 mM of EMS for 20, 40 and 60 minutes. Appropriate dilutions were spread on spore medium and incubated at 28 ºC for three days. The growing colonies were transplanted on spore medium slants for the forward assay of lincomycin productivities.

Assay of antibiotic production

The production of lincomyces of each colony was tested by using *B. subtilis* as the test organisms on Nutrient agar (NA). The antibiotic productivity was examined for the individual colony by inoculation in the broth of production medium. The incubated production medium flasks were examined for the contents of antibiotics by a paper disk diffusion assay. Standard curves were prepared with authentic Lincomycin (Table 1).

Table 1: The inhibition zone diameters using different concentrations of lincomycin.

Lincomycin concentration (µg/ml)	Inhibition zone with *B. subtilis* (mm)
120	9
240	11
410	13
530	15
650	18
850	21

Total DNA isolation from mutant strains

Total DNA from superior mutants was extracted according to i-genomic BYF DNA extraction Mini Kit, iNtRON Biotechnology Inc., South Korea. The concentration and purity of the isolated DNA were assayed according to the UV-absorbance at 260 and 280 nm using spectrophotometer according to Sambrook *et al.*, (1989).

Molecular analysis of novel mutants

Ready-To-Go PCR Beads (GE Healthcare, Lifescience, Illustra, HP7 9NA UK, Product Booklet Code: 27-9559-01) were applied for PCR protocols. Each bead contains all of the important reagents, except primer and DNA template, to run the amplification of 25 µl PCR reactions. Three different primers were used in the present study. The first primer (P1) sequence was 5'-CAT ACC CCC GCC GTT-3'. The second primer (P2) sequence was 5'-GTG TTG TGG TCC ACT-3'. The third primer (P3) sequence was 5'-TGA GTG GTC TAC GTG-3'. All primers were supplied by Operon Technologies Company, Netherlands. To each PCR bead, 12 ng of the used random primer and 40 ng of the

purified DNA sample were added. The total volume of the amplification reaction was completed to 25 µl using sterile distilled water. The amplification protocol was carried out as follows: Denaturation at 95°C for five min. Thirty-five cycles each consists of the following segments: Denaturation at 95°C for one min; primer annealing for two min. according to GC ratio of each primer and incubation at 72°C for two min. for DNA polymerization. At the end, hold the PCR at 4°C till analysis. The amplified DNA products from RAPD analysis were electrophorated on 1.0% agarose gel and 1 X TBE buffer at consistent 100 volt for about 2 hrs. The different band sizes were determined against 100 bp ladder (Vivantis # NL 1407-Malaysia) and the obtained banding profiles were stained with 0.5 µg/ml ethidium bromide and photographed using Gel Documentation System with UV Transeliminator.

RESULTS AND DISCUSSION

Mutation induction response of *S. lincolnensis*

The microorganism *S. lincolnensis* was exposed to UV light and EMS mutagen. When the Spores suspension was irritated to UV-light as mentioned previously in the adopted materials and methods. Data in Table (2) showed that the survival percentages sharply decreased gradually by increasing treatment time. Moreover, data in Table (3) showed the same trend for survival percentages after EMS mutagenesis, since the highest survival percentage was recorded following the exposure time (20 min), followed by those obtained from the spores suspensions exposed to 40 and 60 min.

Table 2: Effect of UV on viability of *S. lincolnensis* NRRL 2936.

Exposure time (min)	Survival	
	No.	%
0	1137	100.00
6	584	51.36
12	205	18.03
15	62	5.45

Table 3: Effect of EMS on viability of *S. lincolnensis* NRRL 2936.

Exposure time (min)	Survival	
	No.	%
0	816	100.00
20	155	19.00
40	39	4.78
60	28	3.43

Mutation induction and lincomycin productivity

The change in DNA nucleotide sequence is basically produced by mutation. Generally, the induction of mutations are done by the use of mutagenic agents that interact with DNA. Since, the natural mutations are rare in comparison of the induced mutations, many type of change in DNA sequence (base pair substitution, insertion, deletion) are induced randomly in a microbial DNA by the application of chemical and physical mutagenic agents. The popular mutagens used for bacterial strain improvement are UV and EMS which typically produce different of point mutations in the bacterial DNA. Mutagenesis of industrial microbial strains is widely used for the enhancement of the antibiotic production by *Streptomyces* (Thoma, 1971; El-Bondkly and Khattab, 2004; Zhihua *et al.*, 2006; Xiang-Jing *et al.*, 2009; Khattab and El-Bondkly, 2006; Khattab, 2011).

Table (4) presents the licomycin production of 20 randomly selected mutants out of the survived isolates following the exposure of *S. lincolnensis* to UV-light for 6 min compared to the untreated wild strain. These results indicated that the majority of the tested mutants produced lincomycin higher than their wild parental strain. Meanwhile, four mutants, i.e., UV-6-5, UV-6-9, UV-6-15 and UV-6-18 proved to have the same efficiency lincomycin productivity of the wild strain. On the other hand, seven mutants produced lincomycin lower than their wild parental strain. The highest lincomycin producer mutant was UV-6-13 since it showed 61.54 percent production higher than the wild strain.

Results presented in Table (5) clearly showed that, out of selected 20 mutants which was exposed to UV-light for 12 min. The results indicated that 13 out of 20 mutants produced lincomycin more than the original strain. The mutants exceeded the wild type strain with variable percentages ranged from 7.69% (UV-12-4) and up to 84.62% (UV-12-8). Moreover, other 7 mutants produced lincomycin lowers than their original parental strain.

Data in Table (6) also indicated that, in spite of no one of the tested mutants lost completely its lincomycin productivity, however, five mutants produced the lower amount of lincomycin of the wild type strain. Meanwhile, three mutants, i.e., UV-15-14, UV-15-15 and UV-15-17 proved to have the same efficiency lincomycin productivity of the wild strain. The highest lincomycin producer mutant was UV-15-6 since it showed 84.62 percent production higher than the wild strain.

Similarly, other authors have been used UV light successfully to induce mutants with improved productivity of antibiotic from *Streptomyces* (El-Bondkly and Khattab, 2004; Zhihua *et al.*, 2006; Xiang-Jing *et al.*, 2009; Khattab and El-Bondkly, 2006; Khattab, 2011). For many reasons, it appeared that UV-induced mutants were more stable through long term of generation and sub-culturing (Thoma, 1971). Also, the obtained results were in agreement with those obtained by Khattab (2011). He improved kanamycin production by *Streptomyces kanamyceticus* using UV-light mutagenesis and the results indicated that, the majority of the resistant mutants to 250 µg/ml of Kanamycin obtained after different UV-doses were produced kanamycin higher than their original parental strain. The highest Kanamycin producer mutant was K12/4 since it showed 66.67 percent production higher than the original strain.

Table 4: Lincomycin productivity of different mutants obtained following exposure of *S. lincolnensis* to UV-light for 6 min.

Mutant No.	Inhibition zone with *B. subtilis* (mm)	% from W.T
W.T	13	100.00
UV-6-1	12	92.31
UV-6-2	14	107.69
UV-6-3	9	69.23
UV-6-4	15	115.38
UV-6-5	13	100.00
UV-6-6	16	123.08
UV-6-7	9	69.23
UV-6-8	11	84.62
UV-6-9	13	100.00
UV-6-10	18	138.46
UV-6-11	8	61.54
UV-6-12	15	115.38
UV-6-13	21	161.54
UV-6-14	14	107.69
UV-6-15	13	100.00
UV-6-16	15	115.38
UV-6-17	9	69.23
UV-6-18	13	100.00
UV-6-19	16	123.08
UV-6-20	8	61.54

Table 5: Lincomycin productivity of different mutants obtained following exposure of *S. lincolnensis* to UV-light for 12 min.

Mutant No.	Inhibition zone with *B. subtilis* (mm)	% from W.T
W.T	13	100.00
UV-12-1	15	115.38
UV-12--2	18	138.46
UV-12-3	9	69.23
UV-12-4	14	107.69
UV-12-5	21	161.54
UV-12-6	15	115.38
UV-12-7	11	84.62
UV-12-8	24	184.62
UV-12-9	9	69.23
UV-12-10	20	153.85
UV-12-11	18	138.46
UV-12-12	16	123.08
UV-12-13	14	107.69
UV-12-14	11	84.62
UV-12-15	8	61.54
UV-12-16	18	138.46
UV-12-17	9	69.23
UV-12-18	22	169.23
UV-12-19	12	92.31
UV-12-20	18	138.46

Table 6: Lincomycin productivity of different mutants obtained following exposure of *S. lincolnensis* to UV-light for 15 min.

Mutant No.	Inhibition zone with *B. subtilis* (mm)	% from W.T
W.T	13	100.00
UV-15-1	18	138.46
UV-15-2	14	107.69
UV-15-3	16	123.08
UV-15-4	8	61.54
UV-15-5	16	123.08
UV-15-6	24	184.62
UV-15-7	9	69.23
UV-15-8	12	92.31
UV-15-9	11	84.62
UV-15-10	18	138.46
UV-15-11	14	107.69
UV-15-12	15	115.38
UV-15-13	16	123.08
UV-15-14	13	100.00
UV-15-15	13	100.00
UV-15-16	21	161.54
UV-15-17	13	100.00
UV-15-18	16	123.08
UV-15-19	22	169.23
UV-15-20	11	84.62

The original strain *S. lincolnensis* was treated with 200 mM of EMS for different periods as mentioned at Materials and Methods. Table (7) presents lincomycin productivity of randomly selected 20 mutants following EMS treatment for 20 min. The results presented in Table (7) showed that this treatment enhanced the lincomycin productivity up to 107.69 percent from the mutant E-20-19 over the original strain {as shown in Figure 1}. The next highest producer mutant was E-20-15 which exceeded their original strain with 92.31 percent. Moreover, extra 10 mutants exhibited higher productivity than the parental strain with percentages ranged from 7.69 (E-20-7) to 84.62 (E-20-12).

Results presented in Table (8) clearly showed that, out of selected 20 mutants which was treated with EMS for 40 min. The obtained results showed an opposite trend since the majority of the tested mutants (17 mutants) out of 20 mutants produced lincomycin more than the original strain. The mutants exceeded the wild type strain with variable percentages ranged from 7.69% (M-40-4) and up to 146.15% (M-40-18) {as shown in Figure 1}. Moreover, other 3 mutants produced lincomycin lower than their original parental strain. The results presented in Table (9) showed that the highest lincomycin productivity (130.77%) was obtained from the isolate S-60-8 (Figure 1). Meanwhile, 17 isolates produced lincomycin higher than the original strain but lower than the superior mutant S-60-8. On the other hand, one mutant (S-60-12) of the tested 20 ones

lost some of their productivity in comparison to their parental strain. Another one mutant (S-60-15) proved to have the same efficiency lincomycin productivity of the wild strain. The obtained results were in agreement with those obtained by Khattab, (2011). He improved kanamycin production by *Streptomyces kanamyceticus* using UV-light mutagenesis and the results indicated that, the majority of the resistant mutants to 250 µg/ml of Kanamycin obtained after different UV-doses were produced kanamycin higher than their original parental strain. The highest Kanamycin producer mutant was K12/4 since it showed 66.67 percent production higher than the original strain.

In general, it could be concluded that the mutagenic treatments with proved to EMS be effective over UV-light for the enhancement of the lincomycin production. Moreover, it is worthy to postulate that using EMS for 40 min. was the highest effective dose since the highest producer mutant (M-40-18) exhibited higher lincomycin productivity than their untreated wild type strain which used commercially at International level for lincomycin production, were also.

Table 7: Lincomycin productivity of different mutants obtained following treatment of *S. lincolnensis* with EMSs for 20 min.

Mutant No.	Inhibition zone with *B. subtilis* (mm)	% from W.T
W.T	13	100.00
E-20-1	18	138.46
E-20-2	11	84.62
E-20-3	13	100.00
E-20-4	11	84.62
E-20-5	8	61.54
E-20-6	13	100.00
E-20-7	14	107.69
E-20-8	20	153.85
E-20-9	16	123.08
E-20-10	16	123.08
E-20-11	18	138.46
E-20-12	24	184.62
E-20-13	21	161.54
E-20-14	13	100.00
E-20-15	25	192.31
E-20-16	11	84.62
E-20-17	21	161.54
E-20-18	16	123.08
E-20-19	27	207.69
E-20-20	18	138.46

Table 8: Lincomycin productivity of different mutants obtained following treatment of *S. lincolnensis* with EMS for 40 min.

Mutant No.	Inhibition zone with *B. subtilis* (mm)	% from W.T
W.T	13	100.00
M-40-1	23	176.92
M-40-2	25	192.31
M-40-3	22	169.23
M-40-4	14	107.69
M-40-5	18	138.46
M-40-6	18	138.46
M-40-7	16	123.08
M-40-8	15	115.38
M-40-9	18	138.46
M-40-10	9	69.23
M-40-11	27	207.69
M-40-12	28	215.38
M-40-13	11	84.62
M-40-14	11	84.62
M-40-15	30	230.77
M-40-16	18	138.46
M-40-17	16	123.08
M-40-18	32	246.15
M-40-19	15	115.38
M-40-20	26	200.00

Table 9: Lincomycin productivity of different mutants obtained following treatment of *S. lincolnensis* with EMS for 60 min.

Mutant No.	Inhibition zone with *B. subtilis* (mm)	% from W.T
W.T	13	100.00
S-60-1	25	192.31
S-60-2	16	123.08
S-60-3	14	107.69
S-60-4	21	161.54
S-60-5	22	169.23
S-60-6	18	138.46
S-60-7	27	207.69
S-60-8	30	230.77
S-60-9	28	215.38
S-60-10	24	184.62
S-60-11	16	123.08
S-60-12	11	84.62
S-60-13	16	123.08
S-60-14	15	115.38
S-60-15	13	100.00
S-60-16	14	107.69
S-60-17	21	161.54
S-60-18	25	192.31
S-60-19	18	138.46
S-60-20	24	184.62

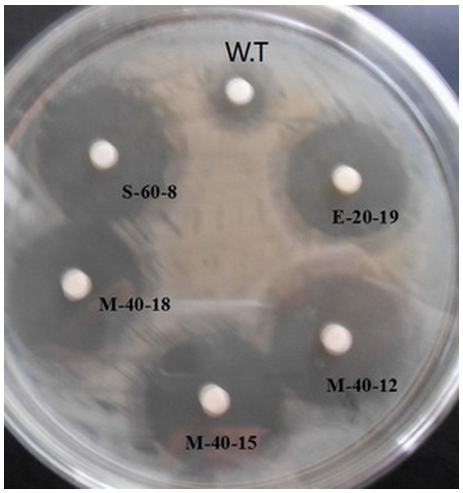

Fig. 1: Lincomycin production of *S. lincolnensis* NRRL 2936 (W.T) against superior mutant strains of E-20-19, M-40-12, M-40-15, M-40-18 and S-60-8.

Finally, we demonstrated improvement of the Lincomycin productivity (approximately over 1100 µg ml^{-1}) of the industrial strain by 3.0-fold by introducing a Lincomycin resistant mutation after EMS mutagenesis. The previous study by Krzek *et al.*, (2000) demonstrated that, the Lincomycin production of wild natural producers (10–100 µg ml^{-1}) and overproducing industrial strains (100–1000 µg ml^{-1}) can be detected by thin-layer chromatography. Also, the obtained superior mutants were higher than the *Streptomyces lincolnensis* ATCC 25466 (the wild type industrial strain) which produced 50 µg ml^{-1} of Lincomycin (Koberska *et al.*, 2008). Lee *et al.*, (2014) found that the Lincomycin Production by *S. lincolnensis* increased by 2~3 fold by optimization of cultivation medium. In addition they further increased the productivity by optimizing the culture condition (agitation speed, impeller type, pH, etc.). The highest level of lincomycin produced was 350 mg/L at 240 h under the best operating conditions. Majerčíková *et al.*, (2015) also studied the glucose effect on lincomycin production by *S. lincolnensis*. The results showed that the preculture medium application and glucose addition had positive effect, resulting in a remarkable increase in the lincomycin production.

Molecular analysis of superior mutants

An attempt was conducted to assay the genetic effects of mutagenic treatments on the DNA nucleotide sequence of the selected mutants compared to the original strain was also considered. Three 15-mer primers were applied using randomly amplified polymorphic DNA (RAPD) technique to detect the molecular variations between five Superior mutants and *S. lincolnensis* W.T. The results presented in Figure (2) presented the random amplified banding patterns of the tested mutants when primer (P1) was used. One bands with sizes of ~990 bp was detected for all tested mutants and the wild strain. Moreover, four additional bands with size of ~250, 450, 550 and 650 bp were occurred (Lane 1) for the wild strain. Three mutants (lanes, 2, 3 and 4) exhibited the same banding pattern of the wild strain. On the other hand, the mutants (lanes 5 and 6) exhibited two additional bands with size of ~300, and 700 bp and lost the band with size of ~250bp.

Using primer (P2) against the tested mutants and the wild strain (Figure 3) exhibited one bands with sizes of ~775 bp was detected for all tested mutants and the wild strain. One very faint band with sizes of 500 bp was detected for the wild strain (Lanes 1). Two very faint bands with sizes of ~300 and 550 bp were detected when the DNA of mutants (Lanes 3 and 4) were used as a templates. On the other hand, the mutants (Lanes 5 and 6) exhibited two very faint amplified bands with sizes of ~400 and 800 bp.

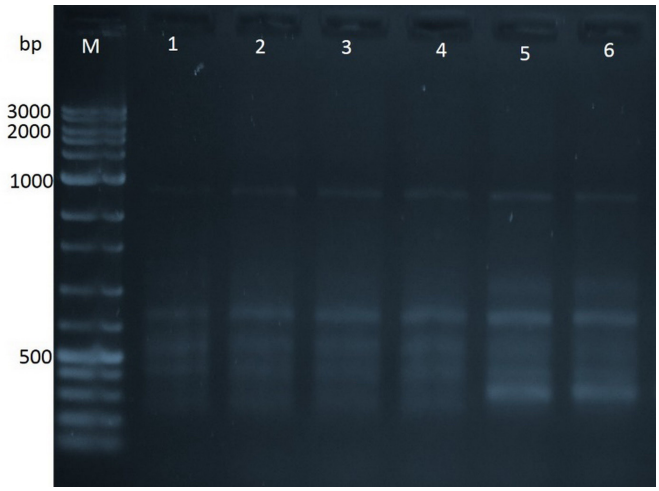

Fig. 2: DNA Photograph amplified profiles by RAPD for five best mutants (Lanes 2 to 6) and *S. lincolnensis* W.T (lane 1) using primer (P1) against VC100 bp plus DNA ladder Vivantis # NL 1407-Malaysia (lane M). Superior mutants sequence as follows: E-20-19, M-40-12, M-40-15, M-40-18 and S-60-8.

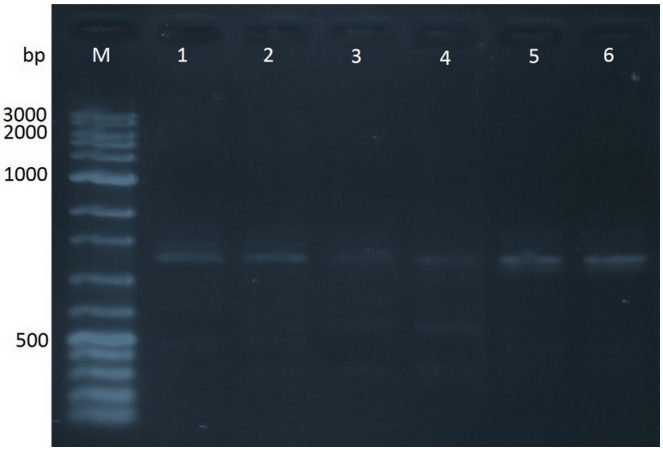

Fig. 3: DNA Photograph amplified profiles by RAPD for five best mutants (Lanes 2 to 6) and *S. lincolnensis* W.T (lane 1) using primer (P2) against VC100 bp plus DNA ladder Vivantis # NL 1407-Malaysia (lane M). Superior mutants sequence as follows: E-20-19, M-40-12, M-40-15, M-40-18 and S-60-8.

With regarding to using primer No. 3 (Figure 4), it was clearly noticed that no amplified bands were detected when the DNA of mutants (Lanes 2 and 3) and the wild strain (Lane 1) were used as a templates. Moreover, the mutant (lane 4) exhibited two very faint amplified bands with sizes of ~400 and 700 bp. Finally, amplified distinct band ~500bp was detected when the DNA of mutants (Lanes 5 and 6) were used as a templates.

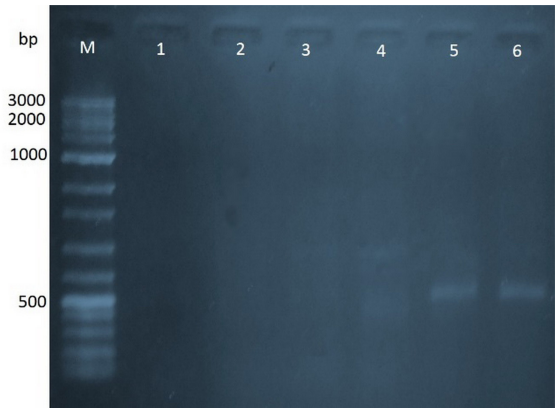

Fig. 4: DNA Photograph amplified profiles by RAPD for five best mutants (Lanes 2 to 6) and *S. lincolnensis* W.T (lane 1) using primer (P3) against VC100 bp plus DNA ladder Vivantis # NL 1407-Malaysia (lane M). Superior mutants sequence as follows: E-20-19, M-40-12, M-40-15, M-40-18 and S-60-8.

The above RAPD variabilities proved that the genetic variations after mutagenesis in *S. lincolnensis* mutants are occurred. Also, many of these variation obtained by RAPD, could be useful for assessment the genetic markers for screening of the new mutants after mutagenesis. The study results were in agreement with those obtained by Subedi *et al*., 2015. Our study is the first to examine the mutagenesis response of *S. lincolnensis* NRRL 2936 and study the probability of change in RAPD profiles in relation to the improvement of Lincomycin production.

CONCLUSION

Improvement in lincomycin production by *S. lincolnensis* mutants after mutagenesis was obtained according this work. Also, differences in some superior mutants RAPD patterns comparison with the wild type strain were obtained by the PCR protocol and these differences at the same time proved as evidence of genetic variability of *S. lincolnensis* mutant strains.

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How to cite this article:
El-Sherbini A and Khattab AA. Induction of novel mutants of *Streptomyces lincolnensis* with high Lincomycin production. J App Pharm Sci, 2018; 8(02): 128-135.