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Strain Improvement of the New Strain of *Bacillus Methylotrophicus* for Enhanced Production of Antimicrobial Metabolites

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ABSTRACT

The present study describes the screening process of a mutant strain of Bacillus methylotrophicus strain Kharuss 0103 isolated from poultry farm showing enhanced production of antibiotics and antagonistic activities against four pathogenic bacteria. The mutant was obtained by treating the new strain of bacteria with physical mutagen (UV rays). B. methylotrophicus strain Kharuss 0103 after exposed to UV were more active against B.subtilis with maximum zone of inhibition of 5 mm through Agar well diffusion method. There was no change in zone of inhibition for E.coli before and after the mutation of this new strain. This result indicated that mutation of this strain is recommended for various applications especially as an antibacterial agent against human pathogens.

Keywords : Agar well diffusion method, B. methylotrophicus strain Kharuss 0103, Mutation, Mutant strain

INTRODUCTION

In the present scenario, drug resistant to human pathogenic bacteria has been commonly reported from all over the world (Piddock KJV *et al*., 1989; Davis J *et al*., 1994). This situation is becoming alarming due to indiscriminate use of antibiotics. It has become essential to search for new antimicrobial agents from other sources. Antibacterial substances are widely produced by the microorganisms including the genera *Bacillus, Penicillium, Streptomyces* etc which produces more than 5000 antibiotics (Todar K *et al*., 2002). Bacteria are responsible for producing various metabolites including antibiotics. Antibacterial compounds isolated from bacteria are inhibitory to many other bacterial strains, which are of considerable ecological significance (Saz A *et al*., 1963). *Bacillus* is the largest antibiotic producing genus having both antibacterial and antifungal compounds. *Bacillus* species produces wide range of cyclic lipopeptides which are active against different microorganism (Kim HS *et al*., 2003). *B.subtilis, B. licheniformis* and *B. amyloliquefaciences* are well studied for their antagonistic activity and production of antibiotics. But there are less report on the antagonistic activity of *B. methylotrophicus*. In the quest of new antibacterial agent, the present study was undertaken to isolate the new strain of *B. methylotrophicus* from poultry farm and to check its antibiotic production efficiency andantagonistic activity against human pathogens by mutation method.

MATERIALS AND METHODS

Sample collection and isolation

Samples (surface soil) were collected from poultry farm of Guduvanchery, Tamil Nadu (India) and were serially diluted in aseptic condition. The isolated bacteria were preserved in slants at 4±2°C.

Identification of bacteria

Identification of bacteria as a new strain (B. *methylotrophicus* strain Kharuss 0103) was done by Morphological, Biochemical and 16S rRNA gene sequencing.

Maintenances of bacterial cultures

E.coli, B.subtilis, Pseudomonas aeruginosa and *Staphylococcus aureus* were obtained from Department of Plant Biology and Biotechnology, Loyola College, Chennai (India) and were preserved in slants after subculturing at 4±2°C.

Preliminary screening of antibacterial activities

Preliminary screening for antibacterial activity of the isolate was checked by cross-streak method. *B. methylotrophicus* strain Kharuss 0103 was streaked as a single line on Nutrient agar and were incubated at 37°C. After incubation period, the overnight grown cultures (*E.coli, B.subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*) were cross streaked perpendicular to the line of growth of *B. methylotropicus* strain Kharuss 0103. After that the plates were incubated at 37°C for 24 hours. Absence of growth adjacent to *B. methylotrophicus* strain Kharuss 0103 indicated inhibition of target culture. *B. methylotrophicus* strain Kharuss 0103 was inoculated in Nutrient broth and was kept in shaker for 24 hours. The culture was centrifuged at 5000 rpm and supernatant was used for testing the antimicrobial activity. The pathogens were swabbed on the Mueller-Hinton agar plates and supernatant was tested by Agar well diffusion method. After 24 hours, the zone of inhibition for each strain was measured.

Affect of mutation (UV irradiation) on antimicrobial activity

For UV irradiation method of Parekh *et.al* (2000) was adopted. 3 ml of overnight broth culture of *B. methylotropicus* strain Kharuss 0103 were exposed to UV irradiation at a distance of 30 cm for 3 minutes. 1 ml of exposed cultures was transferred to 9 ml of Nutrient broth and the tubes were incubated for 24 hours in a shaker. After incubation, tubes were removed and the broth was centrifuged at 2000 rpm for 10 minutes. The supernatant was used to examine the post mutation effect on the strain for the antibacterial activity.

RESULT AND DISCUSSION

A moderate level of antibacterial activity of *B. methylotrophicus* strain Kharuss 0103 were obtained against four pathogens (Table 1). Highest activity with 3 mm of zone of inhibition was shown for *B. subtilis*. Minimum zone of inhibition of 1 mm was shown against *Staphylococcus aureus*. The strain

B. methylotrophicus showing antibacterial activity against tested pathogen was treated with physical mutagens (UV irradiation) to study the effect of mutation on their antibacterial activity. After exposure to UV radiation, a variation in zone of inhibition against all the human pathogens except *E.coli* were seen (Table 2). As compared to control (Fig-a), UV mutated strain at 3 minutes showed an increased trend in the inhibition against *B.subtilis* (+2 mm), *Pseudomonas aeruginosa* (+1 mm) and *Staphylococcus aureus* (+1 mm) (Fig.-b). There was no change in zone of inhibition (2 mm) for *E.coli* before and after mutation. Classical strain improvement for years has allowed for the selection of strains, which are probably altered in the gene regulation and have increased ability to over produce secondary metabolites (Radha Krishna E *et al*., 2011). The zone of inhibition for *B.subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* were increased upon exposure to physical mutagen. This may be due to the activation of genes after mutation which is responsible for the production of antibiotics and its antagonistic activity. Similar results were obtained by Calam C (1964). In our investigation *B. methylotrophicus* strain Kharuss 0103 were more active against *B.subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*. But the activity was less for *E.coli*. This finding favors the work of Katz and Demain (1977) who demonstrated that most *Bacillus* antibiotics are active against gram(+) bacteria. Yan *et al* (2011) observed that *B. methylotrophicus* showed antimicrobial activity against some pathogenic bacteria. In our study *B. methylotrophicus* strain were also found to be effective against human pathogens. Secondary metabolite from microorganism having a diverse chemical structure and biological activities are produced only by some *Bacillus* species (Stachelhaus *et al*., 1995).

CONCLUSION

The finding of present investigation led to conclude that antibiotics production and antagonistic activity of *B. methylotrophicus* strain can be enhanced by mutation. Mutants of this strain are recommended for various applications. This property indicates the possibilities of its use as a potential antibacterial agent against human pathogen.

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TABLE:1. Antimicrobial activity of *B. methylotrophicus* strain Kharuss 0103.

S.NO	Test organisms	Zone of Inhibition (mm)
1	E.coli	2 mm
2	Bacillus subtilis,	3 mm
3	Pseudomonas aeruginosa	2 mm
4	Staphylococcus aureus	1 mm

TABLE:2. Antimicrobial activity of *B. methylotrophicus* strain Kharuss 0103 after exposing to UV.

S.NO	Test organisms	Zone of Inhibition (mm)
1	E.coli	2 mm
2	Bacillus subtilis,	5 mm
3	Pseudomonas aeruginosa	3 mm
4	Staphylococcus aureus	2 mm

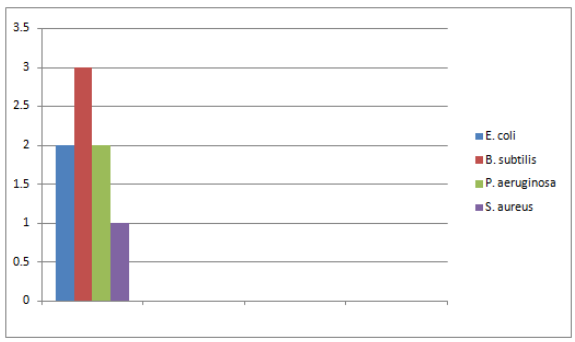

Fig-a: Antimicrobial activity of *B. methylotrophicus* strain Kharuss 0103 against human pathogens (in mm).

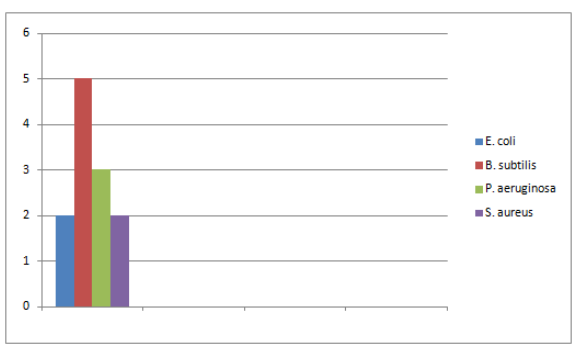

Fig-b: Antimicrobial activity of *B. methylotrophicus* strain Kharuss 0103 after exposing to UV (in mm).

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