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Optimum Condition of Marine Actinomycetes, *Streptomyces* sp. NS 13239 for Growth and Producing Antibiotics

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In previous study, marine actinomycetes producing the antibiotics were investigated to invent new antibiotics from east coast of Korea. The optimum growth conditions of *Streptomyces* sp. NS 13239 were 28℃, pH 7.0 and 3% of NaCl concentration in various media. *Streptomyces* sp. NS 13239 showed strong antimicrobial activity against gram-positive bacteria, specially Methicillin resistant *Staphylococcus aureus* (MRSA), but just weak antimicrobial activity against yeasts and mold. On the other hand, it did not show antimicrobial activity against gram-negative bacteria. The optimum conditions for producing antibiotics were almost consistent with optimum growth conditions except carbon source and nitrogen source.

Key words: Marine actinomycetes, Antibiotics, Antimicrobial activity, Methicillin resistant *Staphylococcus aureus* (MRSA)

Introduction

Since the discovery of penicillin by Fleming (1926), much of the effort has focused on new antibiotics and many antibiotics have invented (Molloy et al., 1971; Ishimura et al., 1983; Kim et al., 1984; Mitsuaki et al., 1988). Fukuda et al. (1990) had isolated antibiotics A80915 with strong antimicrobial activity on gram-positive bacteria from *Streptomyces aculeolatus*. Ubukata et al. (1995) had isolated antibiotics RS-22 A, B and C with antimicrobial activity on molds and gram-positive bacteria from *Streptomyces violaceusniger*. In recent, the anticarcinogenic agents are also isolated from actinomycetes (Zhang et al., 1988; Lam et al., 1990; Tsukamoto et al., 1998).

These antibiotics have been contributed to sterilize pathogens and cure the epidemic diseases, but the abuse of antibiotics caused the drug-resistant bacteria such as Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Staphylococcus aureus* (VRSA) and Vancomycin Resistant *Enterococcus* (VRE). They, especially multi-drug resistant bacteria, have brought serious problems to the medical and the food industry (Knapp et al., 1987; Chambers, 1988). Therefore, new antibiotics that are both highly effective and safe might be of utmost importance for the eradication of the multi-drug resistant bacteria.

In previous paper, we reported that the *Streptomyces* sp. NS 13239 with antimicrobial activity was isolated from marine environment (Shin et al., 20 01). Based on the paper, we further investigated the optimum growth condition for antibiotic production.

Materials and Methods

Media

The Modified Bennett's medium (David, 1976) was used for effects of temperature, pH and NaCl concentration on growth and antibiotic production of *Streptomyces* sp. NS 13239. The Pridham-Gottlieb's basal medium (David, 1976) was used for test of utilization of carbon and nitrogen source. Mueller Hinton medium (Difco) and YM medium (Difco) were used for measurement of antimicrobial activity against bacteria and fungi, respectively.

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Test strain

For the measurement of antimicrobial activity, we used microorganisms that were *Escherichia coil* ATCC 25922, *Vibrio parahaemolyticus* ATCC 2210001, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, three strains of Methicillin Resistant *Staphylococcus aureus* (MRSA), *Aspergillus oryzae* ATCC 11489, *Candida albicans* IPL 76, and *Saccharomyces cerevisiae* CBS 1200. Three strains of MRSA were obtained from hospital of Inje University.

Preparation of crude antimicrobial agent

The *Streptomyces* sp. NS 13239 was inoculated in 100 mL of YM medium and incubated at 28℃ of shaking incubation for 7 days. The culture was centrifuged at 3,000×g for 15 min. The supernatant was concentrated by rotary evaporator (Buchi, 140B, Germany) and then filtered with membrane filter (pore size, 0.45 μm; Millipore Co., Germany). The filtrate was used for crude antimicrobial agents.

Measurement of antimicrobial activity

The antimicrobial activity was measured with paper disk method (Acar and Goldstein, 1991) and defined as the diameter of clear zone around paper disk. Twenty microliters of the crude antimicrobial agent was inoculated on paper disk (φ8 mm).

Optimum conditions for growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239

The optimum conditions of temperature, pH, NaCl concentration, carbon and nitrogen sources for growth of *Streptomyces* sp. NS 13239 were decided by dry weight of the culture (100 mL) after 7 days of incubation.

The optimum conditions for production of antimicrobial agent of *Streptomyces* sp. NS 13239 were decided by paper disk method using MRSA.

The optimum carbon and nitrogen sources were decided by inoculation of *Streptomyces* sp. NS 13239 to Pridham-Gottlieb's basal medium with 2% of each carbohydrate or nitrogen, respectively.

Results and Discussions

Optimum conditions for growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239

1. Temperature

The optimum temperature for growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239 were shown in Fig. 1.

The *Streptomyces* sp. NS 13239 was able to grow at 10 to 40℃ and the reasonable temperatures for growth were 25℃ and 30℃. The optimum temperature for production of antimicrobial agent was 30℃ with 18 mm of clear zone.

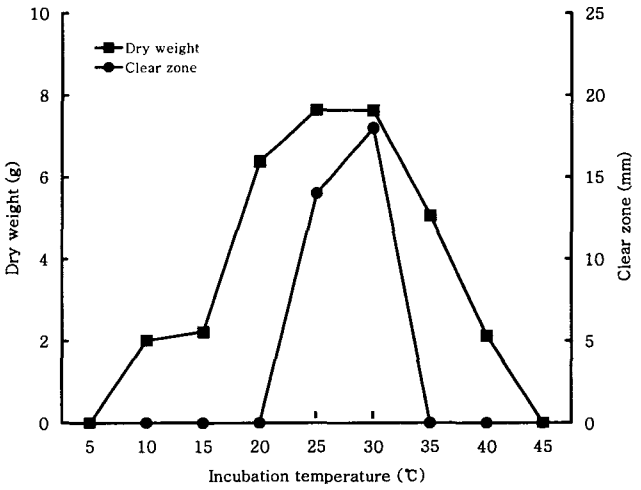

Fig. 1. Effects of temperature on the growth and antimicrobial activity of *Streptomyces* NS 13239 against MRSA by paper disk method.

2. Incubation period

The effects of incubation period on growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239 were shown in Fig. 2.

Two grams of dried weight was obtained on day 1st and its weight was significantly increased on day 7th. The antimicrobial agent was begun to be produced on day 3rd and it showed the highest antimicrobial activity on day 7th.

3. pH

Streptomyces sp. NS 13239 was able to grow at pH 4 to 10 and also showed the antimicrobial activity at the same pH range. The optimum pH for growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239 was 7 (Fig. 3).

4. NaCl concentration

The effect of NaCl concentration on growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239 were shown in Fig. 4.

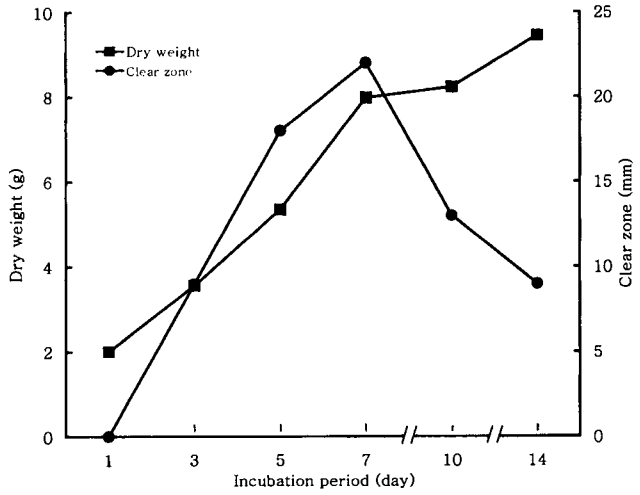

Fig. 2. Effects of incubation period on the growth and antimicrobial activity of *Streptomyces* NS 13239 against MRSA by paper disk method.

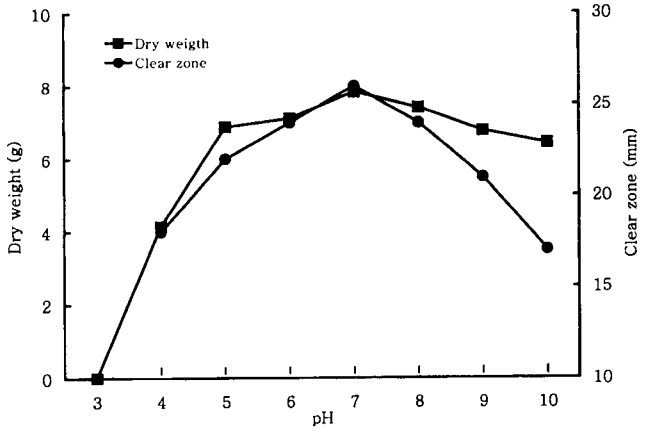

Fig. 3. Effects of pH on the growth and antimicrobial activity of *Streptomyces* NS 13239 against MRSA by paper disk method.

Streptomyces sp. NS 13239 was able to grow at 0 to 8% of NaCl concentration. The production of antimicrobial agent was little different by NaCl concentration. The optimum NaCl concentration for growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239 was 3%. These results suggest that this strain was originated from marine environment or adapted itself to marine environment.

5. Carbon source and nitrogen source

The effects of carbohydrates on growth and production of antimicrobial agent of *Streptomyces* sp. NS 13239 were shown in Fig. 5.

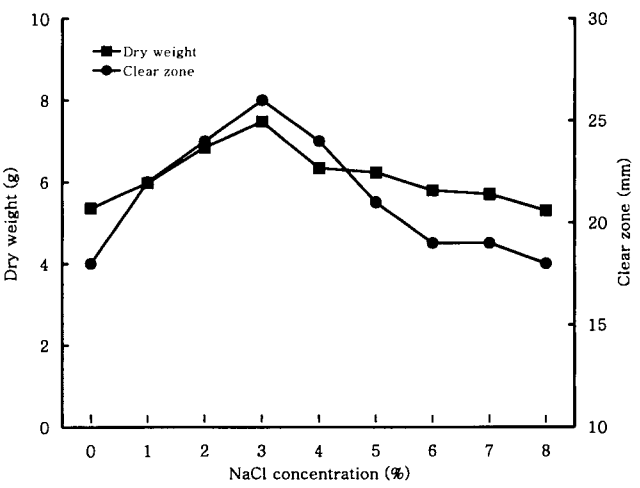

Fig. 4. Effects of NaCl concentration on the growth and antimicrobial activity of *Streptomyces* NS 13239 against MRSA by paper disk method.

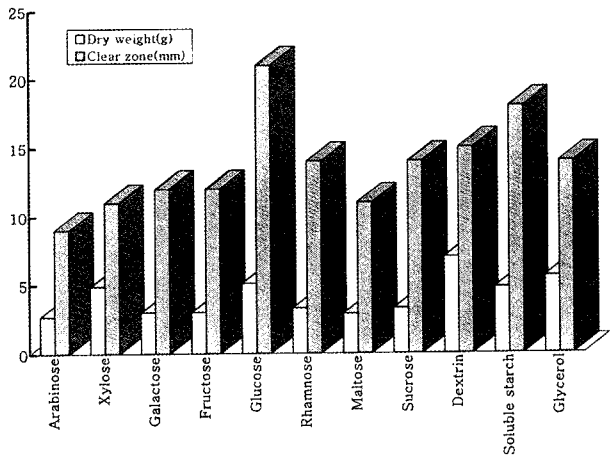

Fig. 5. Effects of carbon sources on the growth and antimicrobial activity of *Streptomyces* NS 13239 against MRSA by paper disk method.

Streptomyces sp. NS 13239 was able to grow very well in medium containing xylose, glucose, dextrin, soluble starch or glycerol, but not arabinose or galactose. The antimicrobial agent was produced well in medium containing glucose or soluble starch, but not arabinose or maltose.

In case of nitrogen sources, *Streptomyces* sp. NS 13239 was able to grow very well in medium containing yeast extract, beef extract and peptone, but not urea. The antimicrobial agent was also produced well in medium yeast extract or beef extract, but not urea (Fig. 6).

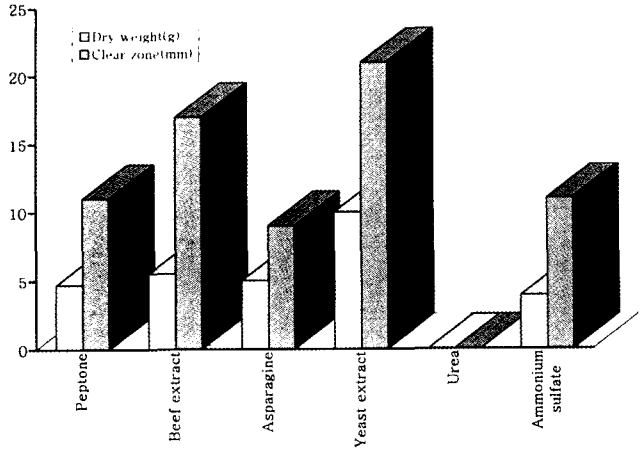

Fig. 6. Effects of nitrogen sources on the growth and antimicrobial activity of *Streptomyces* NS 13239 against MRSA by paper disk method.

Antimicrobial acitivity of *Streptomyces* sp. NS 13239

Antimicrobial activity of *Streptomyces* sp. NS 13239 in optimum condition for production of antimicrobial agent was shown in Table 1.

Table 1. Antimicrobial activity of antibiotic produced by *Streptomyces* NS 13239

Test strains	Clear zone (mm)*
Escherichia coli ATCC 25922	0
Vibrio parahaemolyticus ATCC 2210001	0
Staphylococcus aureus ATCC 25923	28
Bacillus subtilis ATCC 6633	21
Methicillin Resistant *S. aureus* 483	16
S. aureus 716	18
S. aureus 767	17
Aspergillus oryzae ATCC 11489	14
Candida albicans IPL 76	11
Saccharomyces cerevisiae CBS 1200	12

* Clear zone was measured by paper disk method (ϕ 8 mm).

The antimicrobial agent from *Streptomyces* sp. NS 13239 showed strong activity against gram-positive bacteria and a little weak activity against fungi, but not gram-negative bacteria. Especially, it showed strong antimicrobial activity against MRSA. These results support ability to invent some noble antibiotics.

Acknowledgements

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