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Received 10 April 2006; revised accepted 22 June 2006

Secretion of a potent antibiotic by salt-tolerant and alkaliphilic actinomycete *Streptomyces sannanensis* strain RJT-1

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An alkaliphilic actinomycete, *Streptomyces sannanensis* strain RJT-1 was isolated from the alkaline soil of Saurashtra University Campus, Rajkot. The isolate exhibited optimal growth at 5% (w/v) NaCl and pH 9. It was Gram-positive, having filamentous, long thread-like structure and started sporulation after 3 days of incubation. It was capable of producing antibiotic against Gram-positive bacteria, while Gram-negative organisms were not affected. Starch agar was the preferred medium for antibiotic production. Optimum salt and pH for antibiotic production were 3% (w/v) NaCl and 9 respectively. Studies on the nutritional factors showed that the highest antibacterial activity was obtained when glucose and lactose at 1% (w/v) were used as carbon sources. *S. sannanensis* strain RJT-1 displayed higher antibiotic production with inorganic nitrogen sources such as urea, $(NH_4)_2SO_4$ and NH_4NO_3 compared to organic nitrogen supplements. So far only few alkaliphilic actinomycetes have been explored for their antimicrobial potential.

Keywords: Antibiotics, antimicrobial potential, secretion, *Streptomyces sannanensis*.

MICROBES from extreme environments have attracted considerable attention in recent years. This is primarily due to the secret that they hold about the molecular evolution of life and stability of the macromolecules. Majority of the studies on extremophilic organisms, however, have been confined to extremophilic bacteria and actinomycetes are relatively less explored group. A novel, alkaliphilic actinomycetes *Nocardiopsis alkaliphila* sp. nov. was reported[1] to grow optimally at pH 9.5–10. *Nacardiopsis metallicus*[2] exhibited growth in the pH range 7–10.5, whereas in *Bogoriella caseilytica*[3], a new alkaliphilic actinomycete, optimum growth occurred at pH values 9–10. It is widely accepted that alkaliphilic actinomycetes will provide a valuable resource for novel products of industrial interest, including enzymes and antimicrobial agents[4,5]. Pyrocoll, an antibiotic, antiparasitic and antitumour compound was recently detected in novel alkaliphilic *Streptomyces* strain[6]. A new antitherapeutic antibiotic, fattiviracin was detected in *Streptomyces microflavus*[7]. Moreover, few marine actinomycetes have also been recently reported for their antimicrobial activity[8–10]. Some novel antitumour antibiotics, such as chinikomycin and lajollamycin were detected in marine *Streptomyces* spp.[11,12]. These recent examples from the literature highlight the fact that despite extensive exploration of the actinomycetes for their antimicrobial products in the past, the search for novel molecules having unique therapeutic properties continues to be an active area of research. In this context, studies on extremophilic actinomycetes would be a valuable addition. The present communication deals with the isolation and characterization of an alkaliphilic and salt-tolerant actinomycetes, identified as *Streptomyces sannanensis* strain RJT-1. The study focuses on the production of an antibiotic from this organism as a function of salt and pH.

An alkaliphilic strain was isolated from the alkaline soil, collected from the Saurashtra University Campus, Rajkot. Ten grams of alkaline soil was mixed with 1.0 g of $CaCl_2$ and incubated at 45°C. After 7 days, 1 g of the treated soil was added to 25 ml of sterile Actinomyces broth (Hi Media Ltd) containing 5% w/v NaCl. The final pH of the medium was adjusted to 9 by adding separately sterilized 20% Na_2CO_3. The inoculated broth was incubated at 28°C

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under shaking conditions for 4–5 days. The enriched culture was streaked on Actinomyces agar plates (5% w/v NaCl, pH 9). After incubation at 28°C for 6 days, a typical chalky white colony was picked and subcultured until purification. The pure culture was maintained on Actinomyces agar slants (5% w/v NaCl, pH 9) at 4°C. The organism was Gram-positive, having a long filamentous structure. Aerial mycelium was white, whereas substrate mycelium was yellow to yellowish-brown. The organism was identified as *S. sannanensis* based on morphological, physiological and biochemical characteristics by Microbial Type Culture Collection and Gene Bank, IMTECH, Chandigarh. The assigned accession number is MTCC 7038.

S. sannanensis strain RJT-1 could grow up to 7% NaCl concentration with pH 9. It was capable of growing up to 42°C. LL-DAP was present in the cell wall and the organism could utilize glucose, mannitol, galactose, fructose and meso-inositol as the carbon source along with acid production; however, arabinose, xylose, raffinose, rhamnose, salicin and sucrose were utilized by the organism without the production of acid (Table 1). H_2S production and nitrate reduction tests were positive, but methyl red test, Voges-Proskaur test and Indole production were negative for RJT-1. The organism produced certain extracellular enzymes like amylase, protease, and lipase and cellulase.

Antimicrobial activity of *S. sannanensis* was detected using nutrient agar medium (5% w/v NaCl, pH 9). *S. sannanensis* was spotted on nutrient agar and incubated for 4 days till the beginning of sporulation. Thereafter, the molten nutrient agar with activated test culture, i.e. Gram-positive organisms such as *Staphylococcus aureus*, *Bacillus cereus*, *B. megaterium, B. subtilis* and Gram-negative organisms such as *Escherichia coli*, *Proteus vulgaris*, *Shigella dysentry*, *Pseudomonas aeruginosa* and *Salmonella typhosa para B*, was poured on already grown *S. sannanensis*. After incubation for 24 h at 37°C, the zone of inhibition was measured for each test organism. *S. sannanensis* produced antibiotic selectively against Gram-positive organisms, i.e. *S. aureus*, *B. cereus*, *B. megaterium*, *B. subtilis* (Figure 1). However, it did not affect the growth of Gram-negative organisms. Among the Gram-positive organisms, *S. aureus* was more sensitive to the antimicrobial agent; hence fur-

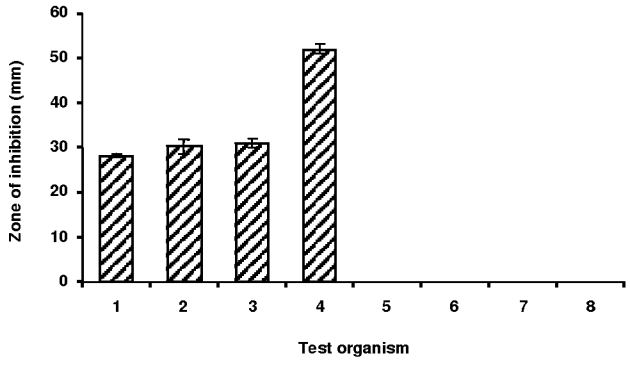

Figure 1. Antibiotic production by *Streptomyces sannanensis* against various test organisms (1, *Bacillus subtilis*; 2, *B. cereus*; 3, *B. megaterium*; 4, *Staphylococcus aureus*; 5, *Escherichia coli*; 6, *Proteus vulgaris*; 7, *Pseudomonas aeruginosa*; 8, *Shigella dysentry*).

Table 1. Biochemical tests for identification of RJT-1

Test	Result
Gram's stain	+
Spore staining (endo spore)	–
Cell shape	Mycelial
Pigments	–
H_2S production	+
MacConkey agar growth	–
Fluorescence	–
Motility	–
Catalase	–
Oxidase	–
Methyl Red test	–
Voges–Proskaur test	–
Indole production	–
Citrate utilization	+
Starch hydrolysis	+
Casein hydrolysis	+
Gelatin hydrolysis	+
Oxidation–fermentation	–
Nitrate reduction	+
Anaerobic growth	–
Urease	+
Growth on	
2% NaCl	+
5% NaCl	+
7% NaCl	+
9% NaCl	–
Growth on	
5 pH	–
8 pH	+
9 pH	+
Growth at	
4°C	–
15°C	+
25°C	+
28°C	+ (Optimum)
37°C	+
42°C	+
50°C	–
Acid production from	
Glucose	+
Arabinose	–
Mannitol	+
Xylose	–
Meso-inositol	+
Raffinose	–
Rhamnose	–
Salicin	–
Sucrose	–
Galactose	+
Fructose	+
Cell-wall amino acids	LL-DAP
Cell-wall sugars	No diagnostic

+, Positive reaction; –, Negative reaction.

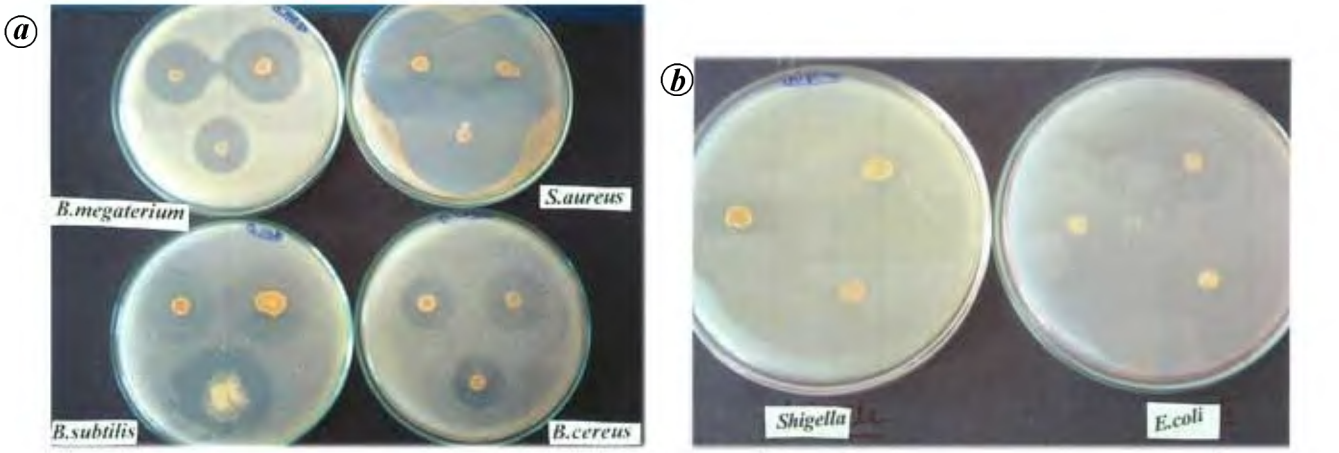

Figure 2. Antibiotic production by *Streptomyces sannanensis* against Gram-positive (*a*) and Gram-negative (*b*) organisms.

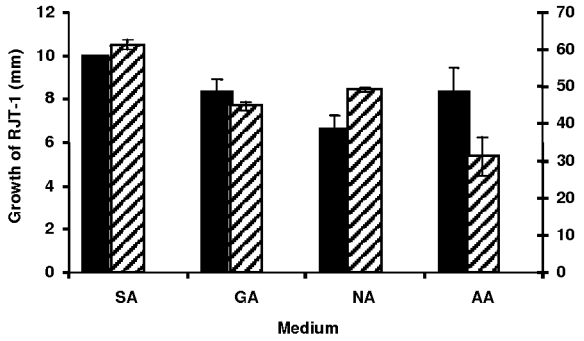

Figure 3. Effect of various media on growth (■) and antibiotic production (▨) by *S. sannanensis* (NA, Nutrient agar; SA, Starch agar; GA, Gelatin agar; AA, Actinomyces agar).

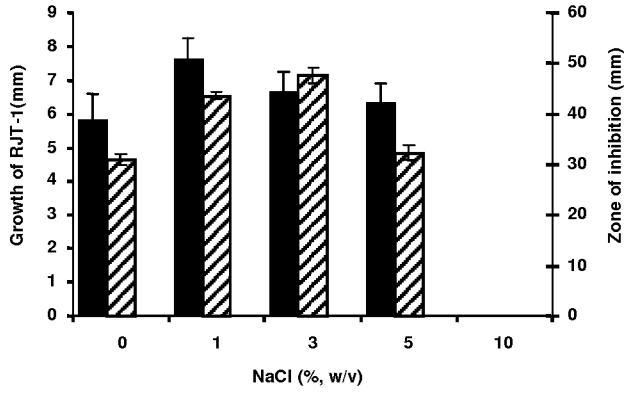

Figure 4. Effect of salt (NaCl, %, w/v) on growth (■) and antibiotic production (▨) by *S. sannanensis*.

ther studies were carried out with it (Figure 2 *a* and *b*). *S. sannanensis* secreted certain valuable enzymes of potential commercial importance, such as protease, amylase, lipase and cellulase (data not shown).

The effect of various media on antibiotic production was examined by inoculating *S. sannanensis* on starch agar, gelatin agar, nutrient agar and actinomyces agar (5% w/v NaCl, pH 9.0). After 4 days, actively growing culture of the test organism, *S. aureus*, was inoculated in all the plates using molten agar method as described above. The zone of inhibition was measured after incubation for 24 h at 37°C. Among various media tried, starch agar was the best for growth as well as antibiotic production (Figure 3).

Optimum salt requirement for antibiotic production was determined by inoculating *S. sannanensis* on starch agar medium supplemented with different salt concentrations (0–5%). After 4 days, *S. aureus* in molten agar was poured in the plates and the zone of inhibition was measured after 24 h incubation at 37°C. Antibiotic production was optimum at 3% NaCl, with slight decrease at 5% (Figures 4 and 5). Recently, a marine actinomycete was isolated from the Sundarbans region of the Bay of Bengal, India[13], which exhibited potent antimicrobial activity against Gram-positive and Gram-negative bacteria, moulds, yeast and several multiple-drug-resistant bacteria. The isolate grew in the presence of 20% (w/v) NaCl, antibiotic production being maximum with 5% (w/v) NaCl in the production medium.

The effect of pH on antibiotic production was determined by inoculating *S. sannanensis* on starch agar plates at different pH values in the range of 7–10. After 4 days, the test organism was inoculated in all plates and the zone of inhibition was measured. Antibiotic production was comparable in the pH range of 7–9, whereas at pH 10, there was no antibiotic production (Figure 6). Similarly, growth was also comparable at the above pH range. Our results are comparable with some *Streptomyces* species recorded to secrete antibiotics against bacteria, fungi and yeast at alkaline pH[14].

A study on the production of antibiotics usually involves a search for optimal media. This is achieved by a systematic study of the suitability of a large number of carbon and nitrogen sources. We explored the effect of carbon

sources on antibiotic production by inoculating *S. sannanensis* on starch agar plates having different sugars like glucose, xylose, arabinose, sucrose and lactose at a concentration of 1% (w/v). The antibiotic production was comparable with glucose, lactose and sucrose followed by xylose and arabinose (Figure 7). This result is quite comparable with *Streptomyces kananmyceticus* M27, for which dextrose proved to be an excellent carbon source

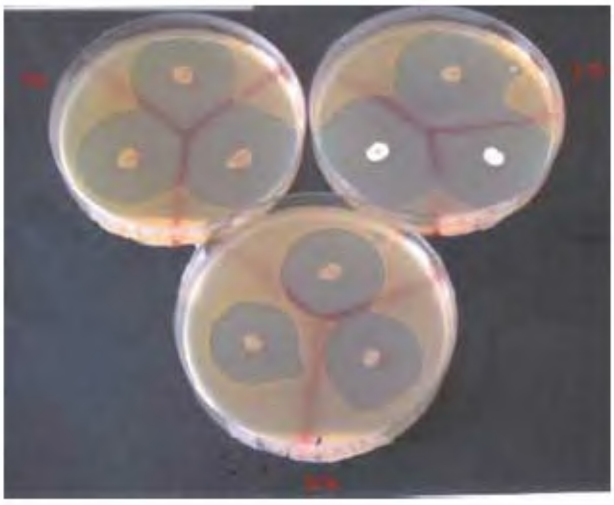

Figure 5. Effect of salt (NaCl, %, w/v) on growth and antibiotic production.

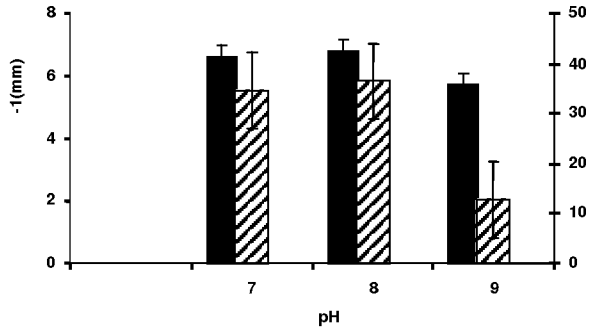

Figure 6. Effect of pH on growth (■) and antibiotic production (▨) by *S. sannanensis.*

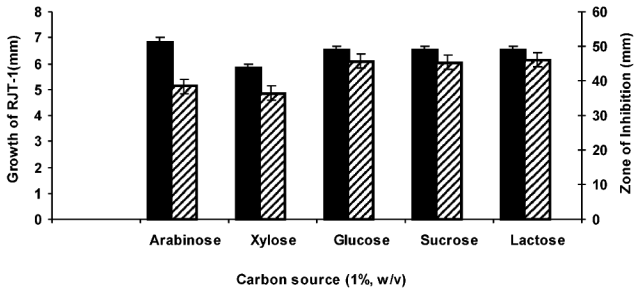

Figure 7. Effect of carbon sources (1%, w/v) on growth (■) and (▨) antibiotic production by *S. sannanensis.*

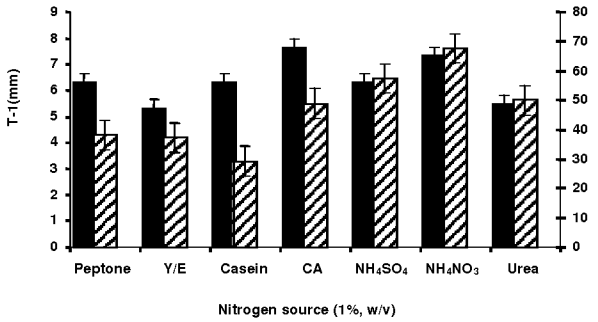

Figure 8. Effect of nitrogen sources (1%, w/v; Y/E, Yeast extract; CA, Casaminoacid) on growth (■) and antibiotic production (▨) by *Streptomyces sannanensis.*

for antibiotic production[15]. *Streptomyces hygroscopicus* D1 produced antibiotic optimally with glycerol as the carbon source[16]. Legator and Gottlieb[17] observed that 1% glycerol supported better chloramphenicol production by *S. venezuelae.*

The effect of nitrogen sources on antibiotic production was also examined in starch agar having different organic sources like peptone, yeast extract, casein and casamino acid, and inorganic nitrogen sources like ammonium sulphate, ammonium nitrate and urea. Inorganic nitrogen sources were better for antibiotic production compared to organic sources. Antibiotic production was favoured with ammonium sulphate, ammonium nitrate and urea (Figure 8). *S. kananmyceticus* M27 also yielded maximum antibiotic production with ammonium sulphate. Hobbs *et al.*[18] reported the carbon and nitrogen sources for actinorhodin production by *S. coelicolor.* Similarly, growth and pristinamycin production in *Streptomyces pristinaespiralis* has been recorded to be governed by nitrogen sources[19]. In *Streptomyces clavuligerus*, amino nitrogen as well as urea support cephalosporin production[20]. Optimization of cultural conditions for antibiotic production has also been attempted[21,22] in *Streptomyces antibioticus* Sr15.4 and *S. californicus* JCM6910.

With the increasing use of antibiotics, the serious problem of antibiotic resistance is gradually increasing. Therefore, intensive search for new antibiotics is going on worldwide. Production of antibiotic as secondary metabolite in excess is controlled by the genetic make up that imparts fullest expression and is profoundly influenced by the kind and quality of nutritional elements and environmental factors. This is also substantiated by our results presented here. Apart from normal actinomycetes, the salt-tolerant and alkaliphilic actinomycetes are much less explored for their antimicrobial potential and extracellular enzymes. The trends and initial results, however, are suggestive of their unique position in the generation of novel antimicrobial substances. It is, therefore, important to pay more attention to extremophilic actinomycetes for new generation of secondary metabolites.

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Received 11 November 2005; revised accepted 4 July 2006

Variation of bioactive components in *Curcuma longa* in Thailand

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Curcuma longa* L. (turmeric) is a popular medicinal herb of Thailand as a spice and a colouring agent. Medicinal uses of the rhizome arise from volatile oil as a carminative and for antifungal activity, and yellow curcuminoids for anti-oxidative and anti-inflammatory properties. In Thailand, *C. longa* is mainly used in forms of capsules/tablets of turmeric powder for herbal medicine, while its extract is popularly used in herbal cosmetics. Thus quality assessment of this plant needs to be controlled for the limits of volatile oil and total curcuminoids contents. This study was undertaken to evaluate the contents of essential oil and total curcuminoids in dried powder of *C. longa* rhizome collected from 13 locations from North, Northeast, Central and South Thailand during January to April 2005. The highest content (8.20 ± 1.66% v/w) of essential oil was found in samples from the North where the climate is cool, while the lowest oil content (7.00 ± 0.00% v/w) was found in samples from the South where it rains all year. In contrast, the highest total curcuminoids content (8.99 ± 0.83% w/w) was found in the southern samples while the lowest content (4.80 ± 1.83% w/w) was found in the northern samples. The total curcuminoids in all samples was found in the limit of 3.07 ± 0.09 to 9.58 ± 0.20% dry weight. The average of volatile oil content was found to be 7.77 ± 1.20% w/w, while the average of total curcuminoids content was found to be 6.24 ± 1.95% w/w. This information will be useful as a guidance for standardization of *C. longa* powder and the extracts, and finding sources of good quality of *C. longa* in Thailand.

Keywords: *Curcuma longa*, curcuminoid content, turmeric oil, Thailand.

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