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Effect of salt and pH on the growth and production of alkaline proteases from Haloalkaliphilic bacteria isolated from saline desert

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ABSTRACT

Saline desert of Kutch is yet unexplored for its microbial diversity and biotechnological potential. Proteases are significant with respect to their applications and cellular roles. A total of 15 bacteria isolated from the Little Rann of Kutch were isolated and screened for their growth and protease secretion at different pH and salt concentrations. Effect of NaCl concentrations and pH was assessed on growth and production of the proteases. Overall, 50 % of the isolates grew in the range of 0-20 % (w/v) NaCl, while nearly 45 % of them optimally produced extracellular proteases at 10 % (w/v) NaCl and 20 % isolates produced protease optimally at 15% (w/v) NaCl concentration. Majority of the isolates secreted protease in pH range 7-11 and nearly 70 % the isolates produced enzyme in the broad range of pH, 7-11 and nearly 6 % isolates produced protease in the range of pH 9-11. Further, around 40% of the isolates produced proteases optimally at pH 9-11. Therefore, the isolates demonstrated a broad range of pH and salt for growth and protease production. The study suggests wide prospective of the applications of these organisms and their enzymes.

Keywords: *Haloalkaliphilic bacteria; saline desert; salt tolerance; enzymatic potential; alkaline protease*

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INTRODUCTION

Deserts being arid regions receive <250 mm average rains. Therefore, a number of studies have been carried out for microbial diversity and biotechnological potential. Despite the large land area of the arid regions, only limited is known about the microbial communities [1, 2].

Further, Indian deserts are least explored for their microbial community and enzymatic potential [2, 3]. The saline desert of Kutch is unique as the north head of the Gulf of Kutch adjoins the Desert of Kutch with the regular flow of saline water during tides or through the water drifted by the south-west winds. Rainfall is limited, so that as water recedes and evaporates, there appears a crust of halite and gypsum crystals which grow in the clay and sands [4]. Thus, the study of microbes from this unique habitat adds to our understanding on the adaptation, ecology and enzymatic potential.

The microflora of the saline ecosystems has attracted a great deal of attention during the last couple of decades. Halophilic and halotolerant bacteria belong to a class of extremophilic organisms that live in habitats of high ionic strength, such as marine water, salt lakes, brines, saline deserts, salterns, saline soils, and salted foods [1, 5-10]. These microbes possess adaptation mechanisms to thrive under the dual extremities of high salinity and alkaline pH [11]. From a biotechnological stand point, enzymes withstanding multitude of the extremity from the haloalkaliphilic bacteria can be quite useful in various applications [6, 12-15].

To explore newer habitats for novel metabolites and bioactive molecules, there is a constant need to explore unexplored or unusual habitats. The arid or semiarid regions hold significance as they harbour an array of unique organisms with unexplored metabolic potential. In this study, we investigated the effect of salt and pH on the growth and protease production in the bacteria isolated from the saline desert of Kutch.

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MATERIALS AND METHODS

Isolates of the organisms

Four different media SP medium consisting, (g/l): (NaCl, 49; KCl, 1; $MgSO_4.7H_2O$, 0.5; $CaCl_2$.$2H_2O$, 0.18; $NaHCO_3$, 0.03; NaBr, 0.115; $FeCl_3.6H_2O$, 0.5; Bacto trypton, 5; Yeast extract, 10; Glucose, 1; Agar, 3%), Reasoner's 2A (R2A) agar (HiMedia Laboratories, India) consisting, (g/l): (Casein acid hydrolysate, 0.5; yeast extract, 0.5; protease peptone, 0.5; dextrose, 0.5; starch, 0.5; KH_2PO_4, 0.3; $MgSO_4$, 0.024; Sodium pyruvate, 0.30; NaCl (5%, w/v)), Soil Extract medium (SE) consisting , (g/l): (Glucose,1; K_2HPO_4, 0.5; Soil extract, 17.75, Agar, 15) and HM medium consisting, (g/l): NaCl, 220; $MgSO_4.7H_2O$, 10; KCl, 5; Sodium citrate, 3; KNO_3, 1; $CaCl_2.2H_2O$, 0.20, Trace minerals, 0.5; Bacto tryptone, 5; Yeast extract, 1; Agar, 3% (w/v) were used to target and isolate diverse microbiota of desert soil.

Total of 15 isolates were obtained from Venasar study site which were further selected for the optimization of protease secretion. Media profile of the isolates is given in Table 1.

Effect of salt on the growth and protease production

Actively growing cultures of 15 bacteria isolates, prepared in their respective isolation medium at pH 8 were used as inoculums for the assessment of the production of the alkaline protease. The regular spots of cultures were inoculated on the gelatin agar medium containing (g/l): gelatin, 30; peptone, 10; agar, 30; NaCl (0%, 5%, 10%, 15% and 20% (w/v)) and pH was kept 8 by adding separately autoclaved Na_2CO_3 in the medium. The inoculated plates were incubated for 72-96 hours at 37°C. Frazier's reagent (g/l: $HgCl_2$, 150gm; Concentrated HCl, 200mL) was poured into the plate for the detection of protease production. The extracellular protease secretion was indicated by the clear zone of substrate utilization surrounding the colonies. The colony diameter and zone of clearance were measured. The ratio of zone diameter to the colony diameter was calculated to estimate the relative enzyme production as a function of the colony size.

Effect of pH on the growth and protease production

Inoculums of the isolates were prepared as described above. The regular spots of cultures were inoculated on the gelatin agar medium containing (g/l): gelatin, 30; peptone, 10; agar, 30; NaCl (5% and 25% (w/v)) with different pH at interval of 1 (7, 8, 9, 10 and 11). The pH 7, 8 and 9 was adjusted with separately autoclaved Na_2CO_3, while pH 10 and 11 was adjusted by autoclaved 1M NaOH. The plates were incubated for 72-96 hours at 37°C. Detection of protease production was followed as described in above section.

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RESULTS

Revival of the isolates

Haloalkalophiles were isolated from the samples collected from the saline desert area from Little Rann of Kutch (Gujrat) on the SP agar, Reasoner's 2A (R2A) agar, Soil Extract medium (SE), HM agar.

Isolates	Isolation Media
VS -1	SP
VS -2	SP
VS -3	SP
VS -5	SP
VS -6	SP
VS -9	SP
VS -10	SP
VS -12	SP
VS -16	R2A
VS -19	R2A
VS -21	SE
VS -22	SE
VS -24	SE
VS -32	HM
VS -35	SP

Table 1 Isolation of all the bacterial isolates of the saline desert of Kutch

Effect of salt on the growth and protease production

The effect of NaCl was studied on the growth and protease production in 15 bacterial isolates. Overall, ~ 50% isolates could grew over a wide range of 0-20% (w/v) NaCl, while ~ 40 % of the isolates could grow in range of 0-15 % (w/v) NaCl and ~ 6% were able to grow in the range of 0-10 % (w/v) NaCl. Ioslates VS-1, VS-2 and VS-35 had optimum growth at 10 % (w/v) NaCl concentration, while VS-3, VS-5, VS-12, VS-19 and VS-21 have shown growth optima at 5 % (w/v) NaCl concentration. On the other hand, ~ 30% isolates produced enzyme in the broad range of 0-15 % (w/v) NaCl and 20 % produced protease in the range of 0-10 % (w/v) NaCl. Interestingly, 13% isolates produced protease in the range of 0-20 % (w/v) NaCl.

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Further, it was observed that 45 % of the isolates secreted protease optimally at 10 % (w/v) NaCl concentration and 30% of them produced protease optimally at 5 % (w/v) NaCl concentration. In addition, ~ 20 % of the isolates produced protease optimally at 15 % (w/v) NaCl concentration. Isolate VS-1 did not produce protease at any of the salt concentration examined. Overall, many isolates have shown broad salt requirement for both, growth and production of the proteases.

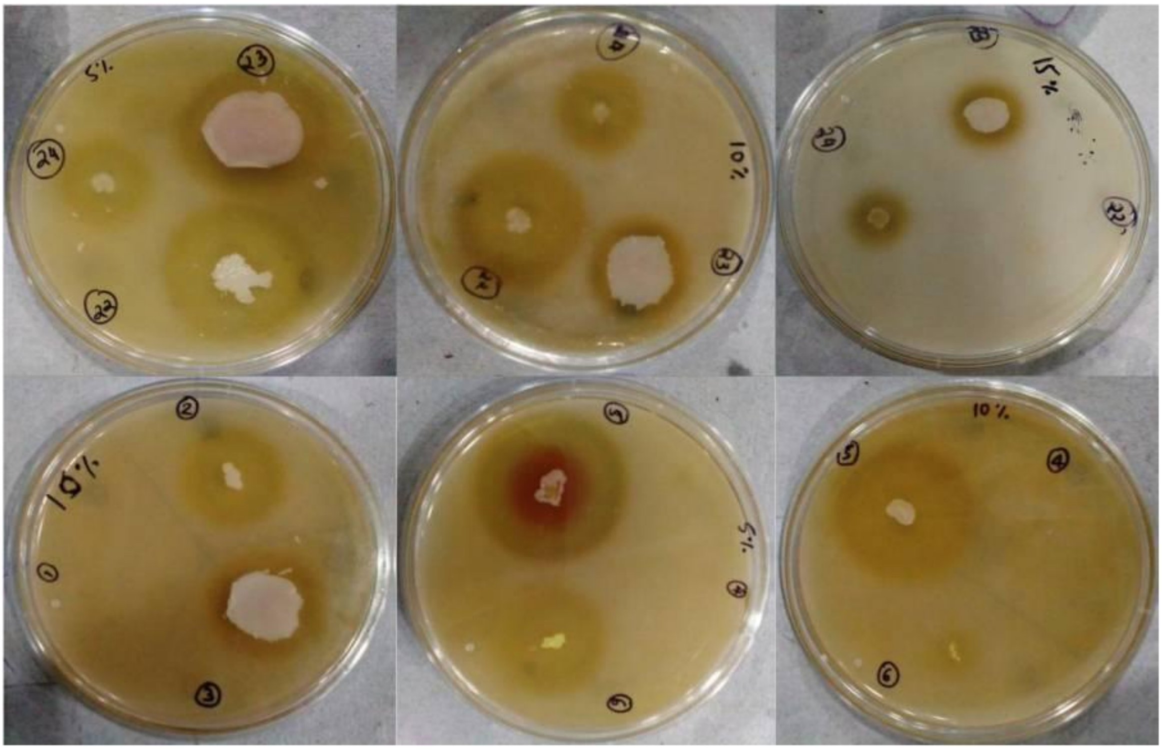

Figure 1 Screening of protease production using a drop spot technique on the gelatine agar plates

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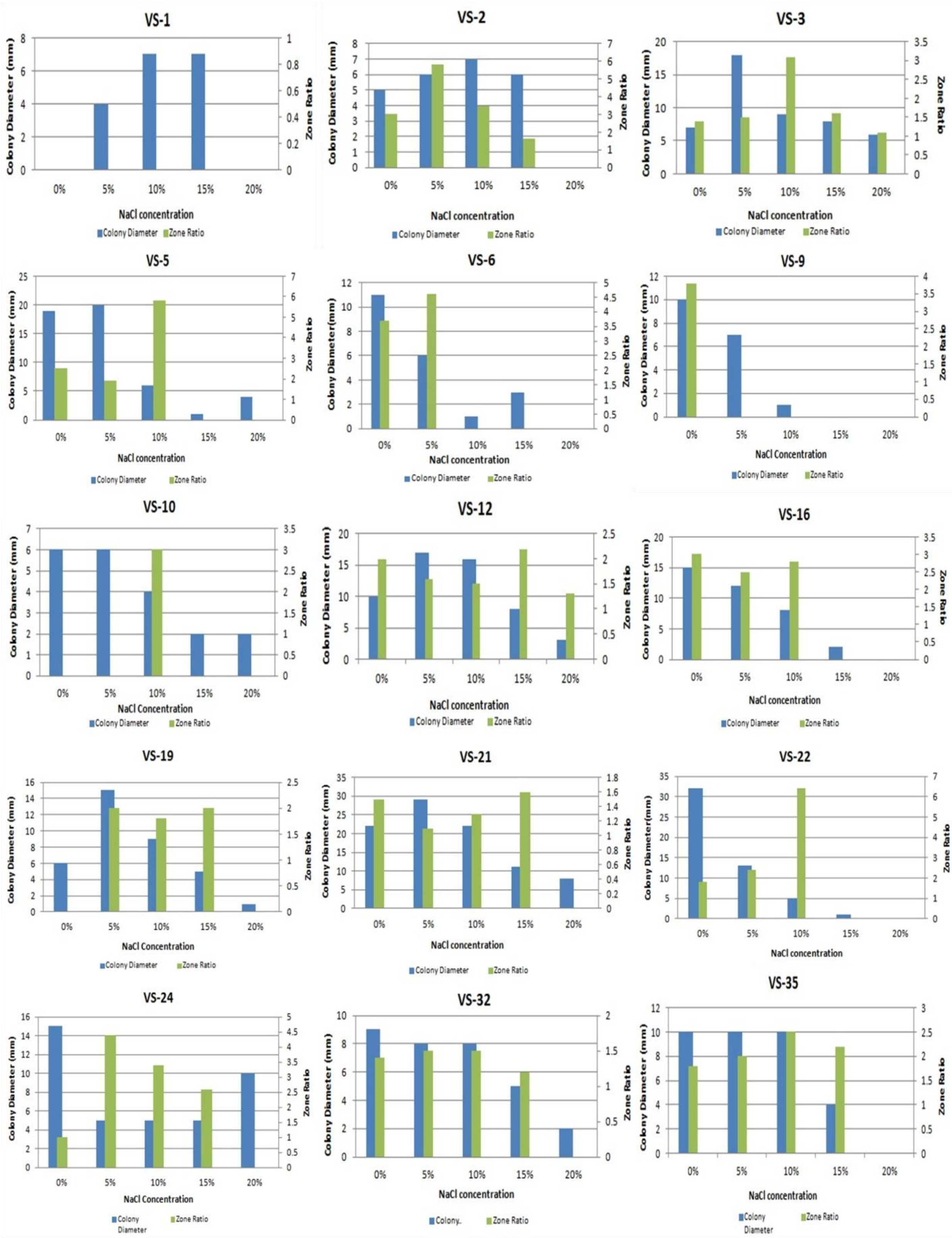

Figure 2 Effect of salt on the growth and production of the proteases from haloalkaliphilic bacteria isolated from saline desert of Kutch

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Effect of pH on the growth and production of the alkaline protease

The effect of pH was examined on the growth and protease production of all the 15 isolates. Overall, 90 % of the isolates could grow in the range of pH 7-11, while 6 % of the isolates grew in the range of pH 7-10. Many of the isolates were able to grow optimally in the pH alkaline. Strains VS-2, VS-10, VS-12 and VS-22 have shown growth optima at pH 9 while strains VS-21 and VS-32 had optimum growth at pH 10 and 11, respectively. On the other hand, ~ 70% of the isolates produced enzyme in the broad range of pH 7-11 and 6 % produced proteases in the range of pH 9-11. Further, it was observed that, 40 % the isolates optimally produced protease at pH 11. Further, 40 % of the isolates optimally produced protease at pH 9. In addition, 6 % of the isolates optimally produced protease at pH 7, pH 8 and pH 9 each. Overall, many isolates have shown broad pH requirement not only for their growth but also for the protease secretion.

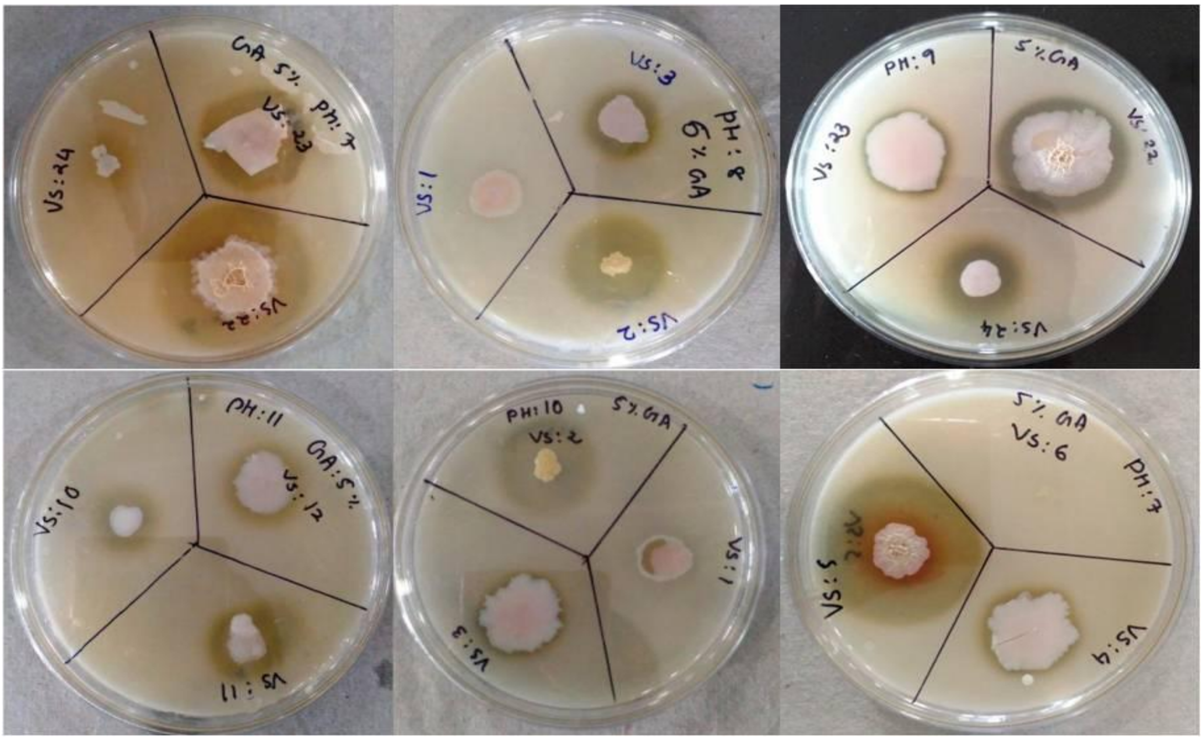

Figure 3 Screening of protease production at different pH using a drop spot technique on gelatine agar plates

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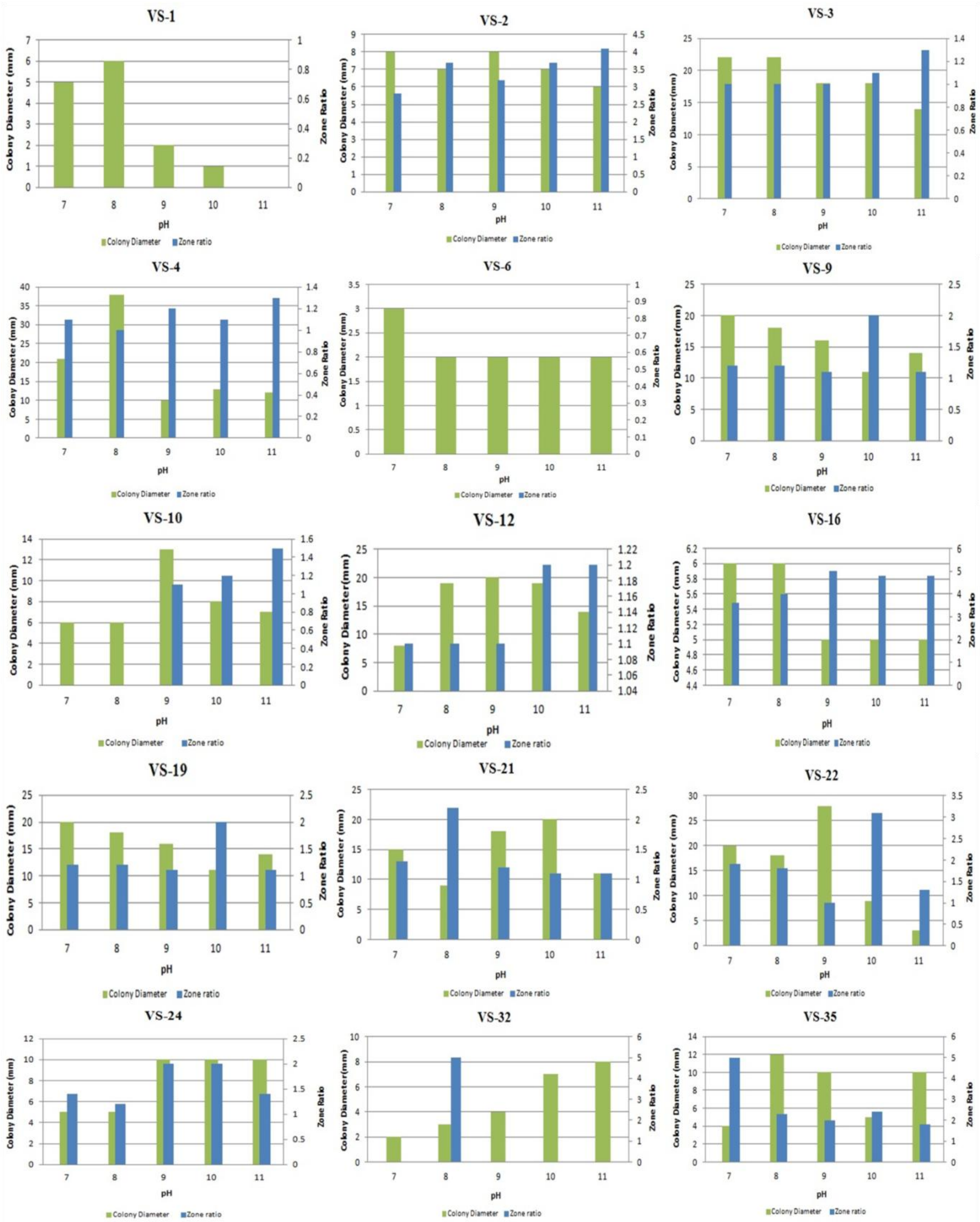

Figure 4 Effect of pH on the growth and production of protease from haloalkaliphilic bacteria isolated from saline desert of Kutch

DISCUSSION

Till now many haloalkaliphilic bacteria and actinomycetes have been studied from various marine environments [15-22]. However, arid environments have been relatively less explored [2,

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10, 23, 24]. Further, microbial community analysis have been carried out in deserts such as, Antacama Desert (Chile), Monegros Desert (Spain), Negev desert (Israel), Gobi desert (Mongolia) and Taklamaken desert (China) Sahara desert (Africa), Sonoran desert (America) while Indian desert areas are not much explored for microbial diversity and their biotechnological application point of view [1, 2].

In view of these facts, we isolated haloalkaliphilic and haloalkalitolerant bacteria from the desert of Kutch, an unique and unexplored arid zone. It has a special and different demography from other deserts of the world because of its location near the sea and low-lying areas, through which marine water enters into the vast area. Consequently, the desert of Kutch is an admixture of the saline, marshy and coastal desert where water and soils are saline. This characteristic makes desert a special habitat from the biodiversity point of view because it harbours its own, unique environment [1, 4]. The study of the microorganisms at the limits of the biological tolerance facilitates to understand the adaptation strategies.

The haloalkaliphilic microorganisms are significant not only due to the necessity for understanding the mechanisms of adaptations, but also due to the huge prospect of their application potential [11]. Efforts have focused on the investigations over the past decade on the enzymes capable to function under harsh conditions [12, 15, 22, 25, 26]. In present study, Most of the isolates were moderately halophilic and alkaliphilic in nature and hence referred as haloalkaliphiles. Majority of the isolates produced proteases at 0-15% (w/v) NaCl, 10% being optimum in most. Similarly, majority of the isolates produced proteases in broad range of pH, 7-11. This is considered to be an excellent feature as far as protease production flexibility is concerned. Further, many isolates optimally produced proteases at pH 10-11, a range which did not exactly correspond with its growth. So, growth and enzyme production were not linked. By and large, there was considerable difference in the production of the protease among the isolates. Rekik et al. (2019) reported alkaline protease from *Bacillus safensis* active over broad range of pH and salt concentrations [27]. Similarly, broad pH and temperature-active protease from *Bacillus subtilis* was reported [28].

Enzyme secretion at such a broad pH and salt concentration makes them interesting candidate for varied applications. It is very likely that the proteases produced from these bacteria will be stable at the range of pH and salt concentrations [29, 30]. Moreover, dual extremities; alkaline pH and salinity further project them as promising candidates for various biotechnological applications [31-34].

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CONCLUSIONS

In this report, effect of salt and pH has been assessed on the protease production in the bacteria isolated from the saline desert of Kutch, Gujarat, India. Not many studies are available on the exploration of microbial diversity and biotechnological potential of the desert ecosystems. In this study, broad tolerance to pH and salt was observed for their growth and the growth was not linked with the protease production. Protease production in such a broad pH and salt concentration range increases their prospective potential in varied industrial applications.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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