DIVERSITY OF CULTURABLE MODERATELY HALOPHILIC BACTERIA PRODUCING EXTRACELLULAR HYDROLYTIC ENZYMES FROM MARINE SEDIMENTS

DIVERSESIDADE DE BACTÉRIAS CULTIVÁVEIS MODERADAMENTE HALOFÍLICAS QUE PRODUZEM ENZIMAS HIDROLÍTICAS EXTRACELULARES DE SEDIMENTOS MARINHOS

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ABSTRACT:: A total of 114 moderately halophilic bacteria were isolated from marine sediment environments. The isolates are belonged to 23 species based on the 16S rRNA sequence analysis. 63, 52, 47, 57, 74, 15 and 4 isolates are able to produce protease, amylase, lipase, pectinase, pullulanase, xylanase, cellulase, respectively. Combined hydrolytic enzyme activity analysis show that 15 strains present 1 hydrolytic activity, 32 strains present 2 hydrolytic activities, 21 strains present 3 hydrolytic activities, 26 strains present 4 hydrolytic activities, 11 strains present 5 hydrolytic activities and 2 strains present 6 hydrolytic activities. Hydrolase activities are widely distributed in a variety of species. The highest rates for production of protease, amylase, lipase, pectinase, pullulanase, xylanase and cellulase were observed in species of B. baekryungensis, Hallobacillus sp., B. pumilus, B. megaterium or P. chungwhensis, B. pumilus, B. baekryungensis, respectively. However, the higher activities of protease, pectinase and pullulanase are frequently produced by the species of Halomonas sp. B. amyloliquefaciens or P. chungwhensis, and Vibrio sp. respectively. This investigation show that the diversity of halophilic bacteria from marine sediments could serve as a potential source of hydrolytic enzymes for industrial applications.

KEYWORDS: Moderately halophilic bacteria. Hydrolytic enzyme. Marine sediment.

INTRODUCTION

Halophilic microorganisms have attracted considerable interest due to their considerable capability of producing compounds with great industrial potential (MARGESIN AND SCHINNER 2001; VENTOSA et al. 1998). One of the most important biotechnological applications of these halophilic bacteria is focused on their production of diverse extracellular enzymes, such as amylase, lipase, caseinase, xylanase, inulinase, pectinase, cellulase, pullulanase, gelatinase, urease, glutaminase and asparaginase (MELLADO et al. 2004), as the bacteria have the desirable physiological properties of stability and solubility at high salt concentrations, which facilitate their use in the areas of food processing, feed additives, biomedical sciences and chemical industries (KULKARNI et al. 1999; NIEHAUS et al. 1999; PANDEY et al. 1999 and 2000; RAO et al. 1998).

Moderately halophilic bacteria usually are defined as a group of halophiles able to grow optimally in media containing 3-15% NaCl (VENTOSA et al. 1998), which are widely distributed in various marine environments and have been frequently isolated from crystallizer ponds, saline soil, salt marshes and solar salterns (OREN et al. 2002). This study aimed to isolate moderately halophilic bacteria from marine sediments, and characterize their capabilities for producing different extracellular hydrolytic enzymes with a view to providing valuable information about their potential utilization in industrial scale process.

MATERIAL AND METHODS

Isolation of moderately halophilic bacteria and culture conditions

Marine sediments of tidal zones were collected with aseptic methods. One gram of each sample was suspended in autoclaved sea water and made an appropriate dilution. One hundred milliliter of each diluted sample was plated on the isolation medium containing (per liter): casamino acid 7.5 g, yeast extract 10 g, MgSO₄·7H₂O 20 g, trisodium citrate 3 g, KCl 2 g, FeSO₄·7H₂O 1.6 mg, NaCl 100 g, agar 15 g, pH 7.2-7.4. The plates were incubated at 30°C for 10 d. Based on the colony characteristics, different bacteria were picked up and inoculated into liquid medium containing 5% NaCl. Pure culture of each isolate was stored in 20% glycerol at -80°C for further identification and characterization.

Identification of the isolates
Extraction of bacterial genomic DNA, amplification and sequencing of 16S rRNA gene sequences were performed as WEI et al. (2015) described. Preliminary identification was performed by BLAST search to suggest the closest relatives against GenBank database and a more precise identification was performed by phylogenetic analysis with type strains of the nearest neighbors. Isolates were regarded as belonging to a species when sequence similarity with the species types strain was at least 97%.

**Determination of extracellular hydrolytic enzyme activity**

Proteolytic activity of the isolates was screened and determined on the medium (per liter) containing skim milk 15 g, yeast extract 2 g, NaCl 100 g, agar 20 g. After incubation at 30°C for 5 d, clear zones around the colonies appearing were taken as the evidence of protease producing strains.

The presence of amylolytic activity on plates was determined on the medium (per liter) containing starch 5 g, yeast extract 2 g, (NH₄)₂SO₄ 1.4 g, K₂HPO₄ 2 g, MgSO₄·7H₂O 0.2 g, NaCl 100 g, agar 20 g, pH 7.2. After incubation at 30°C for 5 d, the plates were flooded with 0.3% I₂-0.6% KI solution; a clear zone around the growth colony indicates the positive strains which through of producing amylase to hydrolyse starch.

Lipolytic activity of the isolates was detected on Tributyrin medium (per liter) containing beef extract 3 g, peptone 10 g, tributyrin 6 ml, NaCl 100 g, agar 20 g (KHUNT; PANDHI 2011). The plates were incubated at 30°C for 5 d. Colonies showing a surrounding clear zone were lipase producers.

Assay of pectinolytic activity by the method described by Rohban et al. (2009) on the medium (per liter) containing (NH₄)₂SO₄ 1.4 g, MgSO₄·7H₂O 0.2 g, K₂HPO₄ 2 g, yeast extract 2 g, peptin 5 g, nutrient solution 1 ml (FeSO₄·7H₂O 5 mg/l, MnSO₄·H₂O 1.6 mg/l, ZnSO₄·7H₂O 1.4 mg/l, CaCl₂ 2 mg/l), NaCl 100 g, agar 20 g. After incubation at 30°C for 5 d, the plates were flooded with 0.3% I₂-0.6% KI solution; a clear zone around the growth colony was identified as the pectinase producing strains.

The method of detection of pullulanase activity was adopted by Rohban et al. (2009) and Ruben et al (1993) with modifications. The medium (per liter) was used as yeast extract 2 g, pullulan 5 g, NaCl 100 g, agar 20 g. After incubation at 30°C for 5 d, the plates were flooded with 0.3% I₂-0.6% KI solution instead of 97% ethanol, a clear zone around the colonies was identified as the pullulanase activity produced by the strains.

Xylanase activity was detected using the medium (per liter) containing yeast extract 2 g, peptone 5 g, MgSO₄·7H₂O 0.5 g, CaCl₂ 0.15 g, xylan 10 g, NaCl 100 g, agar 20 g. After incubation at 30°C for 5 d, the plates were stained with 10 ml 0.1% congo red solution for 20 min, then drained of the dye, a clear zone around the colonies indicate the xylanase activity producers (KAKHKI et al. 2011; ROHBAN et al. 2009).

Cellulase activity of the cultures were screened on the medium (per liter) containing carboxy methyl cellulose (CMC) 5 g, NaNO₃ 1 g, K₂HPO₄ 2 g, KCl 1 g, MgSO₄·7H₂O 0.5 g, yeast extract 2 g, glucose 1 g, NaCl 100 g, agar 20 g, pH 7.2. After incubation at 30°C for 5 d, the plates were poured with 10 ml 0.1% congo red solution and stained with the dye for 20 min, then drained of the dye. A clear zone surrounding the colony shows the cellulase activity producing by the strains (KAKHKI et al. 2011; ROHBAN et al. 2009).

**RESULTS AND DISCUSSION**

**Isolation and identification of moderately halophilic bacteria**

A total of 114 strains were obtained which grew well on the media containing of 5% -15% NaCl. 16S rRNA sequence analyses show that they are grouped into 23 species at a 97% sequence similarity threshold. Out of 114 isolates, 94 isolates (82.5%) are Gram positive, while 20 isolates (17.5%) are Gram negative. 17 species belong to the type of Gram positive bacteria with the dominance of Bacillus baekyangensis (15.8%), Bacillus pumilus (12.3%), Bacillus megaterium (10.5%), Pontibacillus chungwhensis (12.3%) and Halobacillus sp. (9.6%), comparison of 6 species belong to the type of Gram negative bacteria with the dominance of Cobetia marina (8.8%) (Table 1). The result is similar with Babavalian et al. (2013) reported that more Gram positive (61 isolates) than Gram negative (22 isolates) moderately halophilic bacteria were obtained from a largest hypersaline Playa in Iran for produce hydrolytic enzymes.

**Enzymatic activity of isolates**

Halophilic microorganisms have been investigated for their potential biotechnological applications in various fields, such as production of compatible solutes, biopolymers, enzymes, food biotechnology, and biological waste treatment (MARGESIN; SCHINNER 2001). Moreover, increasing concerns focus on the salt tolerant
extracellular hydrolytic enzymes produced by moderately halophilic and halotolerant bacteria (MELLADO et al. 2004; VENTOSA et al. 1998; MORENO et al. 2013). Halophilic and halotolerant bacteria are an excellent source of enzymes exhibiting salt, pH and temperature tolerance (GOMEZ; STEINER 2004; SÁNCHEZ-PERRO et al. 2003).

In this study, seven extracellular hydrolytic enzyme activities were tested among the 114 isolates (Table 1). 107 isolates (93.9%) are able to produce at least one of the hydrolytic activities, whereas 7 isolates (6.1%) are unable to produce any of the tested hydrolytic activities. A total of 63 (49 Gram positive and 14 Gram negative), 47 (41 Gram positive and 6 Gram negative), 57 (48 Gram positive and 9 Gram negative), 74 (60 Gram positive and 14 Gram negative), 15 (all Gram positive), 4 (all Gram positive) isolates are able to produce protease, amylase, lipase, pectinase, pullulanase, xylanase, and cellulase, respectively. Combined hydrolytic enzyme activities were detected and the results show that the isolates have diversities for enzyme production. Fifteen strains present one hydrolytic activity, 32 strains present 2 hydrolytic activities, 21 strains present 3 hydrolytic activities, 26 strains present 4 hydrolytic activities, 11 strains present 5 hydrolytic activities and 2 strains present 6 hydrolytic activities. Among the tested strains, *Halobacillus* sp. HNI111 and *Halomonas* sp. QD55 show the higher protease activity, *B. amyloliquefaciens* BDH11 and BDH27 present the higher amylase activities, *B. megaterium* HNS83 and *B. pumilus* BDH24, HNS77 and HNS81 present the higher lipase activities, *B. amyloliquefaciens* BDH27, *B. cereus* HNI98 and *P. chungwhensis* HNI96 show the higher pectinase activities, *Vibrio* sp. QD45 presents the higher pullulanase activity, *B. pumilus* HNS74 presents the higher xylanase activity, and *B. amylolquefaciens* BDH11 and HNI92 present the higher cellulase activities.

Our results show that the higher rates of production of protease, amylase, lipase, pectinase, and pullulanase, but much lower rates of xylanase and cellulase, are observed among these isolates. This is somewhat different from Rohban et al. (2009), who reported higher rates of production of amylase, lipase, protease, xylanase, and inulinase, but lower rates of pullulanase, pectinase, and cellulase production, among the halophilic isolates isolated from Howz Soltan Lake. We infer that this is due to the different strain species fostered by the different environments. Among the cultured strains, most of them present the combined hydrolytic activities and similar results to those reported by Rohban et al. (2009) and Sánchez-Porro et al. (2003).

### Table 1. Hydrolytic enzyme activity of moderately halophilic isolates

| Species               | Strain number | Accession number | Activity of hydrolytic enzymes (halo diameter, cm) |
|-----------------------|---------------|------------------|-----------------------------------------------|
|                        |               |                  | Protease | Amylase | Lipase | Pectinase | Pullulanase | Xylanase | Cellulase |
| Gram-positive bacteria |               |                  |          |         |        |           |            |          |           |
| BDH9                  | KF933630      | –                | –        | –       | –      | –         | –          | –        |
| BDH15                 | KF933610      | –                | –        | –       | –      | 1.1       | –          | –        |
| BDH25                 | KF933620      | 0.4              | –        | –       | –      | 0.7       | –          | –        |
| QD35                  | KF933691      | 1.2              | –        | –       | –      | –         | –          | –        |
| QD37                  | KF933693      | 1.0              | –        | –       | –      | 0.4       | –          | –        |
| QD40                  | KF933716      | –                | –        | –       | –      | 1.6       | –          | –        |
| Bacillus baekryungensis |               |                  |          |         |        |           |            |          |           |
| QD41                  | KF933695      | –                | –        | –       | –      | 0.5       | –          | –        |
| QD42                  | KF933696      | –                | –        | –       | –      | 0.4       | –          | –        |
| QD54                  | KF933706      | –                | 0.7      | 1.9     | –      | 0.2       | –          | –        |
| QD56                  | KF933708      | –                | –        | 0.4     | 0.2    | 1.1       | –          | –        |
| HNS72                 | KF933669      | 0.4              | –        | –       | 0.2    | –         | –          | –        |
| HNS80                 | KF933677      | 0.4              | 1.5      | 0.8     | 1.5    | –         | –          | –        |
| HNS82                 | KF933679      | –                | –        | –       | –      | –         | –          | –        |
|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| HNS84 | KF933681 | 0.4 | – | 0.7 | – | – |
| HNS85 | KF933682 | – | – | – | – | – |
| HNS87 | KF933684 | 1.2 | – | – | – | 0.2 |
| HNS89 | KF933714 | 0.4 | – | – | – | 0.4 |
| HNI99 | KF933657 | 0.7 | – | – | 0.2 | 0.4 |

**Bacillus algicola**
- QD43 KF933697 – – – – 0.4 – –

**Bacillus cibi**
- BDH3 KF933624 – – 1.2 – – – –
- BDH7 KF933629 – 0.7 – 0.8 1.1 – –
- BDH8 KF933629 – – 2.0 – – – –
- BDH12 KF933608 – – 1.5 – – 0.8 –
- BDH17 KF933612 – 0.2 – – 1.0 – –
- BDH24 KF933619 – – 2.3 0.4 1.2 – –
- HNS61 KF933658 – – 1.6 – – 1.6 –
- HNS64 KF933661 – – 2.0 – – 0.8 –

**Bacillus pumilus**
- HNS69 KF933666 – – 1.1 – 1.4 0.6 –
- HNS70 KF933667 – – 1.5 – – 1.4 –
- HNS74 KF933671 – – 1.4 – – 2.1 –
- HNS75 KF933672 0.2 – 0.6 – – 0.4 –
- HNS77 KF933674 0.2 – 2.4 – – 1.0 –
- HNS81 KF933678 – – 2.3 – – 0.2 –
- HNI91 KF933650 1.2 – – 0.8 – 1.6 –
- BDH11 KF933607 1.2 1.6 – 1.8 1.9 1.2 1.2

**Bacillus amyloliquefaciens**
- BDH27 KF933622 – 1.7 0.7 2.5 2.0 – 0.4
- HNI92 KF933651 – 1.0 0.2 1.9 1.5 0.4 1.2
- BDH1 KF933605 0.2 – – – – – –
- BDH20 KF933615 – – – – – – –

**Bacillus sp.**
- HNS62 KF933659 – – 0.4 – – – –
- HNS67 KF933664 – – 0.8 – – – –
- BDH5 KF933712 1.1 0.8 – 1.2 1.6 –
- BDH6 KF933627 1.0 0.2 0.6 – 1.0 – –
- BDH22 KF933617 1.0 – – – 0.8 – –

**Bacillus aquimaris**
- BDH28 KF933713 1.5 0.7 – 1.0 1.7 –
- HNS65 KF933662 1.2 0.6 0.2 1.5 1.8 – –
- HNS66 KF933663 – – – – – – –
- HNS86 KF933683 1.5 0.6 – – – – –
- BDH4 KF933626 0.4 0.2 1.1 1.0 1.0 – –
- BDH10 KF933606 – 0.4 – 0.8 1.1 – –
- BDH23 KF933618 – 0.8 0.4 1.2 1.5 – –
- HNS68 KF933665 – 0.6 0.2 1.3 1.4 – 0.4
- HNS71 KF933668 – – 2.2 0.2 – – –
- HNS73 KF933670 0.2 – 0.4 0.2 – – –

**Bacillus megaterium**
- HNS76 KF933673 0.2 0.6 0.8 0.7 1.6 – –
- HNS78 KF933675 0.2 – 1.5 0.8 1.4 – –
- HNS79 KF933676 – – 1.8 1.2 – – –
- HNS83 KF933680 – – 2.4 – – 1.6 –
- HNS88 KF933685 – – 1.6 – 1.3 – –
- HNI104 KF933635 – 0.8 – 1.0 1.4 – –
- BDH19 KF933614 0.4 – 0.5 0.4 – – –

**Bacillus cereus**
- HNI98 KF933715 0.6 0.6 – 2.4 – 0.8 –
### Diversity of culturable bacteria

| Strain   | Accession | Gram-positive bacteria | Gram-negative bacteria |
|----------|-----------|------------------------|------------------------|
| Staphylococcus sp. | QD53 KF933705 | 0.4 2.1 0.4 1.0 | QD34 KF933690 1.0 0.6 |
|          | BDH13 KF933609 | 0.4 0.7 1.8 1.8 | QD36 KF933692 1.1 0.4 |
|          | BDH21 KF933616 | 0.6 0.8 0.2 1.4 | QD38 KF933717 1.0 0.7 |
|          | QD63 KF933660 | – – – 0.2 | QD39 KF933694 0.4 0.4 |
|          | HNS90 KF933686 | – 0.4 – 0.2 | QD46 KF933699 – – |
|          | HNI96 KF933655 | 1.1 – 2.5 1.2 | QD48 KF933701 – 0.4 |
|          | HNI97 KF933656 | 1.4 0.8 – 2.0 | QD51 KF933703 1.2 – |
|          | HNI100 KF933631 | 1.0 0.4 0.2 2.2 | QD52 KF933704 1.0 0.8 |
|          | HNI103 KF933634 | – – 0.2 – 0.2 | QD58 KF933710 – 0.6 |
| Virgibacillus chiguensis | HNI105 KF933636 | 0.4 – – 0.2 | HNI95 KF933654 – – |
|          | HNI106 KF933637 | – – 1.0 0.2 | HNI108 KF933639 1.2 0.5 |
|          | HNI113 KF933642 | 1.2 1.3 0.6 1.4 | HNI111 KF933718 2.1 0.4 |
|          | HNI115 KF933644 | 0.6 0.8 0.9 1.0 | HNI114 KF933643 0.4 0.5 |
|          | HNI118 KF933647 | 0.2 0.5 – 0.4 | HNI116 KF933645 0.2 – |
|          | HNI120 KF933649 | – 0.4 – 0.2 | HNI117 KF933646 0.2 0.2 |
|          | BDH16 KF933611 | 1.2 0.4 – 0.3 | BDH18 KF933613 0.6 0.8 |
|          | BDH19 KF933620 | – – – 1.2 | QD31 KF933687 1.4 0.5 |
|          | HNI194 KF933653 | 0.4 0.7 – 0.3 | HNI102 KF933633 0.6 0.4 |
| Pontibacillus chungwhensis | HNI105 KF933636 | 0.4 – – 0.2 | HNI110 KF933639 1.2 0.5 |
|          | HNI106 KF933637 | – – 1.0 0.2 | HNI111 KF933718 2.1 0.4 |
|          | HNI113 KF933642 | 1.2 1.3 0.6 1.4 | HNI114 KF933643 0.4 0.5 |
|          | HNI115 KF933644 | 0.6 0.8 0.9 1.0 | HNI116 KF933645 0.2 – |
|          | HNI118 KF933647 | 0.2 0.5 – 0.4 | HNI117 KF933646 0.2 0.2 |
|          | HNI120 KF933649 | – 0.4 – 0.2 | BDH18 KF933613 0.6 0.8 |
|          | BDH19 KF933620 | – – – 1.2 | QD31 KF933687 1.4 0.5 |
|          | HNI194 KF933653 | 0.4 0.7 – 0.3 | HNI102 KF933633 0.6 0.4 |
| Halobacillus sp | HNI108 KF933639 | 1.2 0.5 – 0.4 | BDH26 KF933621 0.2 – |
|          | HNI111 KF933718 | 2.1 0.4 – 0.7 | HNI111 KF933718 2.1 0.4 |
|          | HNI114 KF933643 | 0.4 0.5 – 0.6 | HNI114 KF933643 0.4 0.5 |
|          | HNI116 KF933645 | 0.2 – – 0.4 | HNI116 KF933645 0.2 – |
|          | HNI117 KF933646 | 0.2 0.2 – 0.5 | HNI117 KF933646 0.2 0.2 |
|          | HNI119 KF933648 | 0.4 0.2 – 0.4 | HNI119 KF933648 0.4 0.2 |
| Planococcus maritimus | HNI110 KF933632 | 1.0 – – 0.2 | HNI101 KF933632 1.0 – |
| Exiguobacterium sp | BDH26 KF933621 | 0.2 0.4 – – | BDH26 KF933621 0.2 0.4 |
|          | HNI109 KF933640 | 0.2 0.5 – – | HNI109 KF933640 0.2 0.5 |
| Paenibacillus sp | HNI110 KF933641 | 0.2 – – 0.4 | HNI110 KF933641 0.2 – |
|          | HNI111 KF933642 | 1.0 – – 0.2 | HNI111 KF933642 1.0 – |
|          | HNI112 KF933643 | 1.0 – – 0.2 | HNI112 KF933643 1.0 – |

**Gram-negative bacteria**

| Strain   | Accession | Gram-positive bacteria | Gram-negative bacteria |
|----------|-----------|------------------------|------------------------|
| QD34 KF933690 | 1.0 0.6 | QD34 KF933690 1.0 0.6 |
| QD36 KF933692 | 1.1 0.4 | QD36 KF933692 1.1 0.4 |
| QD38 KF933717 | 1.0 0.7 | QD38 KF933717 1.0 0.7 |
| QD39 KF933694 | 0.4 0.4 | QD39 KF933694 0.4 0.4 |
| QD46 KF933699 | – – – | QD46 KF933699 – – |
| QD48 KF933701 | – 0.4 | QD48 KF933701 – 0.4 |
| QD51 KF933703 | 1.2 | QD51 KF933703 1.2 – |
| QD52 KF933704 | 1.0 0.8 | QD52 KF933704 1.0 0.8 |
| QD54 KF933710 | – 0.6 | QD54 KF933710 – 0.6 |
| QD59 KF933711 | 0.6 0.6 0.6 | QD59 KF933711 0.6 0.6 |
| HNI193 KF933652 | – – – | HNI193 KF933652 – – |
| Halomonas venusta | QD32 KF933688 | 1.1 0.4 0.4 | QD32 KF933688 1.1 0.4 |
|          | QD55 KF933707 | 2.1 0.3 | QD55 KF933707 2.1 0.3 |
|          | QD57 KF933709 | 0.4 0.6 | QD57 KF933709 0.4 0.6 |
| Alteromonas sp | QD47 KF933700 | – – – | QD47 KF933700 – – |
| Vibrio sp | QD45 KF933698 | – – 0.8 | QD45 KF933698 – 0.8 |

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Enzymatic profiles of species

Enzymatic profiles and the percentage for production of each enzymes associated with species are shown in Figure 1. A higher ratio of hydrolytic activity producing strains are frequently observed within a number of limited species. 18 species are able to produce protease, out of those species, B. baekryungensis (7.9%) and Halobacillus sp. (9.6%) show higher percentages of protease producing strains. However, higher percentages of higher proteolytic activity producing species are appeared in the species of B. aquimaris (2.6%) and Halobacillus sp. (3.0%) (Fig. 1A). 16 species are able to produce amylase. 8.8% of strains belonging to Halobacillus sp. are able to produce amylase, which is greater than all other species. Whereas higher percentages of higher amylolytic activity producing species are appeared in the species of B. amyloliquefaciens (2.6%) and P. chungwhensis (2.6%) (Fig. 1B). 16 species are able to produce lipase. B. pumilus (9.7%) and B. megaterium (8.7%) show the higher percentage of producing lipase, however no higher lipolytic activity producing strains are observed in the species of B. megaterium. B. pumilus (5.3%) show the higher percentage of higher lipolytic activity producing strains (Fig. 1C). 17 species are able to produce pectinase. B. megaterium (8.8%) and Halobacillus sp. (8.8%) show the higher percentage of producing pectinase, however no higher pectinolytic activity producing strains were observed in this two species, and higher percentages of higher pectinolytic activity producing strains were observed in the species of B. amyloliquefaciens (2.6%) and P. chungwhensis (2.6%) (Fig. 1D). 16 species are able to produce pullulanase. B. baekryungensis (10.5%), P. chungwhensis (9.7%) and Halobacillus sp. (9.7%) show the higher percentage of producing pullulanase, and all the higher pullulanase activity producing strains were belonged to the species of B. aquimaris (2.6%) (Fig. 1E). 5 and 2 species are able to produce xylanase and cellulase respectively (Fig. 1F and Fig. 1G). Xylanase production was also found to be limited to species of B. algicola, B. pumilus, B. amyloliquefaciens, B. megaterium, P. chungwhensis and Halobacillus sp., and the higher percentage of producing xylanase is B. pumilus (8.8%) (Fig. 1F). When compared with other enzymes, cellulose producing species is only associated with the two species of B. amyloliquefaciens (2.6%) and B. megaterium (0.9%) (Fig. 1G).

Moderately halophilic bacteria producing protease, amylase, lipase, pectinase, pullulanase and cellulase are widely distributed in the marine sediment environments. The highest rates for production of protease, amylase, lipase, pectinase, pullulanase, xylanase and cellulase are members of B. baekryungensis, Halobacillus sp., B. pumilus, B. megaterium or P. chungwhensis, B. amyloliquefaciens, B. pumilus, and B. baekryungensis, respectively (Fig. 1). Rohban et al. (2009) reported that most amylase, DNase, and lipase producers are members of the genera Oceanobacillus, Halomonas, and Gracilibacillus respectively. Cellulase producers were detected among members of Gracilibacillus, Virgibacillus and Halobacillus. Kakhki et al. (2011) reported that under their conditions Halorubrum is the predominant genus showing the highest rates of amylase, lipase, pullulanase, inulinase and DNase production. Our data is much more similar to the results reported by Berrada et al. (2012), in which Bacillus is the predominant genus showing the higher production rates for amylase, lipase, DNase, protease and cellulase. It is interesting that higher average rates for production of the enzymes by a certain species do not necessarily mean that the higher enzyme-producing activity belonged to a strain from the same species.
Figure 1. Enzymatic profiles of the isolates and comparison of the percentage for production of each enzymes among the strains. A, Protease; B, Amylase; C, Lipase; D, Pectinase; E, Pullulanase; F, Xylanase; G, Cellulase. Red columns indicate the percentage of species able to produce hydrolytic enzymes, blue columns indicate the percentage of species able to produce higher enzymatic activity.
CONCLUSIONS

We isolated 114 moderately halophilic bacteria from marine sediments, which comprising of 94 Gram positive bacteria and 20 Gram negative bacteria. A total of 107 isolates are able to produce at least one of the hydrolytic activities. Most of them present the combined hydrolytic activities including protease, amylase, lipase, pectinase and pullulanase, and only 15 and 4 isolates present xylanase and cellulose activities respectively.

The higher average rates for production of the enzymes by a certain species is inconsistent with the higher enzyme-producing activity belonged to the same species. Both of them are limited to a few different species.

RESUMO: Um total de 114 bactérias moderadamente halofílicas foram isoladas de ambientes de sedimentos marinhos. Os isolados pertencem a 23 espécies com base na análise da sequência 16S rRNA. 63, 52, 47, 57, 74, 15 e 4 isolados são capazes de produzir protease, amilase, lipase, pectinase, pullulanase, xilanase, celulase, respectivamente. A análise da atividade enzimática hidrolítica combinada mostra que 15 cepas apresentam 1 atividade hidrolítica, 32 cepas apresentam 2 atividades hidrolíticas, 21 cepas apresentam 3 atividades hidrolíticas, 26 cepas apresentam 4 atividades hidrolíticas, 11 cepas apresentam 5 atividades hidrolíticas e 2 cepas apresentam 6 atividades hidrolíticas. Atividades de hidrolase são amplamente distribuídas em uma variedade de espécies. As maiores taxas de produção de protease, amilase, lipase, pectinase, pullulanase, xilanase e celulase foram observadas em espécies de B. baekryungensis, Hallobacillus sp., B. pumilus, B. megaterium ou P. chungwhensis, B. amyloliquefaciens, B. pumilus, B. baekryungensis, respectivamente. No entanto, as atividades mais elevadas de protease, pectinase e pullulanase são frequentemente produzidas pelas espécies de Halomonas sp. B. amyloliquefaciens ou P. chungwhensis e Vibrio sp. respectivamente. Esta investigação mostra que a diversidade de bactérias halofílicas de sedimentos marinhos pode servir como uma fonte potencial de enzimas hidrolíticas para aplicações industriais.

PALAVRAS-CHAVE: Bactérias moderadamente halofílicas. Enzima hidrolítica. Sedimento marinho.

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