Lentibacillus jeotgali sp. nov., a halophilic bacterium isolated from traditional Korean fermented seafood

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A novel, Gram-positive, non-motile, endospore-forming and moderately halophilic bacterium, strain Grbi^T, was isolated from a traditional Korean fermented seafood. The organism grew optimally in the presence of 10–15 % NaCl, at 37 °C and pH 8.0. The peptidoglycan of the cell wall consisted of *meso*-diaminopimelic acid, and the predominant menaquinone was MK-7. The major fatty acids of strain Grbi^T were iso-$C_{16:0}$ (36.4 %), anteiso-$C_{15:0}$ (30.3 %) and iso-$C_{14:0}$ (18.2 %). The polar lipids were phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid. The genomic DNA G+C content was 42.5 mol%. Strain Grbi^T was most closely related to the type strain *Lentibacillus kapialis* JCM 12580^T, with which it shared 97.5 % 16S rRNA gene sequence similarity. The DNA–DNA hybridization value between strains Grbi^T and *L. kapialis* JCM 12580^T was 8 %. Based on phenotypic, genotypic and phylogenetic data, strain Grbi^T should be classified as a novel species within the genus *Lentibacillus*, for which the name *Lentibacillus jeotgali* sp. nov. is proposed. The type strain is Grbi^T (=KCTC 13300^T=JCM 15795^T).

Jeotgal is one of the traditional Korean fermented seafoods. There are about 145 types of jeotgal made from salted marine organisms such as fish, molluscs and crustaceans. Jeotgal has been an important source of dietary protein since AD 683 (Suh & Yoon, 1987) and a source of numerous diverse bacteria belonging to the phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria*, as well as lactic acid bacteria and yeast (Kim *et al.*, 2005). Recent studies have reported the isolation of many novel bacteria and archaea from jeotgal: *Bacillus jeotgali* (Yoon *et al.*, 2001a), *Planomicrobium koreense* (Yoon *et al.*, 2001b), *Jeotgalibacillus alimentarius* (Yoon *et al.*, 2001c), *Halomonas alimentaria* (Yoon *et al.*, 2002b), *Psychrobacter jeotgali* (Yoon *et al.*, 2003a), *Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus* (Yoon *et al.*, 2003b), *Bacillus cibi* (Yoon *et al.*, 2005a), *Psychrobacter alimentarius* (Yoon *et al.*, 2005b), *Psychrobacter cibarius* (Jung *et al.*, 2005), *Nesterenkonia jeotgali* (Yoon *et al.*, 2006), *Methylobacterium jeotgali* (Aslam *et al.*, 2007a), *Salinicoccus jeotgali* (Aslam *et al.*, 2007b), *Natronococcus jeotgali* (Roh *et al.*, 2007a), *Halalkalicoccus jeotgali* (Roh *et al.*, 2007b), '*Paenibacillus tyraminigenes*' (Mah *et al.*, 2008), *Alishewanella jeotgali* (Kim *et al.*, 2009) and *Haloterrigena jeotgali* (Roh *et al.*, 2009).

The genus *Lentibacillus* was first proposed by Yoon *et al.* (2002a) to accommodate an aerobic, Gram-variable, endospore-forming, rod-shaped and moderately halophilic bacterium isolated from a salt field of the Yellow Sea in Korea. Its predominant isoprenoid quinone is menaquinone-7; the cell-wall peptidoglycan type is *meso*-diaminopimelic acid; the major polar lipids are phosphatidylglycerol and diphosphatidylglycerol; the major fatty acids are anteiso-$C_{15:0}$ and iso-$C_{16:0}$; and the DNA G+C content is 42–49 mol% (Lee *et al.*, 2008a). In this study, we describe strain Grbi^T as a novel species belonging to the genus *Lentibacillus*, based on phenotypic and chemotaxonomic characterizations and phylogenetic analysis.

Strain Grbi^T was isolated from a traditional Korean fermented seafood called Garibi-jeotgal in Korean, made from scallops. The strain was isolated from the sample using the dilution-plating technique on marine 2216 agar (MA; BBL) plates supplemented with 20 % (w/v) NaCl at 30 °C. The isolate was repeatedly restreaked to obtain a pure culture on MA plates supplemented with 20 % (w/v) NaCl. The requirements and tolerance of various NaCl concentrations (0, 3, 5, 7.5, 10, 15, 20, 25 and 30 %) were determined in broth medium that comprised all of the constituents of MB, except NaCl, supplemented with appropriate concentrations of NaCl. Strain Grbi^T grew in 3.0–20.0 % (w/v) NaCl, with optimal growth occurring in 10–15 % (w/v) NaCl. Growth at different temperatures (4,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Grbi^T is FJ182050.

Figures showing TLC of the polar lipids and amino acid composition of the cell wall hydrolysate of strain Grbi^T are available with the online version of this paper.

10, 15, 20, 25, 30, 37 and 45 °C) was tested on MA supplemented with 10 % (w/v) NaCl. Growth at different pH values (5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0) was examined using MB supplemented with 10 % (w/v) NaCl. The following buffers were used: pH 5.0, 0.1 M CH_3COOH/0.1 M CH_3COONa; pH 6.0, 7.0 and 8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0 and 10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3; pH 11.0, 0.05 M Na_2HPO_4/0.1 M NaOH. Strain Grbi^T grew at 4–45 °C and at pH 6.0–8.0, with optimal growth occurring at 37 °C and at pH 8.0. Unless stated otherwise, all tests were performed at 37 °C at pH 8.0 ± 0.2 on MA supplemented with 10 % NaCl.

Cellular morphology of strain Grbi^T, including cell shape, size, flagella, endospore formation and Gram staining, was observed using a light microscope (ECLIPSE 80*i*; Nikon). Flagella were determined using the staining method (Heimbrook *et al.*, 1989). Motility was examined by the method of Tittsler & Sandholzer (1936) using semisolid agar (Motility Test Medium; BBL). Endospore formation was examined using the spore-staining method (Schaeffer & Fulton, 1933). The Gram reaction was determined using a Gram Stain kit (BBL) according to the manufacturer's instructions and was confirmed by the non-staining method (Buck, 1982). Anaerobic growth was determined by incubation in an anaerobic chamber maintained under an atmosphere of N_2 : CO_2 : H_2 (8 : 1 : 1) on MA supplemented with 10 % NaCl at 30 °C for 7 days. Catalase and oxidase activities were individually determined using a 3 % (v/v) hydrogen peroxide solution and an oxidase reagent (bioMérieux), respectively. Hydrolyses of casein, Tween 80, starch, DNA, tyrosine, cellulose, xanthine and hypoxanthine in 7 days were assayed according to the methods described by Atlas (1993), Gerhardt *et al.* (1994) and Cowan & Steel (1965). API 20NE strips (bioMérieux) were used according to the manufacturer's instructions to examine the enzyme activities of strain Grbi^T. Acid production from carbohydrate as the sole carbon source was determined with API 50CH test strips (bioMérieux) with API 50CHB/E medium salinity adjusted to 10 % (w/v) NaCl, according to the manufacturer's instructions.

Strain Grbi^T and the reference strain *Lentibacillus kapialis* JCM 12580^T were grown on pH 8.0 MA supplemented with 10 % NaCl at 37 °C for quantitative analysis of fatty acids. The cellular fatty acids were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (MIDI, 1999; Sasser, 1990). Total lipids were extracted by the modified method of Xin *et al.* (2000). The amino acid composition of the cell wall hydrolysate was determined using one-dimensional TLC on cellulose sheets (Bousfield *et al.*, 1985). Quinone extraction and identification were according to the method of Komagata & Suzuki (1987).

The 16S rRNA gene sequence of strain Grbi^T was amplified by colony PCR using a PCR Master Mix solution (iNtRON Biotechnology) with two universal primers: forward primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGYTACCTTGTTACGACTT- 3′). The PCR product was purified with a QIAquick PCR Purification kit (Qiagen) and sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems), according to the manufacturers' instructions. The reaction mixtures were analysed with an automated DNA analyser system (PRISM 3730XL DNA analyser; Applied Biosystems). The fragments of the 16S rRNA gene sequence were assembled using SeqMan software (DNASTAR), and pairwise 16S rRNA gene sequence similarities were determined using the EzTaxon server (Chun *et al.*, 2007) to locate phylogenetic neighbours. The 16S rRNA gene sequence of Grbi^T was aligned with 15 reference sequences (Fig. 1), using the multiple sequence alignment program CLUSTAL_X v. 1.83 (Thompson *et al.*, 1997). The phylogenetic relationships of representatives of the genus *Lentibacillus* were determined using the MEGA version 4 software program (Tamura *et al.*, 2007). The neighbour-joining and maximum-parsimony phylogenetic consensus tree was constructed by randomly selecting 1000 bootstrap replicates (Felsenstein, 1985). Chromosomal DNA was extracted using a G-spin DNA extraction kit (iNtRON Biotechnology), and the G + C content was determined using a fluorimetric method employing SYBR Green I and a real-time PCR thermo-cycler (Gonzalez & Saiz-Jimenez, 2002). The genomic DNA of *Escherichia coli* K-12 was used as the calibration reference (Gonzalez & Saiz-Jimenez, 2002). DNA–DNA hybridization was performed using the fluorometric method of Ezaki *et al.* (1989) with modifications (Hirayama *et al.*, 1996).

Colonies of strain Grbi^T were opaque ivory, smooth, circular, raised and 1.0–2.0 mm in diameter after cultivation at 37 °C for 3 days on MA supplemented with 10 % (w/v) NaCl. Cells of strain Grbi^T were Gram-positive, strictly aerobic rods and approximately 0.5–1.0 μm wide and 2.0–4.0 μm in length. Flagella and motility were not observed. Single, terminal, spherical endospores were observed in swollen sporangia. Strain Grbi^T was positive for catalase activity. However, the oxidase reaction with the oxidase reagent *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine was negative. Strain Grbi^T could be differentiated from related species of the genus *Lentibacillus* on the basis of morphological, cultural and physiological characteristics (Table 1).

The cellular fatty acid profile of strain Grbi^T was qualitatively similar to that of the reference strain *L. kapialis* JCM 12580^T (Table 2). The major fatty acids of strain Grbi^T were iso-$C_{16:0}$ (36.4 %), anteiso-$C_{15:0}$ (30.3 %) and iso-$C_{14:0}$ (18.2 %). Major cellular polar lipids were phosphatidylglycerol, diphosphatidylglycerol and one unknown glycolipid (Supplementary Fig. S1, available in IJSEM Online). The diagnostic diamino acid of the cell wall was *meso*-diaminopimelic acid (Supplementary Fig. S2). The predominant isoprenoid quinone was MK-7. The G + C content of the genomic DNA of strain Grbi^T was 42.5 mol%, which falls within the range for the genus

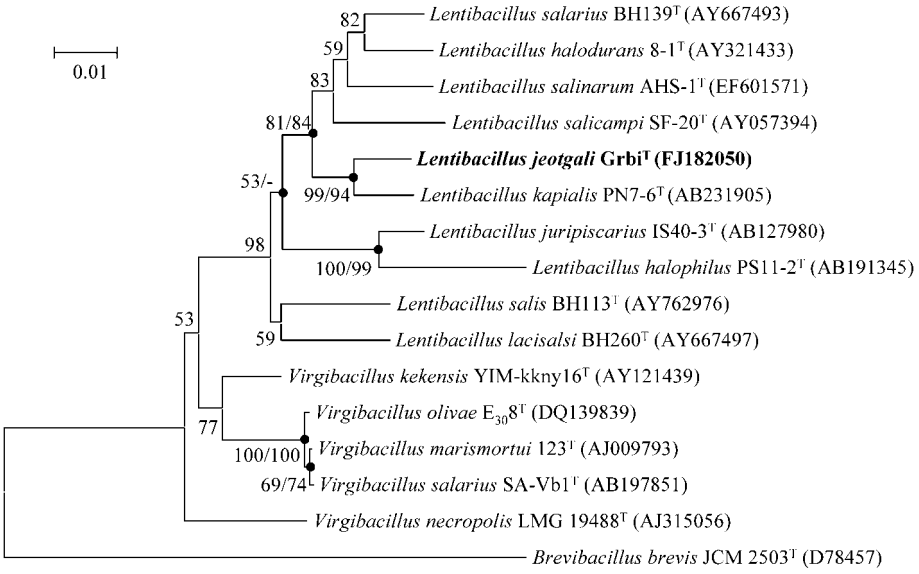

Fig. 1. Phylogenetic consensus tree based on 16S rRNA gene sequences showing the relationships between strain Grbi^T and type strains of the most closely related *Lentibacillus* species. Filled circles indicate generic branches that were present in both phylogenetic consensus trees generated by the neighbour-joining algorithm and the maximum-parsimony algorithm. Numbers at nodes indicate percentage bootstrap values, as calculated by neighbour-joining/maximum-parsimony probabilities. Bootstrap analyses were performed with 1000 repetitions and only values higher than 50 % are shown at the branch points. Bar, 0.01 accumulated changes per nucleotide.

Lentibacillus (Lee *et al.*, 2008a). Thus, the fatty-acid profile, major lipoquinone, cell wall type and DNA G+C content of strain Grbi^T were typical of the genus *Lentibacillus*.

The 16S rRNA gene sequence of strain Grbi^T was a continuous stretch of 1414 bp. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Grbi^T was associated with the genus *Lentibacillus* and located in a clade with the type strain *L. kapialis* JCM 12580^T (Fig. 1). Strain Grbi^T was most closely related to the unidentified halophilic bacterium CM-1 (NCBI accession no. EU673372.1), isolated from sludge of a saline sewage treatment plant, and the type strain *L. kapialis* JCM 12580^T, with which it shared 98.9 % and 97.5 % 16S rRNA gene sequence similarity, respectively. The 16S rRNA gene sequence similarity values between strain Grbi^T and *Lentibacillus salicampi* JCM 11462 ^{T}, *Lentibacillus salinarum* JCM 11311^T, *Lentibacillus salis* KCTC 3936^T, *Lentibacillus juripiscarius* JCM 12147^T, *Lentibacillus salarius* KCTC 3911^T, *Lentibacillus lacisalsi* KCTC 3915^T, *Lentibacillus halodurans* DSM 18342^T and *Lentibacillus halophilus* JCM 12149^T were 96.2, 96.1, 96.0, 95.7, 95.4, 95.2, 95.1 and 94.3 %, respectively. Since two strain pairs with 16S rRNA gene sequence similarity values of less than 97.0 % and DNA–DNA hybridization values of less than 70 % are definitely different species (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987), we performed a DNA–DNA hybridization test only between the newly isolated Grbi^T and *L. kapialis* JCM 12580^T. The genomic DNA hybridization value between strains Grbi^T and *L. kapialis* JCM 12580^T was 8 %, indicating that strain Grbi^T should be classified as a different species to *L. kapialis*.

On the basis of the results of phenotypic, genotypic and phylogenetic studies, strain Grbi^T represents a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus jeotgali* sp. nov. is proposed.

Description of *Lentibacillus jeotgali* sp. nov.

Lentibacillus jeotgali (je.ot.ga′li. N.L. gen. n. *jeotgali* of jeotgal, a traditional Korean fermented seafood).

Cells are non-motile, non-flagellated, Gram-positive, strictly aerobic rods and approximately 0.5–1.0 μm wide and 2.0–4.0 μm in length. Single, terminal, spherical endospores are formed in swollen sporangia. The colonies are opaque ivory, smooth, circular, raised and 1.0–2.0 mm in diameter after cultivation at 37 °C for 3 days on MA supplemented with 10 % (w/v) NaCl. Growth occurs in 3–20 % (w/v) NaCl (optimum 10–15 %), at temperatures ranging from 10 to 45 °C (optimum 37 °C), and in the pH range 6.0–8.0 (optimum pH 8.0). The isolate is catalase-positive, but oxidase- and urease-negative. Hydrolyses aesculin but not gelatin, arginine, casein, Tween 80, starch, DNA, tyrosine, cellulose, xanthine or hypoxanthine. Acid is produced from glycerol, D-ribose, D-galactose, D-glucose, D-fructose, D-mannitol, methyl α-D-mannoside, arbutin, aesculin, maltose, inulin, glycogen and 2-ketogluconate, but not from erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl β-D-xyloside, D-mannose, L-sorbose,

Table 1. Taxonomic characteristics of strain Grbi^T and closely related type strains of the genus *Lentibacillus*

Strains: 1, Grbi^T (data from this study); 2, *L. kapialis* JCM 12580^T (Pakdeeto *et al.*, 2007); 3, *L. salicampi* JCM 11462^T (Yoon *et al.*, 2002a); 4, *L. salinarum* JCM 11311^T (Lee *et al.*, 2008b); 5, *L. salis* KCTC 3936^T (Lee *et al.*, 2008a); 6, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 7, *L. salarius* KCTC 3911^T (Jeon *et al.*, 2005); 8, *L. lacisalsi* KCTC 3915^T (Lim *et al.*, 2005); 9, *L. halodurans* DSM 18342^T (Yuan *et al.*, 2007); 10, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006). All strains were catalase-positive, urease-negative rods. Symbols: +, positive reaction; −, negative reaction; w, weak reaction; ND, data not available; ai, anteiso; i, iso.

Characteristic	1	2	3	4	5	6	7	8	9	10
Spore shape	Spherical	Spherical	Spherical/ oval	Oval	Spherical	Oval	Spherical/ oval	Spherical	Spherical/ oval	Spherical
Pigmentation	−	Red	−	−	Light yellow	−	−	−	−	−
Motility	−	−	+	+	+	−	+	+	−	+
Colony diameter (mm)	1.0–2.0	1.2–3.0	1.0–2.0	0.5–1.2	0.2−0.3	0.9–3.9	ND	ND	4–5	0.2–0.6
Cell size (μm)	0.5–1.0 × 2.0–4.0	0.2–0.4 × 0.8–2.5	0.4–0.7 × 2.0–4.0	0.7–1.2 × 0–4.0	0.4–0.6 × 0.8–2.5	0.4–0.5 × 1.5–6.0	0.2–0.3 × 1.5–3.0	0.4–0.6 × 1.2–3.0	0.5 × 1.5–2.5	0.4–0.6 × 1.0–3.0
Temperature for growth (°C)										
Range	10–45	15–45	15–40	15–45	20–45	10–45	15–50	15–40	22–45	15–42
Optimum	37	37	30	37–40	37	37	30–35	30–32	30	30–37
NaCl concentration for growth (%, w/v)										
Range	3–20	5–30	2–23	3–24	5–15	3–30	1–20	5–25	5–30	12–30
Optimum	10–15	10	4–8	10–12	10	10	12–14	12–15	8–12	20–26
pH for growth										
Range	6.0–8.0	5.0–9.0	6.0–8.0	6.0–9.5	7.0–9.2	5.0–9.0	6.0–8.0	7.0–9.5	6.0–9.0	6.0–8.0
Optimum	8.0	7.0	ND	6.5–7.0	8.0	7.0	7.0–7.5	8.0	7.0–7.5	7.0–7.5
Oxidase activity	−	+	+	+	+	+	−	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	−	−
Hydrolysis of:										
Aesculin	+	−	−	+	−	−	+	−	−	−
Casein	−	−	+	−	−	+	−	−	−	−
Tween 80	−	−	+	−	−	+	−	−	−	−
Acid production from:										
L-Arabinose	−	−	−	−	−	−	+	+	ND	−
Cellobiose	−	−	w	−	−	−	ND	ND	−	−
D-Fructose	+	+	−	−	+	+	+	+	+	−
D-Galactose	+	+	w	w	−	−	ND	ND	−	−
D-Glucose	+	+	+	+	w	+	+	−	+	−
Lactose	−	−	−	−	−	−	+	−	−	−
Maltose	+	−	−	−	−	−	+	−	−	−
D-Mannitol	+	+	−	−	w	−	w	−	−	−
D-Mannose	−	w	w	−	−	−	+	−	+	−
D-Ribose	+	+	−	+	−	+	+	+	−	−
Salicin	−	−	w	−	−	−	−	−	−	−
Sucrose	−	w	−	−	−	w	ND	ND	−	−
Trehalose	−	−	−	−	+	−	w	−	−	−
D-Xylose	−	−	−	−	+	+	+	w	−	−
Major fatty acids	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{17:0}$, $C_{16:0}$
DNA G+C content (mol%)	42.5	41.6	42.4	49.0	46.2	43.4	43	44	43.4	42.4

L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, salicin, cellobiose, D-lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 5-ketogluconate. The major fatty acids are iso-$C_{16:0}$ (36.4 %), anteiso-$C_{15:0}$ (30.3 %) and iso-$C_{14:0}$ (18.2 %). The major cellular polar lipids are phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. The diagnostic diamino acid of the cell wall is *meso*-diaminopimelic

Table 2. Fatty acid composition (%) of strain Grbi^T and *L. kapialis* JCM 12580^T

Strains: 1, Grbi^T; 2, *L. kapialis* JCM 12580^T. All data are from the present study. Strain Grbi^T and the reference strain *L. kapialis* JCM 12580^T were grown on pH 8.0 MA supplemented with 10 % NaCl at 37 °C for quantitative analysis of fatty acids. Values are percentages of total fatty acids. tr, Trace (less than 1.0 %); −, not detected.

Fatty acid	1	2
iso-$C_{14:0}$	18.21	8.57
$C_{14:0}$	tr	tr
iso-$C_{15:0}$	2.12	13.81
anteiso-$C_{15:0}$	30.30	26.67
$C_{15:0}$	tr	tr
$C_{16:1}\omega7c$ alcohol	tr	−
iso-$C_{16:0}$	36.41	29.08
$C_{16:0}$	1.22	1.44
iso-$C_{17:0}$	tr	3.31
anteiso-$C_{17:0}$	9.80	15.44
iso-$C_{18:0}$	tr	tr
anteiso-$C_{19:0}$	−	tr

acid. MK-7 is the predominant isoprenoid quinone. The G+C content is 42.5 mol%.

The type strain is Grbi^T (=KCTC 13300^T=JCM 15795^T), isolated from a traditional Korean fermented seafood.

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